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Mixed silages of sugarcane and forage peanut treated with *Lactobacillus buchneri*

Silagens mistas de cana-de-açúcar e amendoim forrageiro tratadas com Lactobacillus buchneri

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Abstract
There is evidence for the beneficial effects of forage peanut on the nutritive value and fermentation profile of silages; however, its effects on sugarcane silage have not been determined. The objective of the study was to evaluate the chemical composition, fermentation profile, microbial composition, and dry matter recovery (DMR) of sugarcane silage containing various amounts of forage peanut (*Arachis pintoi* cv. Belmonte) (0%, 25%, 50%, and 75% on a fresh matter basis), treated or untreated with *Lactobacillus buchneri*. A completely randomized 4 × 2 factorial design was used with three replications. The interaction between forage peanut levels and inoculant influenced the concentrations of dry matter, crude protein, neutral detergent fiber and acid detergent fiber, organic acids and ethanol, populations of lactic acid bacteria and yeast, gas and effluent losses, and DMR. Forage peanut levels had effects on dry matter, hemicellulose, acid detergent insoluble nitrogen, pH, and ammonia nitrogen. Increasing proportions of forage peanut increased the protein content and decreased the fiber content in the silage, while also reducing the production of ethanol and effluent. We recommend the inclusion of 40%–75% forage peanut in the sugarcane ensilage to improve the chemical composition and fermentation profile. Furthermore, inoculation with *L. buchneri* associated with forage peanut increases the concentration of antifungal acids in the silage and decreases the yeast population and ethanol production.
Keywords: Chemical composition; Dry matter recovery; Ethanol; Microorganisms; Organic acids

Resumo
O objetivo do estudo foi avaliar composição química, perfil fermentativo, população de microrganismos e recuperação de matéria seca (RMS) de silagem de cana-de-açúcar contendo níveis crescentes (0, 25, 50 e 75%, na base da matéria natural) de amendoim forrageiro (*Arachis pintoi* cv. Belmonte), tratadas ou não com *Lactobacillus buchneri*. Usou-se o esquema fatorial 4×2, no delineamento inteiramente casualizado, com três repetições. Verificou-se efeito de interação níveis de amendoim forrageiro e inoculante para teores de matéria seca, proteína bruta, fibra em detergente neutro e ácido, ácidos orgânicos e etanol, população de bactérias láticas e leveduras, perdas por gases e por efluente e RMS. Houve efeito de níveis de amendoim forrageiro no teor de hemicelulose, nitrogênio insolúvel em detergente ácido, pH e nitrogênio amoniacal. Verificou-se que o aumento de níveis de amendoim forrageiro incrementou teor de proteína e diminuiu teor de fibra, além de reduzir a produção de etanol e de efluente. Recomenda-se inclusão de 40% a 75% de amendoim forrageiro na ensilagem de cana-de-açúcar para melhorar a composição química e o perfil de fermentação. A inoculação com *L. buchneri* associada ao amendoim forrageiro aumenta a concentração de ácidos antifúngicos na silagem e decresce a população de leveduras e a produção de etanol.
Palavras-chave: Ácidos orgânicos; Composição química; Etanol; Microrganismos; Recuperação de matéria seca

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Introduction

Sugarcane (*Saccharum* spp.) is a forage resource traditionally used to feed ruminants. To facilitate its handling and optimize labor utilization, its ensilage is recommended. However, abundant yeast and soluble carbohydrates in sugarcane silages result in intense alcoholic fermentation and dry matter (DM) loss[1].

Bacterial additives can act as auxiliaries in sugarcane silage fermentation processes. In particular, *Lactobacillus buchneri* reduces the yeast population and increases aerobic stability[1,2] via acetic acid production[3,4]. As an alternative to additives, mixtures of legumes and grasses have been evaluated at the time of ensiling, with the aim of improving the nutritional and/or fermentative characteristics of the silage[5]. *Arachis pintoi* 'Belmonte' originated from a non-seeding accession collected from the area of Belmonte, Bahia, Brazil and was the first *A. pintoi* cultivar released for vegetative propagation[6]. Recently, it was found that forage peanut improves the nutritive value and fermentation profile of silages of Marandu signal grass[7].

