

Research Paper

Effects of adding agro-industrial by-products and bacterial inoculant at ensiling on nutritional quality and bacterial colonization of Tifton 85 [*Cynodon dactylon* (L.) Pers.] silages

Efectos de agregar subproductos agroindustriales e inoculante bacterial al momento de ensilar en la calidad nutritiva y la colonización bacterial de ensilajes de Tifton 85 [Cynodon dactylon (L.) Pers.]

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Abstract

The objective of this study was to evaluate effects of adding agro-industrial by-products (soybean hulls and corn-processing residue) and bacterial inoculant to Tifton 85 forage at ensiling on nutritional quality and bacterial colonization of resulting silages. The design was completely randomized in a 3 × 2 factorial scheme, with 6 treatments and 4 replicates. Treatments were: Tifton 85 forage; Tifton 85 + soybean hulls; Tifton 85 + corn-processing residue; Tifton 85 + bacterial inoculant; Tifton 85 + soybean hulls + inoculant; and Tifton 85 + corn-processing residue + inoculant. Inclusion of by-products increased dry matter and organic matter percentages of silages, while addition of soybean hulls improved crude protein concentration in silage. Total digestible nutrients in silages containing by-products were higher than in straight Tifton 85 silage. Addition of by-products increased in vitro dry matter and organic matter digestibilities of resulting silages. Most treatments showed aerobic stability up to 144 hours after exposure to air, except for Tifton 85 + corn-processing residue without inoculant, which became unstable by 120 hours of exposure. Addition of by-products at ensiling of Tifton 85 forage appears beneficial but there seems little benefit in adding bacterial inoculant. More studies on a larger scale are needed to confirm these preliminary results, while feeding studies would determine any improvement in animal performance when fed silage containing by-products.

Keywords: Digestibility, fodder conservation, gas production, *Lactobacillus*, pH, tropical grass.

Resumen

El objetivo de este estudio fue evaluar los efectos de la adición de subproductos agroindustriales (cáscara de soja y residuos del procesamiento de maíz) e inoculante bacteriano al forraje Tifton 85 al momento de ensilar, sobre la calidad nutricional y la colonización bacteriana de los ensilajes resultantes. El diseño fue completamente al azar en un esquema factorial 3 × 2, con 6 tratamientos y 4 repeticiones. Los tratamientos fueron: forraje Tifton 85; Tifton 85 + cáscaras de soja; Tifton 85 + residuo de procesamiento de maíz; Tifton 85 + inoculante bacteriano; Tifton 85 + cascarilla de soja + inoculante; y Tifton 85 + residuo de procesamiento de maíz + inoculante. La inclusión de subproductos aumentó los porcentajes de materia seca y materia orgánica de los ensilajes, mientras que la adición de cáscaras de soja mejoró la concentración de proteína cruda en el ensilaje. Los nutrientes digestibles totales en los ensilajes que contenían subproductos fueron más altos que

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en el ensilaje de solo Tifton 85. Además, la adición de subproductos aumentó la digestibilidad in vitro de la materia seca y de la materia orgánica de los ensilajes resultantes. La mayoría de los tratamientos mostraron estabilidad aeróbica hasta 144 horas después de la exposición al aire, excepto Tifton 85 + residuo de procesamiento de maíz sin inoculante, que se volvió inestable a las 120 horas de exposición. La adición de subproductos en el ensilaje del forraje Tifton 85 parece beneficiosa, pero parece haber poco beneficio en la adición de inoculante bacteriano. Se necesitan más estudios a mayor escala para confirmar estos resultados preliminares, mientras que los estudios de alimentación determinarían cualquier mejora en el rendimiento de los animales cuando se alimentan con ensilaje que contiene subproductos.

Palabras clave: Conservación de forrajes, digestibilidad, *Lactobacillus*, pasto tropical, pH, producción de gas.

Introduction

Grasses of the *Cynodon* genus are widely used to produce conserved forages, due to rapid growth rate, high dry matter production and elevated nutritional value (Souza et al. 2006). Among these grasses Tifton 85 Bermuda grass [*Cynodon dactylon* (L.) Pers.] is of interest, as it has a higher neutral detergent fiber digestibility and lower lignin concentration than other cultivars of the genus (Mandebvu et al. 1999).

