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Research Paper

Chemical composition, fermentation profile, microbial population and dry matter recovery of silages from mixtures of palisade grass and forage peanut

Composición química, perfil de fermentación, población microbiana y recuperación de materia seca en ensilajes de Urochloa brizantha *y* Arachis pintoi

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Abstract

The study evaluated chemical composition, fermentation profile, microbial population and dry matter recovery of silages made from mixtures of palisade grass (*Urochloa brizantha* cv. Marandu) and forage peanut (*Arachis pintoi* cv. Belmonte). The experiment was conducted and analyzed in a complete randomized factorial design using 5 levels of each forage (0, 25, 50, 75 and 100% on a fresh matter basis), with and without microbial inoculant and 3 replications. The crude protein concentration increased linearly (P<0.05) and fiber concentration decreased linearly (P<0.05) as forage peanut level in silage increased. There was a positive quadratic effect (without inoculant) and positive linear effect (with inoculant) on lactic acid concentration (P<0.05) and a positive quadratic effect (P<0.05) on lactic acid bacteria population with increasing forage peanut levels in silage. The main effects of the addition of forage peanut to palisade grass at ensiling were improvement in the chemical composition and fermentation profile of the grass silage. We recommend adding 25–75% forage peanut to palisade grass prior to ensiling to improve the quality of the resulting silage but there is little merit in adding microbial inoculant to the forage at ensiling. Feeding studies with animals would verify potential benefits in production from inclusion of legume with grass at ensiling, while studies with addition of energy sources at ensiling would determine any further benefits to be achieved in silage quality.

Keywords: Ammonia nitrogen, *Arachis pintoi*, effluent, microbial inoculant, organic acids, pH, *Urochloa brizantha*.

Resumen

En la Universidade Federal de Viçosa, Minas Gerais, Brasil, se evaluaron la composición química, el perfil de fermentación, la población microbiana y la recuperación de materia seca en ensilajes de diferentes mezclas de *Urochloa brizantha* cv. Marandu y *Arachis pintoi* cv. Belmonte (maní forrajero). El diseño experimental fue factorial (5 × 2) completamente al azar, utilizando cinco niveles de cada especie (0, 25, 50, 75 y 100% con base en materia fresca), con y sin inoculante microbiano, y tres replicaciones por tratamiento. La concentración de proteína cruda aumentó linealmente (P<0.05), mientras que la concentración de fibra disminuyó linealmente (P<0.05) con niveles crecientes de maní forrajero. Los niveles crecientes de maní forrajero presentaron un efecto cuadrático positivo (sin inoculante) y lineal positivo (con inoculante) en la concentración de ácido láctico (P<0.05), y cuadrático positivo (P<0.05) en la población de bacterias acido-lácticas. Los principales efectos de la adición de maní forrajero a la gramínea al momento

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de ensilar fueron el mejoramiento en la composición química y el perfil de fermentación del ensilaje, mientras que la adición de inoculante microbiano mostró pocos beneficios. Los mejores resultados se obtuvieron con pasto *U. brizantha* cv. Marandu en mezcla con 25–75% de maní forrajero, sin aplicación de inoculante. Se requieren estudios complementarios de alimentación con ganado para confirmar los beneficios potenciales de esta mezcla, igualmente estudios para incrementar la calidad de las mezclas mediante la adición de fuentes de energía al momento de ensilar.

Palabras clave: Ácidos orgánicos, efluente, inoculante microbiano, nitrógeno amoniacal, pH.

Introduction

In tropical regions, pasture areas of the genus *Urochloa*, including *Urochloa brizantha*, with potential for silage production of reasonable quality have been established. According to Dawo et al. (2007), the production of tropical grass silage intercropped with legumes may be a strategy to increase dry matter yields and nutritive value of diets for ruminants. Recently, Silva et al. (2018) found that palisade grass (*Urochloa brizantha* cv. Xaraés) and stylo (*Stylosanthes capitata* mixture with *S. macrocephala* cv. Campo Grande) mixed silages had good nutritive value and fermentation profile. In addition, the introduction of legumes into production systems has several benefits, such as increasing nutritive value, voluntary intake and performance of livestock, as well as contributing to lower greenhouse gas emissions (Lüscher et al. 2014).