Sugarcane has an intensive fermentation process. The addition of forage peanut, a culture with a lower soluble carbohydrate content and higher buffering capacity, has the potential to reduce the fermentation intensity, with a slower decrease in the pH of the ensiled material, thereby increasing the production of organic acids with antifungal effects and minimizing ethanol production.

The objective of this study was to evaluate the chemical composition, fermentation profile, microorganism population, and dry matter recovery (DMR) of sugarcane silage with increasing levels of forage peanut (0%, 25%, 50%, and 75%, based on fresh matter), with and without the inclusion of *L. buchneri*.

Materials and Methods

The trial was performed in two areas (area 1 and area 2) at the Animal Science Department of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil. Forage peanut (*Arachis pintoi* 'Belmonte') was harvested on the same day as sugarcane 'RB 76-5418.' Both forage crops were already established and received adequate management for each species in separate areas.

The soil in area 1 with sugarcane was classified as eutrophic red-yellow Argisol[8]. Sugarcane received organic fertilization with dry cattle manure and unknown dose. The soil in area 2 with forage peanut was classified as dystrophic red-yellow latosol, according to the soil taxonomy[8]. The forage peanut was established approximately 10 years before the study and was eventually used for the production of experimental silages, with unknown fertilization management and without grazing. A factorial trial (4 × 2) with a completely randomized design and three replications was performed. The treatments consisted of sugarcane ensiled with four levels of forage peanut (0%, 25%, 50%, and 75%, fresh matter basis), with or without the addition of the microbial inoculant *L. buchneri* NCIMB 40788 (Silomax Cana, 2.5 × 10^{10} CFU/g, Matsuda, Brazil).

Sugarcane was manually harvested, and forage peanut was harvested at the beginning of flowering using a brush cutter (STIHL®), both at a height of 5 cm above ground level. Both forages were harvested on the same day and were chopped in a stationary ensilage machine (JF 60, Maxxium, São Paulo, Brazil) adjusted to obtain an average particle size of 1–2 cm. The forage was subjected to treatment with the inoculant. Five grams of inoculant were applied to each ton of natural material and diluted in 1 L of water for application, following the manufacturer's recommendations, with a back sprayer with a capacity of 5 L. The untreated material (0%) received a volume of water equal to the amount of inoculant.

The forage was ensiled in plastic buckets with a capacity of 20 L, and compaction was performed, generating an average density of 695 kg/m³. The tops of the buckets contained a Bunsen valve to allow the escape of fermentation gases. A cotton bag containing 4 kg of sand was placed at the bottom of each bucket to enable the estimation of effluent loss. After ensiling, the silos were stored for 60 days. Subsequently, gas and effluent losses and total dry matter losses were calculated according to Jobim et al.[9]

The microbial groups were enumerated from 25 g of each sample, to which 225 mL of phosphate-buffered saline was added to obtain a 10^{-1} dilution[10]. Successive dilutions were performed with the aim of 10^{-3} to 10^{-7} dilutions for the detection of lactic acid bacteria (LAB), 10^{-2} to 10^{-6} for enterobacteria, and 10^{-1} and 10^{-5} for molds + yeasts, in forage samples before ensilage. For silages samples, dilutions ranging from 10^{-2} to 10^{-6} were prepared for LAB and dilutions from 10^{-2} to 10^{-6} were obtained for enterobacteria, molds, and yeasts.

The selective culture media in Petri dishes were MRS agar® (Difco Laboratories, Detroit, MI, USA) for LAB, with incubation for 48 h at 37°C, and Violet Red Bile (VRBGA; Oxoid, Basingstoke, United Kingdom) for enterobacteria, incubated for 24 h at 37°C, both using the pour plate technique. For molds and yeasts, the Petrifilm system (3M Microbiology Products, St. Paul, MN, USA) was used after incubation for 3 and 5 days at 25°C for yeasts and molds, respectively. Plaques with between 30 and 300 colony-forming units (CFUs) were considered eligible for counting. To determine pH values, 25 g of silage was collected from each silo and

100 mL of distilled water was added. After resting for 1 h, pH was determined using a potentiometer (Tecnal, São Paulo, Brazil).