Forage from *Cynodon* species is often ensiled, as an alternative to hay making because of reduced drying time (Arriola et al. 2015). However, some characteristics of these species, such as low levels of soluble carbohydrates and high humidity at the ideal vegetative stage for harvesting, may limit the ability to produce quality silages (Evangelista et al. 2000). In this context, addition of absorbent material, such as ground cereals and agro-industrial by-products, can reduce moisture content, thus avoiding losses due to undesirable fermentation, effluent production and deterioration (Paziani et al. 2006), as well as improving chemical and nutritional composition of silage.

Additives with high pectin levels, such as soybean hulls, have high water-absorption capacity, and even in forage with high moisture content, pectin can make water unavailable, thus hindering development of undesirable microorganisms (Rodrigues et al. 2005). By-products obtained during maize cleaning and milling are also high-quality ingredients (Strazzi 2015), which can be added to forage at ensiling to help reduce moisture concentration and increase nutrient concentration of resulting silage. Bacterial inoculants are also used to improve fermentative characteristics of silage by increasing prevalence of lactic acid bacteria in the epiphytic population, increasing production of lactic acid and promoting a drop in pH (Adesogan et al. 2004; Bernardes and Chizzoti 2012).

Our hypothesis is that simultaneous addition of agro-industrial by-products and bacterial inoculant at ensiling of forage will improve nutritional characteristics and bacterial colonization of resultant silage. Thus, the

objective of this study was to evaluate effects of adding agro-industrial by-products and bacterial inoculant to Tifton 85 forage at ensiling on nutritional quality and bacterial colonization of silage produced after 60 days of storage.

Materials and Methods

The experiment was carried out in Marechal Cândido Rondon (24°31'51" S, 54°01'02" W; 392 masl), in the state of Paraná, Brazil. The animal experimentation protocol was approved by the local Ethics Committee on Animal Use (case no. 06411).

Experimental design and treatments

The design was completely randomized with 6 treatments distributed in a 2 × 3 factorial arrangement. Treatments evaluated were silages made from: Tifton 85 forage (TS); Tifton 85 forage + soybean hulls (TSSH); Tifton 85 forage + corn-processing residue (TSCR); Tifton 85 forage + inoculant (TSI); Tifton 85 forage + soybean hulls + inoculant (TSSHI); and Tifton 85 forage + corn-processing residue + inoculant (TSCRI). Each treatment had 4 replicates, totaling 24 experimental silos.

Forage was harvested from 1.6 ha of Tifton 85 grown on a red clayey eutrophic Latosol with a clay texture (Santos et al 2018) and chemical characteristics as shown in Table 1. At 43 days of regrowth forage was harvested at 5 cm from ground level with a shredder coupled to a tractor and ensiled according to the various treatments. Soybean hulls and corn-processing residue (Table 2) were added to their respective treatments at 100 g/kg fresh forage, aiming to elevate dry matter (DM) concentration of ensiled material to 300 g DM/kg. Inoculant used consisted of *Lactobacillus plantarum* with manufacturer-guaranteed levels of 4.0×10^{10} colony forming units (CFU) per gram, *Pediococcus acidilactici* (1.0×10^{10} CFU/g), cellulase and a carrier. It was applied to chopped forage using a pressure sprayer at a concentration of 2 g inoculant/t fresh forage.

Table 1. Chemical analysis of soil in the area used to produce Tifton 85 forage.

(H ₂ O)	P	OM	K	Ca ²⁺	Mg ²⁺	Al ³⁺	(H+Al)	SB	CEC	BS	Clay
PH	(mg/dm ³)			(cmol _c /dm ³)						(%)	
5.30	36.1	23.2	0.71	5.19	1.89	0.00	4.96	7.79	12.7	61.1	58.1

OM = organic matter; H+Al = potential acidity; SB = sum of bases; CEC = cation exchange capacity; BS = base saturation.

Table 2. Chemical composition (g/kg dry matter) of Tifton 85 forage, soybean hulls and corn-processing residue.

Variable	Fresh Tifton 85 forage	Soybean hulls	Corn-processing residue
Dry matter (g/kg as ensiled)	247	882	890
Organic matter	910	952	895
Crude protein	132	112	109
Neutral detergent fiber	748	648	491
Acid detergent fiber	382	464	213

Subsequently, the forage was homogenized and stored in experimental PVC silos (10 cm in diameter and 50 cm long), equipped with a Bunsen-type valve. A 5 cm layer of autoclaved, dry sand was placed in the bottom of the silo covered by a layer of cotton cloth to drain liquids. Compaction was performed with a wooden stick to a compaction density of approximately 1.58 kg fresh forage per silo (0.003925 m³) resulting in a calculated specific mass of approximately 402 kg fresh forage/m³. Caps were applied to the experimental silos and sealed with adhesive tape before silos were stored at room temperature for 60 days.