Arachis pintoi cv. Belmonte originated from a non-seeding accession collected in the area of Belmonte, Bahia, Brazil and was the first *A. pintoi* cultivar released for vegetative propagation (Paganella and Valls 2002). The possibility of ensiling forage peanut and obtaining appropriate chemical composition and fermentative characteristics was investigated by WingChing-Jones and Rojas-Bourrillón (2006), who suggested ensiling it as a means of incorporating an ingredient with moderate protein concentration in the total ration at low cost.

Kung Jr et al. (2003) recommended the use of microbial inoculants to reduce losses when ensiling tropical grasses, since homolactic bacteria compete with the existing microflora of epiphytic microorganisms, thus increasing fermentation efficiency. This view was supported by Muck (2010), who reported that inoculants based on homolactic bacteria have been the predominant additives for use when ensiling, with beneficial effects on both fermentation and storage efficiency.

The objective of this study was to evaluate the chemical composition, fermentation profile, microbial population and dry matter recovery of silages made from mixtures of palisade grass and forage peanut with or without microbial inoculation.

Materials and Methods

Silage material and treatments

The trial was performed at the Animal Science Department of the Federal University of Viçosa (Universidade Federal de Viçosa - UFV), Minas Gerais, Brazil (20º45' S, 42º51' W; 657 masl), where mean annual rainfall is 1,341 mm, of which 86% occurs between October and March.

Palisade grass (*Urochloa brizantha* cv. Marandu) forage was harvested at 60 days of regrowth and forage peanut (*Arachis pintoi* cv. Belmonte) at the beginning of flowering, at 5 cm from ground level, using a steel blade brush cutter (STIHL®). The palisade grass and forage peanut presented dry matter yields of 7.2 t/ha and 2.6 t/ha, respectively.

Silage making

After harvesting, the forages were chopped separately into particles of approximately 2 cm using a stationary forage harvester, before being weighed and mixed in the following proportions of palisade grass and forage peanut, respectively: 100:0, 75:25, 50:50, 25:75 and 0:100 by weight (fresh matter). Each mixture was then halved and one half was inoculated with microbial inoculant, while the other remained un-inoculated. The inoculant was Sil-All® 4x4 water soluble (Alltech, Paraná, Brazil) and contained: *Lactobacillus plantarum*, *Pediococcus acidi-lactici*, *L. salivarius* ssp. *salivarius* and *Enterococcus faecium*; enzymes (xylanase, amylase, cellulase and hemicellulolytic enzyme); silicone dioxide; and saccharose. The inoculant was added at the recommended rate of 5 g/t fresh forage, diluted in deionized water and applied using a 2-L hand sprayer by spraying uniformly onto the forage that was constantly hand-mixed. Untreated material received a volume of water equal to the amount of inoculant.

After the inoculant was applied, the forage mixtures were ensiled in 20-L plastic silos equipped with snap-on lids fitted with a Bunsen valve that enabled gas release

only from fermentation. At the bottom of each silo, 4 kg of sand was placed inside a cotton bag to capture the effluent. Compression of forage was performed to give a mean density of 580 kg/m^3 (fresh matter). There were 3 replications of each treatment giving 30 silos, which were weighed at the beginning of the experiment, and stored in a covered area at 25 ± 1 ºC for 60 days. After 60 days, each silo was weighed and evaluated for effluent loss and recovery of dry matter, according to techniques described by Jobim et al. (2007).

Chemical and microbial analyses

Buffering capacity (BC) of silages was determined as described by Playne and McDonald (1966). Concentrations of water-soluble carbohydrate (WSC) in forage and of residual water-soluble carbohydrate (RWSC) in silage were determined according to the technique described by Silva and Queiroz (2002). The fermentation coefficient (FC) of the forage was calculated according to the following equation proposed by Weissbach and Honig (1996) and cited by Oude-Elferink et al. (2000): FC = DM + 8 × (WSC/BC),

where:

DM is dry matter (g/kg);

WSC is water-soluble carbohydrate (g/kg); and

BC is buffering capacity (meq HCl/100 g DM).