To determine organic acid contents, an aqueous extract was prepared using 20 g of fresh material, 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. A 20 mL aliquot of this filtered material was centrifuged at 25,000 × *g* for 25 min at -20°C[11] to quantify the organic acids according to Siegfried et al.[12]. The organic acids used for the standard calibration curve were acetic acid, propionic acid, butyric acid, and lactic acid, all at an initial concentration of 10 mmol/L, except for acetic acid, which had an initial concentration of 20 mmol/L. The samples were analyzed on an Ultimate 3000 Dual Chromatograph (Dionex, Sunnyvale, CA, USA) coupled to a Shodex RI-101 refractive index detector (Showa Denko; Kawasaki, Kanagawa, Japan) at 45°C and equipped with a 300 × 7.8 mm Rezex ROA ion exchange (Phenomenex; Torrance, CA, USA) maintained at 45°C. The mobile phase was 4.2 mmol/L sulfuric acid (H_2SO_4) and 0.35 mmol/L sodium-free ethylenediaminetetraacetic acid at a flow rate of 0.7 mL/min.

To determine the chemical composition of the silages, approximately 400 g of samples were collected from each silo, pre-dried in an oven with forced air ventilation at 55°C until reaching a constant weight, and then ground in a "Willey" knife mill with a 1 mm sieve. Samples were subjected to DM analyses[13] following method 930.15; crude protein (CP) was evaluated by the determination of total nitrogen according to the Kjeldahl method[13] following method 976.05; neutral detergent fiber corrected for protein (NDFp)[14] and acid detergent fiber (ADF)[13] were obtained following method 973.18; and acid detergent-insoluble nitrogen (ADIN) was evaluated[15]. Water-soluble carbohydrates were extracted in 80% ethanol, according to the methodology described by Silva and Queiroz[16], and the ammoniacal nitrogen (NH_3-N, %TN) content was determined according to Bolsen et al.[17] Table 1 shows the chemical composition and microbial populations in forage containing sugarcane and forage peanut mixtures, without the microbial inoculant, before ensiling.

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

Table 1. Chemical composition (%DM) and microbial populations (CFU/g) in sugarcane and forage peanut mixtures, before ensiling

	Forage peanut level (%)			
	0	**25**	**50**	**75**
DM	17.56	18.15	19.41	19.78
CP	4.00	10.89	14.41	17.05
NDFp	58.28	58.36	59.23	56.59
ADF	40.13	35.46	33.16	33.79
ADIN	17.40	18.49	16.09	17.03
WSC	23.11	19.86	9.12	8.51
pH	5.06	5.11	5.35	5.54
LAB	7.85	7.32	7.73	7.39
MOL+YEA	5.67	5.89	5.96	6.21
ENT	6.83	6.76	6.88	7.46

Results

With respect to the chemical composition of silages, the interaction between the forage peanut proportion and microbial inoculant had effects on the DM, CP, NDFp, and ADF contents. The forage peanut level had an effect on HEM and ADIN contents (Table 2). Despite the effect of the proportion of forage peanut, we did not obtain an equation to effectively describe the DM content, with average of 19.2%.

The CP content of the silages increased linearly as the level of forage peanut in the ensiled sugarcane increased, varying from 4.26% to 16.4% and from 3.90% to 16.1%, without and with inoculant, respectively. There was a linear decrease in the NDFp content of sugarcane silages with increasing levels of forage peanut, from 55.3% to 49% and from 56.9 to 45.9%, without and with inoculant, respectively. The ADF in uninoculated silage also showed a linear reduction with increasing levels of forage peanut. In the presence of inoculant, a quadratic model was fit to the ADF data, with a minimum ADF content of 30.3% for 54.5% forage peanut in the silage.