Data collection and analytical procedures

Silos were opened after 60 days of storage and a 5 cm layer of silage from both ends of the silo was discarded and remaining material was homogenized and sampled for analysis. For evaluation of aerobic stability, 300 g samples of silage from each silo were selected and packed in plastic flasks at room temperature. Temperatures of silage in plastic flasks plus room temperature were measured with a digital probe thermometer daily at 14:00 h for 6 days (144 hours) after opening. An increase of 2 °C above room temperature was considered as loss of aerobic stability (O'Kiely et al. 2001).

Evaluation of hydrogen potential (pH) was performed immediately after silo opening, as described by Cherney and Cherney (2003). For analysis of ammoniacal nitrogen (NH₃-N), 200 g samples were pressed in a

hydraulic press for juice extraction. Extracted liquid was used to determine NH₃-N by the potassium hydroxide distillation method, proposed by Fenner (1965) and adapted by Vieira (1980).

For chemical analysis, samples were dried in a forced-air oven (55 °C for 72 h) and ground through a 1 mm sieve screen in a Wiley mill (Star FT-80/2, Fortinox, Piracicaba, SP, Brazil). Samples were analyzed according to AOAC (1990) methodology for DM (method 934.01), ash (method 938.08) and crude protein (CP; method 981.10), while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991) as adapted to the Ankom²²⁰ Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Amounts of organic matter (OM) were calculated as the difference between ash and total DM. Acid detergent insoluble protein (ADIP) was obtained from protein analysis of ADF residue. Total digestible nutrient concentration (TDN) was estimated according to the equation described by Kunkle and Bates (1998):

$$\text{TDN} = \text{OM} \times (26.8 + 0.595 \times \text{IVOMD})$$

where:

TDN = total digestible nutrients;

OM = organic matter; and

IVOMD = in vitro organic matter digestibility.

Dry matter recovery (DMR) was estimated according to the method of weighing silos and dry matter before and after ensiling, described by Jobim et al. (2007):

$$\text{DMR} = \left(\frac{\text{FMop} \times \text{DMop}}{\text{FMcl} \times \text{DMcl}} \right) \times 100$$

where:

FMop = forage mass at opening;

DMop = dry matter % at opening;

FMcl = forage mass at closing; and

DMcl = dry matter % at closing.

For in vitro procedures, ruminal fluid was collected from 3 Jersey steers fitted with ruminal cannulae. Animals were grazing Tifton grass pasture and received a concentrate mix of ground corn, soybean meal and minerals.

In vitro gas production was measured using the technique described by Theodorou et al. (1994) and adapted by Mauricio et al. (1999) by means of a wireless computerized system Ankom RF-Gas production

system (Ankom Technology, Macedon, NY, USA). Pressure of gases produced in the bottles was measured every 10 min for 48 h and then converted to volume. Volume of accumulated gas was corrected for fermented DM. Kinetic parameters of ruminal fermentation were estimated following the bicompartamental model proposed by Schofield et al. (1994).

In vitro dry matter digestibility (IVDMD) was estimated using the technique of Tilley and Terry (1963) and adapted by Holden (1999) using a Daisy^{II} Incubator (Ankom Technology, Macedon, NY, USA). In vitro organic matter digestibility (IVOMD) was determined by burning the remaining residue after incubation. To determine in vitro cell wall digestibility (IVCWD), we used the technique of Goering and Van Soest (1970).

Bacterial population was determined using culture techniques according to Silva et al. (1997). Briefly, 225 mL of sterile distilled water was added to 25 g of silage. The solution was stirred and considered as dilution 10^{-1} . From this solution, we pipetted 1 mL in successive dilutions of 10^{-2} to 10^{-8} , using test tubes containing 9 mL distilled water. Subsequently, diluted extracts were placed in Petri dishes, using 0.1 mL of inoculum per plate seeded on the surface and 1 mL for plates seeded at depth.