To determine chemical composition, fresh forage and silage samples were dried in an oven at 55 °C until constant weight, and then ground in a Wiley mill with a 1-mm sieve. These samples were used to determine the concentrations of: DM (AOAC 2005; method number 930.15); crude protein (CP; from total N) according to the Kjeldahl method (AOAC 2005; method number 976.05); acid detergent-insoluble nitrogen (ADIN) according to Licitra et al. (1996); acid detergent fiber (ADF) and lignin according to AOAC (2005; method number 973.18); and ash- and protein-free neutral detergent fiber (NDFap) according to Licitra et al. (1996) and Mertens (2002).

To conduct the microbial counts, 25 g of fresh forage was transferred into a sterile container with 225 mL of sterile solution (Ringers Solution®) to obtain a dilution of 10^{-1} and was then homogenized for 4 min in an industrial blender. Serial dilutions were prepared with MRS (Man, Rogosa and Sharp) agar (*Lactobacillus* MRS Broth®, Difco Laboratories, Detroit, MI, USA) to determine lactic acid bacteria (LAB) numbers, after incubation at 37 ºC for 48 hours, and to determine enterobacteria numbers, after incubation at 37 °C for 24 hours in VRB (Violet Red Bile) agar (Difco Laboratories, Detroit, MI, USA) using the pour-plate technique. Mold and yeast numbers were determined using 3M™ Petrifilm™, after incubation at 25 °C for 3 and 5 days for yeast and mold, respectively. The mold and yeast colony-forming units (cfu) were enumerated separately, according to their macromorphological features, using values between 30 and 300 cfu for counting, and the results obtained were transformed into log x in order to achieve a normal distribution. Duplicate samples were assessed for each species.

Chemical composition, buffering capacity (BC), fermentative capacity (FC) and microbial population of fresh palisade grass, forage peanut and their mixtures prior to inoculation, are shown in Table 1.

Table 1. Chemical and microbial composition of fresh *Urochloa brizantha* cv. Marandu (palisade grass) and *Arachis pintoi* cv. Belmonte (forage peanut) prior to inoculation and ensiling.

Parameter	Palisade grass	Forage peanut
Dry matter (g/kg)	262	210
Crude protein (g/kg DM)	51.4	179
NDF (g/kg DM)	775	472
NDFap (g/kg DM)	729	375
ADF (g/kg DM)	455	338
ADIN (g/kg DM)	100	134
Lignin (g/kg DM)	26.8	58.5
Lignin:ADF ratio	0.06	0.17
WSC (g/kg DM)	15.2	49.8
BC (meq HCl/100 g DM)	4.25	7.14
Fermentative capacity	22.7	35.6
LAB (log cfu/g FM)	5.90	6.63
Enterobacteria (log cfu/g FM)	6.49	8.01
Molds + yeasts (log cfu/g FM)	5.76	6.85

NDF = neutral detergent fiber; NDFap = ash- and protein-free neutral detergent fiber; ADF = acid detergent fiber; ADIN = acid detergent-insoluble nitrogen; WSC = water-soluble carbohydrate; BC = buffering capacity; LAB = lactic acid bacteria; FM = fresh matter; log = denary logarithm of the numbers; cfu = colony-forming unit.

To determine pH, 25 g of silage from each silo was homogenized in 225 mL of distilled water in an industrial blender for 1 min and pH was immediately measured with a pH meter. For determination of NH_3-N concentration (expressed as % of total nitrogen, TN), the extract was filtered through filter paper and the filtrate was used according to Bolsen et al. (1992).

To determine organic acids in the silages, 25 g of silage was homogenized with 225 mL distilled water in an industrial blender for 1 min. The aqueous extracts were filtered, acidified with 20% metaphosphoric acid solution and centrifuged for 15 min, according to Kung Jr (1996). Analysis of organic acids was performed using high-performance liquid chromatography (HPLC) of Shimadzu-BIORAD mark, SPD-10 model, C18 column, reverse phase at a wavelength of 210 nm.