The hemicellulose content, although affected by levels of forage peanut, was not fitted by a regression equation, with an average value of 18.0%. The ADIN content showed quadratic behavior as a function of increasing forage peanut levels, with a maximum level of 17.2% for 62.9% forage peanut in the mixture. The pH and ammonia nitrogen content (NH_3-N %TN) were affected by the levels of forage peanut. There were effects of the interaction between the forage peanut level and inoculant on the concentrations of lactic, acetic, propionic, butyric acids, and ethanol (Table 3).

Table 2. Chemical composition (%DM) of mixed silage of sugarcane and forage peanut, without (NI) and with (I) *L. buchneri*, and respective regression equations

Inoculant	Forage peanut level (%)				EPM	P-value		
	0	25	50	75		P	I	P × I
			DM		0.17	0.16	0.02	<0.01
NI	18.71	19.44	17.91	19.34				
I	20.26	19.37	19.63	18.69				
			CP		0.95	<0.01	0.06	0.02
NI	3.71	8.95	12.73	15.96				
I	3.91	8.13	11.72	16.27				
			NDFp		0.81	<0.01	0.37	<0.01
NI	54.72	53.25	52.57	47.94				
I	56.78	54.69	47.11	47.11				
			HEM		0.55	<0.01	0.44	0.41
NI	17.28	19.61	18.12	15.71				
I	18.87	22.10	16.75	15.69				
			ADF		0.55	<0.01	<0.01	<0.01
NI	37.44	33.64	34.44	32.23				
I	37.91	32.59	30.36	31.41				
			ADIN		0.47	<0.01	0.44	0.81
NI	11.99	15.60	16.53	17.33				
I	13.10	16.72	16.45	17.17				
			Regression Equation					r²
			CP					
NI			Y=4.262+0.16201P					0.98
I			Y=3.90367+0.16259P					0.99
			NDFp					
NI			Y=55.27233-0.08411P					0.52
I			Y=56.914-0.14644P					0.98
			HEM					
			Y= 18.02					
			ADF					
NI			Y=36.66167-0,05933P					0.67
I			Y=37.9195-0.27822P+0.00255P²					0.94
			ADIN					
			Y=12.73067+0.14336P-0.00114P²					0.62

DM: dry matter; CP: crude protein; NDFp: neutral detergent fiber corrected for protein; HEM: hemicellulose; ADF: acid detergent fiber; ADIN: acid detergent-insoluble nitrogen; P = forage peanut level; I = microbial inoculant; P × I = interaction of forage peanut level and microbial inoculant; SEM = standard error of the mean.DM: dry matter; CP: crude protein; NDFp: neutral detergent fiber corrected for protein; HEM: hemicellulose; ADF: acid detergent fiber; ADIN: acid detergent-insoluble nitrogen; P = forage peanut level; I = microbial inoculant; P × I = interaction of forage peanut level and microbial inoculant; SEM = standard error of the mean.

Table 3. Fermentation profile characteristics of mixed silage of sugarcane and forage peanut, without (NI) and with (I) *L. buchneri*, and respective regression equations