For determination of enterobacteria numbers, samples were seeded at depth on plates with Violet Red Bile Agar and incubated at 35 °C for 24 h. For analysis of *Clostridium* spp., samples were seeded on the surface in plates with Reinforced Clostridial Agar and incubated under anaerobic conditions at 35 °C for 24 h, using an incubator with a CO₂ gas system (TE 399 Tecnal; Tecnal Laboratory Equipment, Piracicaba, SP, Brazil). For lactic acid bacteria, samples were seeded on Man, Rogosa and Sharpe Agar and incubated for 48 h at 37 °C. After incubation times were reached, plates containing 25–250 colonies were selected for counting. Colony counting was performed using a Quebec Counter (CP 608, Phoenix Lufarco, Araraquara, SP, Brazil) and values were transformed into log base 10.

Statistical analyses

All statistical analyses were performed using the MIXED procedure of SAS (Statistical Analysis System, version 9.2; SAS Institute Inc., Cary, NC, USA). The experimental design was completely randomized in a 2 × 3 factorial scheme (inoculant × by-products). The mathematical model adopted was:

$$Y_{ijk} = \mu + I_j + B_k + IB_{jk} + \varepsilon_{ijk},$$

where:

Y_{ijk} = temperature value at a given aerobic exposure period;

μ = overall mean;

I_j = fixed effect of inoculant;

B_k = fixed effect of by-product;

IB_{jk} = effect of interaction between inoculant and by-product; and

ε_{ijk} = random error.

Data were submitted to analysis of variance and when significant, effects of agro-industrial by-product, inoculant and their interaction were compared using the Tukey test. Significance was reported at $P < 0.05$.

Silage temperature during aerobic exposure was analyzed as repeated measurements over time. The mathematical model adopted was:

$$Y_{ijkl} = \mu + I_i + B_j + IB_{ij} + \delta_{ijk} + T_l + BT_{jl} + IBT_{ijl} + \varepsilon_{ijkl};$$

where:

Y_{ijkl} = temperature value at a given aerobic exposure period;

μ = overall mean;

I_i = fixed effect of inoculant;

B_j = fixed effect of by-product;

IB_{ij} = effect of interaction between inoculant and by-product;

δ_{ijk} = random effect of silo;

T_l = fixed effect of aerobic exposure period;

BT_{jl} = interaction effect between by-product and aerobic exposure period;

IBT_{ijl} = interaction effect between inoculant, by-product and aerobic exposure period; and

ε_{ijkl} = random error.

Covariance structure was chosen by considering the lowest Akaike Information Criterion. Structures of covariance tested included variance compounds (VC), compound symmetry (CS), first-order autoregressive (AR (1)) and unstructured (UN).

Results

Chemical composition and in vitro nutritional evaluation

There were significant interactions between by-product and inoculant for concentrations of organic matter (OM) and acid detergent insoluble protein (ADIP) only. Addition of by-products increased DM concentration in silages ($P < 0.05$), with highest values occurring in TSCR followed by TSSH and then TS ($P < 0.05$; Table 3). Organic matter concentration was greater for TSCR and TSSH than for TS ($P < 0.05$) without inoculant but in the presence of inoculant OM for TSSH was greater than

for both TSCR and TS. Crude protein concentration for TSSH was greater than for TS independent of inoculant ($P<0.05$). Concentration of ADIP in the absence of inoculant was greater for TS and TSSH than for TSCR, while in the presence of inoculant ADIP was similar for all silages. However, adding inoculant reduced ADIP for TS and TSSH but raised ADIP for TSCR ($P<0.05$). Neutral detergent fiber and ADF were influenced by type of by-product used, with lowest values for TSCR regardless of inoculant ($P<0.05$). Total digestible nutrient values for TSSH and TSCR were higher than for TS ($P<0.05$) with no effect of inoculant. Dry matter recovery was not influenced by addition of inoculant or by-product ($P>0.05$).

Regarding in vitro gas production (Table 4), production of gas from the rapidly degradable (Fraction A) and slowly degradable (D) fractions was higher in TSSH than in TS ($P<0.05$) with no effect of inoculant ($P>0.05$). However, total gas production (A + D) followed the order TSSH>TSCR>TS ($P<0.05$). There were interactions between by-product and inoculant for degradation rates of rapidly (B) and slowly degradable (E) fractions and time for bacterial colonization (C) ($P<0.05$). For B, by-product had no significant effect ($P>0.05$), while inoculant

addition slowed rate of degradation for TS ($P<0.05$). In absence of inoculant, E for TS exceeded those for TSSH and TSCR, while there was no effect of by-product when inoculant was added. For TS adding inoculant slowed rate of degradation ($P<0.05$). In absence of inoculant, lag time for bacterial colonization (C) for TS was greater than for TSSH and TSCR ($P<0.05$), while in presence of inoculant, by-product had no effect ($P>0.05$). Interestingly adding inoculant shortened time for colonization for TS but lengthened it for TSCR ($P<0.05$).