Statistical analyses

The experiment was analyzed as a complete randomized factorial (5 × 2) design using increasing fresh matter levels of *A. pintoi* cv. Belmonte (0, 25, 50, 75 and 100%) in silages of *U. brizantha* cv. Marandu, with and without microbial inoculant, and 3 replicates per treatment. The results were subjected to analysis of variance, with the means of the quantitative factors subjected to regression analysis, selecting equations with a coefficient of determination >0.5, and the means of the qualitative factors were compared using the F-test with 5% probability for a type I error using the statistical program SAEG 9.1 (UFV 2007)

Results

Chemical composition of silages

The concentrations of DM, CP, ADIN, ADF, lignin and RWSC were affected (P<0.01) by increasing levels of forage peanut (Table 2). The concentrations of DM and ADF decreased linearly, while CP, ADIN and lignin increased linearly with increasing levels of forage peanut in silage (Table 3). NDFap concentration was affected by an interaction between forage peanut level and microbial inoculant (P<0.05) (Table 2), decreasing linearly with increasing forage peanut level, with and without microbial inoculant (Table 3). The RWSC concentration was affected (P<0.01) by increasing level of forage peanut (Table 2), but no statistical model was adjusted to the RWSC data since mean concentration was only 14 g/kg DM (Table 3).

Treatment with microbial inoculant affected ADIN (P<0.01), NDFap (P<0.01), lignin (P<0.01) and RWSC (P<0.05) concentrations (Table 2).

Fermentation profile, microbial population and effluent loss

There was a significant interaction between forage peanut level and microbial inoculant with respect to pH (P<0.05) and organic acids (P<0.01) (Table 4). The pH of silage increased linearly as forage peanut level increased (P<0.05), in both un-inoculated and inoculated silages (Table 3). While NH_3-N percentage was affected (P<0.05) by increasing level of forage peanut (Table 4), no statistical model adjusted to the NH_3-N data, which showed a mean of 93.5 g/kg total N (Table 3).

Table 2. Chemical composition of silages made from *Urochloa brizantha* cv. Marandu (palisade grass) and *Arachis pintoi* cv. Belmonte (forage peanut) and their mixtures without and with microbial inoculant.

	Forage peanut level (%)						Significance			CV (%)
	0	25	50	75	100	Mean[1]	L	MI	L×MI	
					Dry matter (g/kg)					
C	265	258	243	226	207	240	**	NS	NS	0.9
I	269	261	243	226	208	241				
					Crude protein (g/kg DM)					
C	54.7	79.2	106	134	164	108	**	NS	NS	4.0
I	59.3	82.1	102	131	162	107				
					ADIN (g/kg DM)					
C	75.1	95.3	106	121	125	104a	**	**	NS	5.1
I	69.7	82.8	99.1	106	123	96.2b				
					NDFap (g/kg DM)					
C	706	633	543	462	388	546a	**	**	**	3.1
I	584	509	441	483	396	488b				
					ADF (g/kg DM)					
C	455	434	409	384	359	408	**	NS	NS	2.1
I	449	432	416	388	356	408				
					Lignin (g/kg DM)					
C	31.1	38.5	47.6	54.6	62.1	44.8a	**	**	NS	7.1
I	24.2	33.1	46.2	47.6	53.0	40.8b				
					RWSC (g/kg DM)					
C	12.5	18.8	8.8	34.2	11.9	17.2a	**	*	NS	51.7
I	3.5	21.6	2.6	16.3	9.7	10.7b				

NDFap = neutral detergent fiber corrected for ash and protein; AIDN = acid detergent-insoluble nitrogen; ADF = acid detergent fiber; RWSC = residual water-soluble carbohydrate; C = Control (un-inoculated); I = inoculated; L = forage peanut level; MI = microbial inoculant; L × MI = interaction between forage peanut level and microbial inoculant. [1]Means within the same column and parameter followed by different letters differ significantly at P<0.05.