Inoculant	Forage peanut level (%)				SEM	P-value		
	0	25	50	75		P	I	P × I
			pH		0.07	<0.01	0.74	0.14
NI	3.41	3.46	3.68	4.23				
I	3.44	3.52	3.61	4.26				
			NH_3-N (%TN)		0.46	<0.01	0.86	0.56
NI	9.75	4.22	4.67	7.48				
I	8.55	4.70	4.75	7.80				
			Lactic acid (%DM)		0.45	<0.01	<0.01	<0.01
NI	8.16	13.38	8.02	7.95				
I	6.44	6.39	8.90	10.25				
			Acetic acid (%DM)		0.26	<0.01	<0.01	<0.01
NI	8.13	6.02	6.53	6.07				
I	3.89	6.05	7.12	7.64				
			Butyric acid (%DM)		0.00	<0.01	<0.01	<0.01
NI	0.03	0.04	0.06	0.05				
I	0.02	0.02	0.02	0.03				
			Propionic acid (%DM)		0.01	0.02	0.58	<0.01
NI	0.65	0.53	0.63	0.60				
I	0.51	0.61	0.63	0.70				
			Ethanol (%DM)		0.23	<0.01	0.01	<0.01
NI	5.85	3.65	3.73	2.91				
I	4.97	4.70	3.07	2.46				
			Regression Equation					r²
			pH					
			Y= 3.44383-0.00568P+0.000216P²					0.96
			NH_3-N (%TN)					
			Y= 9.04033-0.24582P+0.00305P²					0.79
			Lactic acid (%DM)					
NI			Y=9.37					
I			Y= 5.905+0.05573P					0.87
			Acetic acid (%DM)					
NI			Y= 7.95433-0.07249P+0.000664P²					0.71
I			Y= 4.325+0.04927P					0.89
			Butyric acid (%DM)					
NI			Y= 0.03367+0.00034667P					0.60
I			Y= 0.02017-0.00012667P+0.000004P²					0.72
			Propionic acid (%DM)					
NI			Y= 0.6295-0.00282P+0.000036P²					0.14
I			Y= 0.526+0.00231P					0.77
			Ethanol (%DM)					
NI			Y= 5.34833-0.035P					0.77
I			Y= 5.17667-0.03763P					0.92

NH_3-N: ammoniacal nitrogen; TN: total nitrogen; DM: dry matter; P = forage peanut level; I = microbial inoculant; P × I = interaction of forage peanut level and microbial inoculant; SEM = standard error of the mean.

The pH values showed a quadratic behavior, with a minimum value of 3.41 for 13.1% forage peanut in the silage. The NH_3-N %TN content also showed quadratic behavior, with a minimum value of 4.10% obtained with the addition of 40.3% forage peanut. Regression models did not adequately fit the lactic acid content, with an average of 9.37% without inoculant. With inoculant, it showed a linear increase with increasing levels of forage peanut.

The acetic and propionic acid contents in the uninoculated silage were described by a quadratic function, with minimum values of 5.98% acetic acid for 54.6% of forage peanut and 0.57% propionic acid for 39.2% forage peanut. In the presence of the inoculant, the behavior was linear for both acids, with contents ranging from 4.32% to 8.02% for acetic acid and from 0.53% to 0.75% for propionic acid with increasing levels of forage peanut. The butyric acid content showed a linear trend in the absence of the inoculant, ranging from 0.03% to 0.06%. With the use of the inoculant, the butyric acid content showed a quadratic effect, with a minimum content of 0.02% in sugarcane silage with 15.8% forage peanut.

Ethanol decreased linearly, irrespective of the use of the inoculant, ranging from 5.35% to 2.71% and from 5.22% to 2.35% for 0% and 75% forage peanut levels in the silage, respectively. There was an interaction effect of forage peanut level and the microbial inoculant on LAB and yeast populations (Table 4). There was no effect of the treatments on mold populations.

Table 4. Populations of microorganisms of mixed silage of sugarcane and forage peanut, without (NI) and with (I) *L. buchneri*, and respective regression equations

Inoculant	Forage peanut level (%)				SEM	P-value		
	0	25	50	75		P	I	P × I
	LAB (log ufc/g)				0.11	<0.01	0.09	<0.01
NI	7.27	6.76	6.98	7.75				
I	7.23	7.56	6.02	7.48				
	YEA (log ufc/g)				0.15	<0.01	<0.01	<0.01
NI	3.95	3.31	3.25	3.30				
I	4.27	5.29	3.46	3.38				
	MOL (log ufc/g)				0.08	0.26	0.11	0.85
NI	2.30	2.75	2.15	2.50				
I	2.74	3.00	2.65	2.45				
	Regression Equation							r^2
	LAB							
NI	$Y = 7.16415 – 0.0296616P + 0.000518842P^2$							0.75
I	$Y = 7.32722 – 0.0431144P + 0.000585634P^2$							0.75
	YEA							
NI	$Y = 3.95151 – 0.20393P1/2 + 0.0148857P$							0.99
I	$Y = 4.32318 + 0.468372P1/2 – 0.0706733P$							0.72

LAB: lactic acid bacteria population; YEA: yeast population; MOL: mold population; P = forage peanut level; I = microbial inoculant; P × I = interaction of forage peanut level and microbial inoculant SEM = standard error of the mean.