In vitro dry matter digestibility and IVOMD were influenced by use of by-products ($P<0.05$), being higher for TSSH and TSCR than for TS, with no effect of inoculant ($P>0.05$) (Table 4). In vitro cell wall degradability (IVCWD) followed the order TSSH>TS>TSCR ($P<0.05$), with no effect of inoculant.

Bacterial colonization and aerobic stability

There were significant by-product \times inoculant interactions for hydrogen potential (pH) and concentration of $\text{NH}_3\text{-N}$, plus numbers of lactic acid bacteria, enterobacteria and *Clostridium* spp. ($P<0.05$; Table 5). While pH in TSCR was lower than in other silages regardless of the use or

Table 3. Chemical composition and dry matter recovery (g/kg DM) of Tifton 85 silages following addition of agro-industrial by-products and bacterial inoculant at ensiling.

Variable	Inoculant	By-product			s.e.m.	Inoculant.	By-product	Inoc. \times By-pr.
		TS	TSSH	TSCR				
DM (g/kg fresh silage)	Without	237c ¹	294b	310a	3.44	0.20	<0.01	0.79
	With	240c	303b	314a				
OM	Without	898bA	914aA	910aA ²	1.08	0.10	<0.01	<0.01
	With	901bA	914aA	904bB				
CP (g/kg CP)	Without	110b	119a	118ab	1.74	0.12	0.01	0.27
	With	109b	118a	110ab				
ADIP	Without	66.1aA	64.5aA	44.6bB	1.91	0.13	<0.01	<0.01
	With	56.4aB	58.2aB	53.2aA				
NDF	Without	675a	689a	601b	5.96	0.18	<0.01	0.36
	With	693a	684a	618b				
ADF	Without	397b	422a	346c	3.43	0.89	<0.01	0.48
	With	402b	415a	345c				
TDN	Without	577b	646a	649a	8.73	0.76	<0.01	0.45
	With	586b	662a	634a				
DMR	Without	911	956	988	18.6	0.13	0.24	0.06
	With	979	986	962				

¹Means within rows followed by different lower-case letters are different ($P<0.05$). ²Means within columns and variables followed by different upper-case letters are different ($P<0.05$). TS = Tifton 85 silage; TSSH = TS + soybean hulls silage; TSCR = TS + corn residue silage; DM = dry matter; OM = organic matter; CP = crude protein; ADIP = acid detergent insoluble protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; TDN = total digestible nutrients; DMR = dry matter recovery.

Table 4. In vitro gas production (mL/100 mg fermented dry matter), degradation rates and in vitro digestibility (g/kg dry matter) of Tifton 85 silages as affected by addition of agro-industrial by-product and bacterial inoculant.

Variable	Inoculant	By-product			s.e.m.	Inoculant.	By-product	Inoc. × By-pr.
		TS	TSSH	TSCR				
A (mL)	Without	4.68b ¹	8.56a	6.84ab	0.62	0.73	0.03	0.45
	With	5.63b	7.23a	6.46ab				
B (DR of A mL/h)	Without	0.16A ²	0.11A	0.15A	0.016	0.08	0.75	0.04
	With	0.09B	0.13A	0.11A				
C (lag time/h)	Without	5.05aA	2.14bA	0.19bB	0.57	0.54	<0.01	<0.01
	With	2.17aB	2.30aA	2.03aA				
D (mL)	Without	11.9b	17.8a	14.6ab	0.87	0.83	<0.01	0.85
	With	12.0b	16.7a	14.9ab				
E (DR of D mL/h)	Without	0.05aA	0.04bA	0.04bA	0.002	0.02	<0.01	<0.01
	With	0.04aB	0.04aA	0.04aA				
A+D (mL)	Without	16.6c	26.3a	21.4b	0.89	0.65	<0.01	0.42
	With	17.6c	24.0a	21.4b				
IVDMD	Without	606b	702a	714a	14.8	0.62	<0.01	0.60
	With	619b	730a	699a				
IVOMD	Without	629b	736a	747a	16.1	0.68	<0.01	0.58
	With	642b	766a	728a				
IVCWD	Without	632b	711a	588c	8.04	0.45	<0.01	0.36
	With	627b	685a	596c				