Table 3. Regression equations for silages made from *Urochloa brizantha* cv. Marandu (palisade grass), *Arachis pintoi* cv. Belmonte (forage peanut) and their mixtures without and with microbial inoculant.

Variable	Regression equation	r^2/R^2
Dry matter (g/kg)	$Y = 271.033 – 0.610133X$	0.98
Crude protein (g/kg DM)	$Y = 54.1667 + 1.0608X$	0.99
NDFap[1] (g/kg DM)	$Y = 707.655 – 3.2232X$	0.98
NDFap[2] (g/kg DM)	$Y = 563.126 – 1.50958X$	0.66
ADF (g/kg DM)	$Y = 454.187 – 0.929467X$	0.97
ADIN (g/kg total N)	$Y = 74.7867 + 0.510133X$	0.96
Lignin (g/kg DM)	$Y = 28.7533 + 0.3008X$	0.95
RWSC (g/kg DM)	$\overline{X} = 14$	
pH[1]	$Y = 4.14067 + 0.00608X$	0.96
pH[2]	$Y = 4.30333 + 0.00421333X$	0.97
NH_3/TN (%NH_3 of total N)	$\overline{X} = 93.5$	
Lactic acid[1] (g/kg DM)	$Y = 19.3696 + 0.237867X – 0.00170056X^2$	0.81
Lactic acid[2] (g/kg DM)	$Y = 10.3608 + 0.140792X$	0.91
Acetic acid[1](g/kg DM)	$Y = 10.6319 + 0.10101X$	0.87
Acetic acid[2] (g/kg DM)	$Y = 15.3107 - 0.108578X + 0.00156997X^2$	0.58
Propionic acid[1] (g/kg DM)	$\overline{X} = 4.8$	
Propionic acid[2] (g/kg DM)	$Y = 4.00847 + 0.0349003X$	0.91
Butyric acid (g/kg DM)	$\overline{X} = 0.8$	
Lactic acid bacteria (log cfu/g FM)	$Y = 7.63156 + 0.00725874X – 0.0000936216X^2$	0.50
Molds and yeasts[1] (log cfu/g FM)	$Y = 2.85373 + 0.0113453X$	0.80
Molds and yeasts[2] (log cfu/g FM)	$Y = 2.81131 + 0.0228047X – 0.000154406X^2$	0.86
DM recovery[1] (%)	$\overline{X} = 86.5$	
DM recovery[2] (%)	$\overline{X} = 85.6$	

NDFap = neutral detergent fiber corrected for ash and protein; ADF = acid detergent fiber; ADIN = acid detergent-insoluble nitrogen; RWSC = residual water-soluble carbohydrate; NH_3/TN = ammonia N as a percentage of total nitrogen; X = 0, 25, 50, 75 and 100% forage peanut in the mixture with palisade grass. [1]Control (un-inoculated); [2]Inoculated.

Table 4. Fermentation parameters of silages made from *Urochloa brizantha* cv. Marandu (palisade grass), *Arachis pintoi* cv. Belmonte (forage peanut) and their mixtures without and with microbial inoculant.

	Forage peanut level (%)						Significance			CV (%)
	0	25	50	75	100	Mean[1]	L	MI	L×MI	
					pH					
C	4.14	4.29	4.45	4.61	4.74	4.44b	**	**	*	0.9
I	4.29	4.41	4.52	4.63	4.71	4.51a				
					NH_3/Total N (%)					
C	73.0	101	83.7	162	92.3	102	*	NS	NS	29.6
I	90.7	76.1	104	90.4	61.2	84.5				
					Lactic acid (g/kg DM)					
C	19.7	23.2	27.9	27.5	26.1	24.9a	**	**	**	7.1
I	11.2	12.7	17.9	19.9	25.2	17.4b				
					Acetic acid (g/kg DM)					
C	11.6	12.2	14.4	20.0	20.3	15.7	**	NS	**	4.9
I	13.8	16.8	13.0	13.9	21.3	15.8				
					Propionic acid (g/kg DM)					
C	4.50	5.20	4.80	4.80	4.80	4.82b	**	**	**	6.4
I	4.20	4.70	5.70	6.40	7.70	5.74a				
					Butyric acid (g/kg DM)					
C	0.80	0.70	0.90	0.70	0.90	0.80	**	NS	**	3.7
I	0.70	0.80	0.80	0.70	0.80	0.76				