A quadratic model fitted LAB growth, obtaining minimum population of 6.7 CFU/g for 28.6% forage peanut without microbial inoculant and 6.5 CFU/g for 36.8% forage peanut with the inoculant. A quadratic root model fitted yeast population growth, with populations ranging from 3.95 to 3.30 CFU/g and from 4.32 to 3.01 CFU/g for sugarcane silage with 0% and 75% forage peanut, without and with microbial inoculant, respectively. Mold populations were not affected by the treatments, with an average value of 2.56 CFU/g. We detected effects of the interaction between forage peanut level and the inoculant on effluent and gas losses and DMR (Table 5).

Effluent production decreased linearly with increasing forage peanut levels, from 97.2 to 14.2 kg/t FM and 96.1 to 1.72 kg/t FM without and with inoculant, respectively. Gas losses, without and with *L. buchneri*, exhibited quadratic behavior. Without inoculant, the minimum value was 2.30% for 50.5% forage peanut in the silage; with the use of inoculant, the minimum value was 2.94% for 58.1% forage peanut. Regression equations were not adequately fitted to DMR values obtained without the microbial inoculant. With the inoculant, DMR values exhibited a linear trend, ranging from 83.8% to 96.0% with 0% to 75% forage peanut in the sugarcane silage, respectively.

Table 5. Effluent and gas losses and dry matter recovery of mixed silage of sugarcane and forage peanut, without (NI) and with (I) *L. buchneri*, and respective regression equations

Inoculant	Forage peanut level (%)				SEM	P-value		
	0	25	50	75		P	I	P × I
	Effluent losses (kg/t FM)				7.49	<0.01	0.02	<0.01
NI	85.13	92.34	32.47	12.92				
I	101.77	64.81	15.79	13.25				
	Gas losses (%)				0.72	<0.01	<0.01	<0.01
NI	8.99	3.11	2.87	3.64				
I	12.35	6.11	3.03	3.79				
	DM Recovery (%)				1.12	0.03	<0.01	<0.01
NI	95.73	97.73	88.78	95.82				
I	82.65	88.10	94.93	92.93				
	Regression Equation							r^2
	Effluent losses							
NI	$Y= 97.19067-1.10597P$							0.83
I	$Y= 96.09033-1.25832P$							0.88
	Gas losses							
NI	$Y= 8.79483-0.25679P+0.00254P^2$							0.83
I	$Y= 12.38783-0.32535P+0.0028P^2$							0.98
	DM Recovery							
NI	$Y= 95.01$							
I	$Y= 83.79152+0.16308P$							0.72

FM: fresh matter; DM: dry matter. P = forage peanut level; I = microbial inoculant; P × I = interaction of forage peanut level and microbial inoculant; SEM = standard error of the mean.

Discussion

The low DM contents obtained in the silages were related to low DM contents obtained for sugarcane and forage peanut before ensiling (Table 1). The linear relationship between the forage peanut level and CP content can be explained by the high content of this nutrient in the legume, as observed by Gomes et al.[18] Thus, the protein content of sugarcane silage can be improved by the addition of forage peanut, with an increase of up to 4.12 times for a legume content of 75%, which is nutritionally favorable for ruminants. Gomes et al.[18] observed an increase in the CP content by up to 2.4 times when ensiling *Urochloa brizantha* 'Marandu' with forage peanut comprised 75% of total fresh matter.

As the levels of forage peanut in the silage increased, the NDFp and ADF contents in the DM of the silages decreased (Table 2). When undesirable fermentation is frequent, such as alcoholic fermentation in sugarcane, the fiber content increases in silage DM as soluble carbohydrates are reduced[1], resulting in effluent losses[21]. Furthermore, the high proportion of indigestible NDF in sugarcane makes it difficult to solubilize the cell wall during fermentation in the silo and in the rumen[19]. Therefore, silages with lower NDF contents can provide higher fiber digestibility and consequently reduce the limiting effect on DM intake.