¹Means within rows followed by different lower-case letters are different (P<0.05). ²Means within columns and variables followed by different upper-case letters are different (P<0.05). TS = Tifton 85 silage; TSSH = TS + soybean hulls silage; TSCR = TS + corn residue silage; A = gas volume produced from rapidly degradable fraction; B = degradation rate of rapidly degradable fraction (Fraction A) in mL per hour; DR of A = degradation rate of fraction A; C = lag time for bacterial colonization; D = gas volume produced from slowly degradable fraction; E = degradation rate of slowly degradable fraction (fraction D) in mL per hour; DR of D = degradation rate of fraction D; IVDMD: in vitro dry matter digestibility; IVOMD = in vitro organic matter digestibility; IVCWD = in vitro cell wall digestibility.

Table 5. Hydrogen potential (pH), ammonia nitrogen concentration (NH₃-N, g/kg total N) and bacterial colonization (log CFU/g) of Tifton 85 silages as affected by addition of agro-industrial by-products and bacterial inoculant.

Variable	Inoculant	By-product			s.e.m.	Inoculant.	By-product	Inoc. × By-pr.
		TS	TSSH	TSCR				
pH	Without	5.10aA ¹	5.54aA ²	3.56bB	0.12	0.96	<0.01	<0.01
	With	5.11aA	4.85aB	4.23bA				
NH ₃ -N	Without	62.7aA	79.0aA	22.1bA	7.02	0.52	<0.01	0.02
	With	72.2aA	48.9abB	31.5bA				
Lactic acid bacteria	Without	7.00aA	7.19aA	5.84bB	0.18	<0.01	<0.01	<0.01
	With	7.28aA	6.98aA	7.15aA				
Enterobacteria	Without	4.23aA	4.17aA	0.50bA	0.64	0.29	<0.01	0.02
	With	4.74aA	1.33bB	1.12bA				
<i>Clostridium</i> spp.	Without	7.06aA	7.17aA	5.94bB	0.21	0.04	<0.01	0.04
	With	7.18aA	7.14aA	6.98aA				

¹Means within rows followed by different lower-case letters are different (P<0.05); ²Means within columns and variables followed by different upper-case letters are different (P<0.05). TS = Tifton 85 silage; TSSH = TS + soybean hulls silage; TSCR = TS + corn residue silage; Inoc = inoculant; By-pr = by-product.

not of inoculant, adding inoculant lowered pH in TSSH and raised it in TSCR ($P < 0.05$). Concentration of $\text{NH}_3\text{-N}$ followed a similar pattern with TS and TSSH producing higher levels than TSCR in the absence of inoculant, while only TS exceeded TSCR with inoculant ($P < 0.05$). Adding inoculant had an effect only for TSSH, where inoculant lowered $\text{NH}_3\text{-N}$ concentration ($P < 0.05$). Populations of lactic acid bacteria, enterobacteria and *Clostridium* spp. were lower for TSCR than for TS and TSSH in absence of inoculant ($P < 0.05$). However, in treatments with inoculant addition, populations of enterobacteria were the only ones affected by by-products with $\text{TS} > \text{TSSH}$ and TSCR ($P < 0.05$).

Silo temperatures showed an interaction between inoculant, by-product and aerobic exposure period ($P < 0.05$). All silages were still aerobically stable at 144 hours after exposure to air (Figure 1), except for TSCR without inoculant, which broke stability by 120 hours.

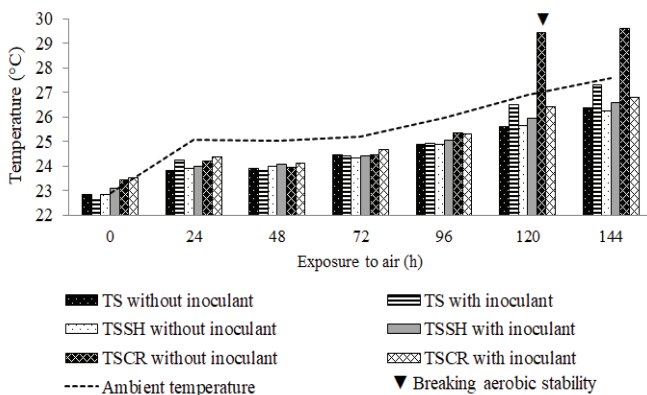


Figure 1. Aerobic stability of Tifton 85 silage (TS), Tifton 85 + soybean hulls silage (TSSH) and Tifton 85 + corn-processing residue silage (TSCR) with or without addition of bacterial inoculant during 144 hours of exposure to air.