C = Control (un-inoculated); I = inoculated; L = forage peanut level; MI = microbial inoculant; L × MI = interaction between forage peanut level and microbial inoculant. NH_3/Total N = ammonia nitrogen as a proportion of Total N. [1]Means within the same column and parameter followed by different letters differ significantly at $P<0.05$.

In the absence of microbial inoculant, concentration of lactic acid showed a positive quadratic relationship (P<0.01; Table 3) with increasing forage peanut level in silage, and estimated maximum value was 27.7 g/kg DM with 75% forage peanut (fresh matter); in the presence of microbial inoculant, lactic acid concentration increased linearly with increasing forage peanut level (P<0.01; Table 3). In contrast, a linear increase (P<0.01; Table 3) in acetic acid concentration was observed in the absence of microbial inoculant, while with microbial inoculant, there was a negative quadratic effect (P<0.01; Table 3), with an estimated maximum value of 13.4 g/kg DM with approximately 35% forage peanut (fresh matter). In the absence of microbial inoculant, an average concentration of 4.8 g propionic acid/kg DM was recorded, while with microbial inoculant, propionic acid concentration increased linearly with increasing forage peanut level (P<0.01; Table 3). While butyric acid concentration was affected (P<0.01) by increasing levels of forage peanut, no statistical model adjusted to the butyric acid data which had a mean of 0.8 g/kg DM (Table 3).

Population of LAB was affected by increasing levels of forage peanut (P<0.05; Table 5). There was a quadratic relationship between forage peanut level and LAB population (P<0.05; Table 3) with a calculated maximum value of 7.77 log cfu/g FM with 38.2% forage peanut (fresh matter). Microbial inoculant had no effect (P>0.05) on the LAB population (mean 7.64 log cfu/g FM).

Populations of molds + yeasts were affected by an interaction between level of forage peanut and microbial inoculant (P<0.01; Table 5). There was a linear increase (P<0.01; Table 3) in mold + yeast populations in silages without microbial inoculant, ranging from 2.85 to 2.87 log cfu/g FM with increasing forage peanut level. However, in inoculated silages, there was a quadratic effect (P<0.01; Table 3), with an estimated maximum value of 3.65 log cfu/g FM with 73.8% forage peanut (fresh matter). No enterobacteria were detected in the silages.

Effluent losses were similar (mean 4.56 kg/t FM; P>0.05) for all silages and the total DM recovery was affected by a significant interaction between level of forage peanut and microbial inoculant (P<0.01; Table 5). However, no statistical model was adjusted to the total DM recovery data where means were 86.5% (un-inoculated) and 85.6% (inoculated) (Table 3).

Table 5. Microbial population, effluent losses and dry matter recovery of silages made from *Urochloa brizantha* cv. Marandu (palisade grass), *Arachis pintoi* cv. Belmonte (forage peanut) and their mixtures without and with microbial inoculant.

	Forage peanut level (%)						Significance			CV (%)
	0	25	50	75	100	Mean	L	MI	L×MI	
						LAB (log cfu/g FM)				
C	7.57	7.67	7.76	7.82	7.39	7.64	*	NS	NS	2.2
I	7.71	7.84	7.70	7.55	7.39	7.64				
						Molds + yeasts (log cfu/g FM)				
C	2.83	3.23	3.27	3.82	3.95	3.42	**	NS	*	5.5
I	2.82	3.26	3.60	3.62	3.56	3.37				
						Effluent losses (kg/t FM)				
C	3.91	3.94	6.36	4.14	3.62	4.39	NS	NS	NS	30.9
I	4.69	5.48	4.01	3.40	6.10	4.73				
						DM recovery (%)				
C	83.9	88.9	87.9	84.4	87.6	86.5	**	*	**	1.1
I	86.6	87.3	86.0	82.9	85.1	85.6				

LAB = lactic acid bacteria; FM = fresh matter; log = denary logarithm of the numbers; cfu = colony-forming unit; C = Control (un-inoculated); I = inoculated; L = forage peanut level; MI = microbial inoculant; L × MI = interaction between forage peanut level and microbial inoculant.