The pH value obtained for exclusive sugarcane silage (3.44) (Table 3) was similar to the values reported by Cardoso et al.[20] and Jesus et al.[21] of 3.41 and 3.39, respectively, and indicated a good fermentation quality of silages, mainly due to the relatively low contents of butyric acid and acetic and propionic acids in inoculated silages. Sugarcane silages with the addition of legumes generally have higher pH values, as observed by Pereira et al.[22] in analyses of sugarcane and pigeon pea mixed silages, which were also fitted by a quadratic equation.

Legumes have greater buffering capacity due to the presence of cations (K^+, Ca^{2+}, and Mg^{2+}) that are neutralized when they come into contact with organic acids, preventing a sharp reduction in pH during the fermentation process[23], which could explain the higher pH values in the silage with the highest proportion of forage peanut, a characteristic of silages of forage species in the family *Poaceae*. Generally, silages inoculated with some strains of *L. buchneri* have slightly elevated pH values due to the degradation of lactic acid into acetic acid[24,25]. However, in our study, the pH was not affected by the

inoculant.

All mean NH_3-N values, regardless of the use of inoculant and forage peanut addition, remained below the 10% limit recommended by Kung Jr. et al.[26] for good quality silage, indicating low activity of clostridial bacteria, which result in proteolysis and butyric acid production. As a result, butyric acid concentrations were also minimal (Table 3). Clostridial activity is one of the main indicators of undesirable fermentation, causing losses of DM and energy, while also affecting the intake of silage by animals[27]. Other microorganisms, such as yeasts and bacilli, can also produce small amounts of butyric acid[28].

The ethanol concentration was negatively related to acetic and propionic acid contents (Table 3) in inoculated silages. This result is interesting and provides evidence for the interactive effect of *L. buchneri* and forage peanut on sugarcane silage by increasing acetic acid production, considering that in sugarcane silages, the population of acetic acid-producing bacteria tends to be minimal due to substrate competition with yeasts[21].

Due to the buffering capacity of the legume, there was an increase in heterofermentative species, supported by the positive effect of the inoculant on the concentrations of acetic and propionic acid, which have antifungal properties and inhibit yeast growth, according to Danner et al.[29], consequently decreasing ethanol production. With reduced yeast activity, lactic acid also showed a linear increase due to the decrease in its use for alcoholic fermentation, according to Pahlow et al.[30]

Some strains of *L. buchneri* do not have the ability to reduce acetyl phosphate to ethanol, possibly due to the lack of acetaldehyde dehydrogenase, and thus increase the concentration of acetic acid as the final fermentation product[31]. Although *L. buchneri* does not produce propionic acid, in the conversion of lactic acid to acetic acid, 1,2-propanediol is also formed, which can be a precursor for the conversion to propionic acid and 1-propanol by the epiphytic microorganism *L. diolivorans*[32], explaining why silages treated with *L. buchneri* can show an increase in the propionic acid content in the silage[33].

The fungicidal effect of acetic and propionic acid is due to their lipophilicity. In an acidic environment, these acids can permeate the yeast cell membrane; within the cell, at neutral pH, the dissociation and release of protons and counter-ions results in acidification of the intracellular environment and affects pH homeostasis, lipid organization, and cell membrane function, which can lead to the death of these microorganisms[29,34].

According to Kung Jr. et al.[26], in good quality silage, lactic acid concentrations should be 60–80 g/kg DM, acetic acid should be up to 30 g/kg MS, propionic acid should be up to 5 g/kg DM, and butyric acid concentrations should be less than 5 g/kg DM. Although the mean values for butyric acid in the present study were within the appropriate range, values exceeding the recommended range were obtained for acetic, lactic, and propionic acids (Table 3). This may be due to the high concentrations of soluble carbohydrates in these silages, resulting in high organic compound production during fermentation (mainly lactic acid, acetic acid, and ethanol), which may correspond to up to 22% of the DM of sugarcane silages, according to Daniel et al.[35] This differs from other silages adopted in animal feed, such as corn silage, which is generally used as a parameter for the average values of acids produced.