Discussion

This study has produced valuable information on benefits of adding soybean hulls and corn-processing residue to Tifton 85 forage prior to ensiling and any additional benefit of adding bacterial inoculant. Inclusion of corn-processing residue and soybean hulls promoted an increase in both DM and OM of the silages. According to Rotz and Muck (1994), DM concentration of around 300 g/kg may reduce potential for undesirable fermentation and effluent production, which would reduce losses during storage. Andrade et al. (2012) added corn meal and soybean hulls to elephant grass at ensiling and showed that these additives were good options for increasing DM percentage, improving fermentation standard and reducing losses via effluent.

Crude protein concentration was not affected markedly by addition of agro-industrial by-products. This is scarcely surprising as CP% of Tifton 85 silage was 11.0 %, while CP% of soybean hulls was only 11.2 % and of corn-processing residue was 10.9 %. Despite this, CP% of the silages ranged from 10.9 to 11.9 %, which is adequate for feeding to non-lactating animals, although lactating cows would need protein supplements, if fed solely on these silages. Neres et al. (2014) obtained a similar result, when evaluating benefits of additives to Tifton 85 forage ensiled at 38 days of vegetative growth. These authors found no differences in CP% between treatments with soybean hulls added and Control (17.6 and 17.3 %, respectively).

With regard to ADIP concentrations in the silages, values for all treatments with inoculant plus TSCR without inoculant were lower than for TS and TSSH without inoculant. Acid detergent insoluble protein is lignin-associated protein that occurs due to non-enzymatic reactions with heating of the ensiled mass (Maillard reactions) and reduces protein digestibility (Van Soest 1994). According to Kung Jr et al. (2018), when excessive amounts of air are retained in forage mass at ensiling, temperatures increase and can reach above 45–60 °C. When this occurs for a prolonged period, it may lead to protein being heat-damaged with increase in ADIP. However, in the present study all treatments were ensiled at the same pressure with similar specific mass, so it is difficult to associate elevated ADIP levels in some treatments with heat damage due to presence of additional air in those silages.

Neutral detergent fiber and ADF concentrations in silage were influenced by type of by-products used, with lowest levels in TSCR treatments. Lower fiber concentrations in corn-processing residue would have been a contributing factor, as portions of corn grain are included in this by-product. As a result of composition of silages with by-product additives, TDN concentrations in these silages were higher than in straight Tifton 85 silage.

Dry matter recovery was not influenced by treatment and values varied between 911 and 988 g/kg. In evaluating Tifton 85 silages with different additives, Neres et al. (2014) also found no benefit from additives, with mean values of 813 g/kg, well below those obtained in this study. Santos et al. (2014) evaluated the use of bacterial inoculant in guinea grass silages (*Megathyrsus maximus* syn. *Panicum maximum*) and obtained higher DMR in treatments with homofermentative inoculant and attributed this outcome to better fermentation profile in these silages resulting from inhibition of undesirable microorganisms. Higher DMR values are desirable as they indicate lower losses during the ensiling process (Quaresma et al. 2010).

In vitro gas production from feedstuffs can be used to estimate their nutritional value (Silva et al. 2014), based on total volume of gases produced by fermentation of nutrients. In terms of gas production from rapidly degradable fraction (A), highest values occurred when soybean hulls were used, with intermediate values following addition of corn-processing residue, indicating that addition of agro-industrial by-products at ensiling of tropical grasses can increase concentration of non-fibrous carbohydrates in resulting silage.

In slowly degradable fraction (D), TSSH again had higher gas production regardless of use or not of inoculant, which is consistent with greater in vitro cell wall digestibility (IVCWD) for these treatments. Higher NDF degradability allows better microbial fermentation, which in turn can increase energy availability of the diet (Arroquy et al. 2014). Higher proportion of gas production from fraction D, relative to fraction A, is due to characteristics of the forage, in which fibrous fractions predominate, coupled with low levels of soluble carbohydrates characteristic of tropical grasses (Adesogan et al. 2004).