Discussion

Estimated dry matter (DM) concentrations in ensiled material ranged from 262 to 210 g/kg DM, with concentrations declining with increasing levels of forage peanut. However, this difference was not enough to affect the effluent loss. Although McDonald et al. (1991) recommended 30 g DM/kg fresh forage as the minimum value in forage at ensiling to minimize loss of effluent, effluent losses in our study can be considered low for a perennial tropical grass and legume. Despite the reduction in DM concentration of silage with the addition of forage peanut, the average value of 262 g/kg for palisade grass before ensiling meets the minimum value of 260 g/kg recommended by Haigh (1999) for good-quality silage production. In contrast, the DM concentration observed in forage peanut (210 g/kg FM) was lower than this recommendation. WingChing-Jones and Rojas-Bourrillón (2006) also recorded a low DM concentration (200 g/kg FM) for 2 cultivars of forage peanut harvested at 12 weeks of regrowth. Wilting forage before ensiling would have overcome this issue but all silages in our study produced limited amounts of effluent, even without wilting.

According to Mahanna (1993), concentration of desirable water-soluble carbohydrates (WSC) in forages before ensiling should fall in the range of 40–60 g/kg, if DM concentration in forage is <350 g/kg FM and good fermentation is expected. In our study, the WSC concentration in palisade grass (15.2 g/kg DM) was much lower than that for forage peanut (49.8 g/kg DM) before ensiling. The WSC concentration in forage is critical for the production of good quality silage because it is the main source of nutrients for the growth of microorganisms that produce lactic acid. However, tropical species usually have a low concentration of WSC because higher temperatures increase metabolic activity and the synthesis of structural compounds, causing decrease in WSC (Van Soest 1994). Other authors have also found low WSC concentrations in *U. brizantha* cv. Marandu, e.g. Bernardes et al. (2005) (11 g/kg DM) and Arroquy et al. (2014) (21.4 g/kg DM).

The differing concentrations of WSC in the 2 forages were reflected in the differing fermentation coefficient (FC), which was lower in palisade grass (22.7) than in forage peanut (35.6). According to Oude-Elferink et al. (2000), forages with insufficient fermentable substrate or low DM concentration have a FC <35. The ensiling potential of 10 tropical forage legumes and one tropical forage grass was evaluated by Heinritz et al. (2012) and FC ranged from 30 to 68 for legumes, while for *Brachiaria* (now: *Urochloa*) grass hybrid cv. Mulato II FC was 52. In our study, FC for cv. Marandu was much lower than that reported for Mulato II, possibly due to lower WSC and DM concentrations. Addition of energy sources, e.g. sucrose or molasses, at ensiling is practised in some situations to increase fermentable energy supply for bacteria (Heinritz et al. 2012; Bureenok et al. 2013; Rosa et al. 2018).

It is important to highlight that the chemical composition of palisade grass silage was improved by the inclusion of forage peanut due to the higher CP concentration in the legume. Including 25–75% forage peanut with grass at ensiling increased CP concentration in the resulting silage by 41–133%. Qu et al. (2013) also reported that CP% in silage made from intercropped corn and lablab bean [*Lablab purpureus* (L.) Sweet] was greater and fiber concentration was lower than those of corn monoculture.

The lower NDFap and ADF concentrations observed with increasing proportion of forage peanut in the silage are due to lower concentrations of these cell wall constituents in the legume compared with grass. In contrast, lignin concentration and lignin:ADF ratio increased with increasing proportion of forage peanut in the ensiled material, which could contribute to a reduction in silage digestibility (Van Soest 1994). While we did not determine digestibility in the present study, Cardoso et al. (2018) found higher in vitro DM degradability for forage peanut than for *Urochloa decumbens* cv. Basilisk.