An acetic acid content above 2% in DM is desirable for silages with a high content of soluble carbohydrates, e.g., sugarcane, as they cause less yeast activity. This is in addition to the low total amount of CO_2 released as a by-product of the formation of acetic acid relative to the amount released during alcoholic fermentation[1], reducing gas losses and improving aerobic stability after opening the silos. The addition of forage peanut acts as an alternative to reduce yeast activity during the fermentation process in sugarcane silage (Table 4), as microorganisms are one of the main issues in the ensiling of this crop[20].

In all silages in this study, no enterobacteria were detected, which can be explained by the acidity of the medium. The silos were opened 60 days after ensiling. According to Luis and Ramirez[36], these bacteria normally multiply until approximately the seventh day of fermentation, when they are replaced by lactic groups, which are more resistant to pH reductions.

The DM content, type of fermentation, and silage density (compaction) influence effluent production[21]. As the density (695 kg/m^3) was similar among all treatments and the DM content showed little variation at the time of ensiling (±2%), it is believed that variation in effluent losses is due to variation in fermentative microorganisms, especially yeasts[37].

According to Kung Jr. et al.[38], the ensiled mass must contain a DM content of 30% to 35% to guarantee minimum effluent losses and to maintain the nutritive value of the silage, via the inhibition of undesirable microorganisms, such as *Clostridia*. Even though the DM contents of all silages in the present study were below this range, the addition of forage peanut was sufficient to reduce effluent losses, reducing material leaching (Table 5). The silages in the present study showed less loss (Table 5) than that of sugarcane silages reported in other studies, even though the DM content at the time of ensiling was higher[39,40]. Therefore, the losses are reasonable for sugarcane-forage peanut mixed silages.

Gas losses are directly related to the activity of heterofermentative microorganisms in the ensiled mass, mainly yeasts, where they convert soluble carbohydrates and lactic acid into acetic acid, ethanol, CO_2, and heat[20,41]. The results for gas losses in the present study may be explained by the effects on the yeast population and the reduction in ethanol production, resulting in reduced gas

production with the addition of up to 58% forage peanut in the sugarcane silage. Ren et al.[42] have shown that reductions in gas losses and a higher DMR indicate that undesirable secondary fermentations were not as frequent during fermentation, which is positive from a nutritional point of view, resulting in more adequate conservation of the material.

In some studies, effects of inoculation with *L. buchneri* on the losses and DMR of sugarcane silages have not been observed[20,39], while other studies have supported the effectiveness of *L. buchneri* [1,40]. These conflicting results can be attributed to differences among strains, including differences in metabolism and the ability to survive in the silage environment, even for strains of the same species[43]. Therefore, these results indicate that forage peanut can be included in sugarcane silages to reduce ethanol production and losses by gases and effluent, with a consequent increase in DMR. This provides a complementary strategy to generate associative effects with the action of the heterofermentative inoculant, in addition to improving the DMR of the silage.

Conclusions

The inclusion of 40%–75% forage peanut in the ensilage of sugarcane improves the chemical composition and fermentation profile. Inoculation with *L. buchneri* associated with forage peanut increases the concentrations of antifungal acids in the silage and decreases the yeast population and ethanol production. Further studies, particularly those focused on animal diets, would improve our understanding of the beneficial effects of this strategy.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: K. G. Ribeiro, T. C. da Silva and O. G. Pereira; *Data curation*: *D. R. da Costa; Formal analysis:* T. C. da Silva; *Investigation*: D. R. da Costa and L. L. Cardoso; *Methodology*: K. G. Ribeiro and T. C. da Silva; *Project administration*: K. G. Ribeiro; *Supevision*: K. G. Ribeiro and O. G. Pereira; *Validation*: K. G. Ribeiro; *Visualization*: D. R. da Costa and K. G. Ribeiro; *Writting (original draft)*: D. R. da Costa; *Writting (review & editing)*: K. G. Ribeiro and G. F. de L. Cruz.

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