In terms of total gas production (A+D), inclusion of soybean hulls produced the highest values, followed by corn-processing residue, with lowest values for TS treatments. These results are consistent with IVDMD and IVOMD, which also increased following inclusion of soybean hulls and corn-processing residue. Agro-industrial by-products improved nutritional value of Tifton 85 silage, indicating their potential for improving animal performance when silages are fed.

Regarding IVCWD, best results were with TSSH (698 g/kg DM), followed by TS (635 g/kg DM) and TSCR (592 g/kg DM). Soybean hulls have high NDF digestibility (Zambom et al. 2001) and are an appropriate additive when making tropical grass silages, since this characteristic of fodder is correlated with DM intake and milk production (Oba and Allen 1999).

Addition of corn-processing residue, regardless of use or not of inoculant, promoted lower silage pH, which may be related to greater concentration of available substrates for lactic acid bacteria. It is important to note that only TSCR silages (with and without inoculant) remained below pH of 4.7, described by Kung Jr et al. (2018) as the maximum acceptable limit for grass silages. Andrade et al. (2012) evaluated elephant grass silages containing additives at 100 g by-product per kg fresh forage and pH of silages containing corn residue was lower than for those containing soybean hulls (3.49 vs. 4.3, respectively).

Concentration of $\text{NH}_3\text{-N}$ normally found in grass silages varies from 80 to 120 g/kg total N (Kung Jr et al.

2018); however, in our study, all treatments remained below these values, indicating good silage quality. Formation of $\text{NH}_3\text{-N}$ occurs due to a group of proteolytic clostridia (*Clostridium* spp.), which develop when pH is above 5.0 (Driehuis 2013). This relationship between pH level and development of proteolytic clostridia can be evidenced in this study, since lowest $\text{NH}_3\text{-N}$ values occurred for TSCR with and without inoculant and for TSSH with inoculant, which also had pH below 5.0. However, it is important to note that populations of clostridia evaluated in the present study were high for all treatments. This may have occurred because some species of non-proteolytic clostridia tolerate pH values down to 4.2 (Driehuis 2013), which would explain the high population of these microorganisms in silages with lesser $\text{NH}_3\text{-N}$ concentration.

Regarding lactic acid bacteria, TSCR without inoculant had the lowest population. Low pH values for this treatment are indicative of more-intense fermentation soon after ensiling, which may have caused reduction in concentration of substrates and later in the population of lactic acid bacteria. Use of bacterial inoculant containing *Lactobacillus plantarum* and *Pediococcus acidilactici* increased the population of lactic acid bacteria in TSCR, but did not show this effect for TS and TSSH. By comparison, Neres et al. (2013) evaluated Tifton 85 without and with addition of soybean hulls, corn grits or inoculant at ensiling and found no differences in populations of lactic acid bacteria in resulting silages.

Concomitant with lactic fermentation, enterobacteria are also present during initial stages of fermentation and compete with lactic acid bacteria for nutrients, reducing silage quality. Rapid pH decline in the ensiled mass to values below 4.5 is desirable to inhibit these microorganisms (Driehuis 2013). In fact, in the present study, smallest population of enterobacteria was observed in treatments with lower pH (TSCR with and without inoculant and TSSH with inoculant), while treatments that showed pH above 5.0 (TS with and without inoculant and TSSH with inoculant) had higher counts of enterobacteria (>4 log CFU/g).

There was no loss of aerobic stability in silages, except for TSCR without inoculant, which warmed to 2 °C above room temperature by 120 hours of exposure to air. Penetration of air into the silage mass results in growth of yeasts, which assimilate lactate causing an increase in temperature and pH (Kung Jr et al. 2018). This increase in pH allows growth of aerobic bacteria and fungi that increases temperature further, causing deterioration in silage quality (Muck 2013). Thus, loss of aerobic stability in TSCR without inoculant may be related to higher

availability of substrate for aerobic microorganisms, since lowest pH values were found in this treatment, indicating higher production of lactic acid.

Results of the present study showed that inclusion of soybean hulls and corn-processing residue with Tifton 85 forage at ensiling improved nutritional value of resulting silage, but only corn-processing residue improved bacterial colonization. Addition of these by-products to this forage at ensiling could be considered desirable but there seems little merit in including bacterial inoculant. Further studies on a larger scale should be conducted to confirm these initial laboratory findings and feeding studies with the various silages would determine if apparent improvement in silage quality was reflected in improved animal performance.

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