A fact related to fermentation profile of the silages was the increase in pH values with increasing levels of forage peanut, probably due to the higher buffering capacity of legumes (McDonald et al. 1991; Kung Jr et al. 2018), as found by Heinritz et al. (2012), who registered an average pH >5.0 for tropical legume silages.

The ammonia nitrogen (NH_3-N) concentrations obtained in silages in this study were within the recommended ranges for good quality silage. According to Kung Jr et al. (2018), plant and microbial proteolytic processes lead to changes in nitrogenous compounds in silages, and the fermentation results in an increase in NH_3-N (usually less than 100–150 g/kg total N). Furthermore, higher than normal levels of soluble N and NH_3-N in wet legume silages are usually a result of proteolytic activity from clostridia. However, we did not verify this in our study.

According to Kung Jr et al. (2018), typical concentrations of lactic acid in commonly fed silages range from 20 to 40 g/kg DM. While acetic acid usually ranges from 10 to 30 g/kg DM, propionic acid is usually undetectable or at very low concentrations (<10 g/kg DM) in good silages and butyric acid should not be detectable in well-fermented silages. In our study, the estimated lactic acid concentrations in the various silages were around 20 g/kg DM (except in inoculated palisade grass, when, based on the respective equation in Table 3, there was up to 50% forage peanut), while acetic acid concentrations were between 10 and 20 g/kg DM, propionic acid concentration was below 10 g/kg DM, and butyric acid concentration was 0.8 g/kg DM.

The ratio of lactic acid to acetic acid is also commonly used as a qualitative indicator of fermentation and this ratio should be 2.5:1 to 3.0:1 in good quality silage (Kung Jr et al. 2018). However, according to these authors, silages with very high lactic acid:acetic acid ratios may sometimes be more aerobically unstable than those with normal ratios because low concentrations of acetic acid may not be sufficient to inhibit lactate assimilating yeasts. In contrast, lactic acid:acetic acid ratios below 1.0 are usually an indication of abnormal fermentations. In our study, the ratio of lactic acid to acetic acid was below 2.5, indicating that the silage would not be considered good quality silage; however, the values were above 1.0, indicating that there was not an abnormal fermentation, except for inoculated palisade grass (lactic:acetic ratio = 0.67).

The LAB population found in palisade grass and forage peanut before ensiling was higher than that reported by Muck (1996) (5.0 log cfu/g FM), under temperate climate conditions, as adequate for the occurrence of good fermentation in silage. In our silages, LAB population exceeded 7.0 log cfu/g FM.

While analyses indicate that DM recovery in the silages was affected by an interaction between increasing level of forage peanut and inoculant, losses were quite inconsistent and failed to follow a definite pattern, with overall means for DM recovery of 86.5 and 85.6%, for un-inoculated and inoculated silages, respectively. This finding is in agreement with the results reported by Cezário et al. (2015) that addition of microbial inoculant did not improve DM recovery (mean of 85%) in palisade grass silages. The authors attributed this to variation in the population of epiphytic bacteria and fungi pre-existing in the forage that could interact with the microbial inoculant. According to Muck (2010), the inefficiency of many commercial inoculants in wet tropical grass silages may result from the inclusion of inappropriate species of lactic acid bacteria or species unable to effectively compete with epiphytic flora when applied at low doses.

Conclusions

The addition of forage peanut to palisade grass during the ensiling process improved the chemical composition and fermentation profile of resulting silage over that of pure grass silage, while adding microbial inoculant at ensiling produced no significant benefit to the resulting silage. We recommend adding 25–75% forage peanut to palisade grass (FM basis) prior to ensiling to produce better quality silage than that from pure grass, with higher levels of legume having the potential to support higher production levels in animals because of increased CP concentration. Feeding studies with animals should be conducted to determine production benefits to be obtained from the mixed silage. Since the mixed silages studied are still not of good quality, further studies to enhance quality by adding energy sources at ensiling seem warranted.

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