Rev. Bras. Saúde Prod. Anim., Salvador, v.21, 01 - 13, e210212020, 2020 **ISSN 1519 9940**
http://dx.doi.org/10.1590/S1519-9940210212020

Nutritive value of sugarcane silages with different bacterial additives and fermentation periods

Valor nutritivo de silagens de cana-de-açúcar com diferentes aditivos bacterianos e períodos de fermentação

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ABSTRACT

The objective of this study was to evaluate the nutritive value of sugarcane silage with or without inoculation with *P. acidipropionici* or *L. buchneri,* over three fermentation periods. The experimental design was completely randomized in a 3 x 3 inoculant by fermentation period factorial arrangement (without inoculant, inoculant 1, inoculant 2; x three fermentation periods, 10, 60, 90 days). Values of pH, dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEM) and lignin were determined and *in situ* DM degradability profiles were modelled for parameters *a*, *b* and *c*, potential degradation (A) and effective degradability (ED). The 90 day fermentation yielded a lower pH for both inoculants. There was an interaction between inoculant and fermentation period ($P < 0.05$) for DM content, with a reduction in silage DM without the additive at 90 days. The CP, HEM, ADF and lignin contents of sugarcane were not influenced by the treatments. The addition of *P. acidipropionici* provided the lowest NDF content at 10 days and presented a higher fraction *a*, potential degradation and ED. At 60 days, there was no variation in soluble fraction, the control silage showed a higher fraction *b*, higher potential degradation and ED. At 90 days of fermentation, *L. buchneri* silages presented a higher fraction *a*, degradation rate and DE and a higher *b* value was obtained in the silage without inoculant. Inoculants are effective in maintaining the silage DM content and nutritional value during prolonged fermentation periods.
Keyword: dry matter, inoculant, ruminal degradation

RESUMO

Objetivou-se avaliar o valor nutritivo de silagens de cana-de-açúcar com ou sem inoculante, em diferentes períodos de fermentação. O delineamento experimental foi o inteiramente casualizado em arranjo fatorial 3 x 3 (sem inoculante, inoculante 1 e inoculante 2 x três períodos de fermentação, 10; 60 e 90 dias). Analisou-se pH, matéria seca (MS), proteína bruta (PB), fibra em detergente neutro (FDN), fibra em detergente ácido (FDA), hemicelulose (HEM) e lignina; e degradabilidade *in situ* da MS, quanto aos parâmetros *a*, *b* e *c*, degradação potencial (A) e degradabilidade efetiva (DE). Houve diferença para o pH, o período de 90 dias apresentou menor média para ambos inoculantes. Houve interação

inoculante x período de fermentação (P<0,05) para o teor de MS, com redução na silagem sem aditivo aos 90 dias. Os teores de PB, HEM, FDA e lignina não foram influenciados pelos tratamentos. A bactéria *P. acidipropionici* proporcionou menor teor de FDN aos 10 dias e apresentaram maior fração *a*, degradação potencial e DE. Aos 60 dias não houve variação na fração solúvel, a silagem controle apresentou maior fração *b*, maior degradação potencial e DE. Aos 90 dias de fermentação, as silagens com *L. buchneri* apresentaram maior fração *a*, taxa de degradação e DE e obteve-se maior valor de *b* na silagem sem inoculante. Os inoculantes são eficientes em manter os teores de MS das silagens durante períodos prolongados de fermentação e manter o valor nutricional do material ensilado.

Palavras-chave: degradação ruminal, inoculante, matéria seca

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a forage alternative for ruminants during the dry season and is often used in natural form through daily manual harvests (Schmidt et al., 2011). Inherent in this form of harvest is the need for hiring labor for cutting, shredding, chopping, loading and frequent transport, which all increase costs. An alternative is to ensile the sugarcane to be able to use it more cost-effectively.

The principle of ensiling is the transformation of soluble carbohydrates in the substrate into lactic acid during fermentation by lactic acid bacteria in an anaerobic environment. However, the chemical characteristics of the soluble carbohydrates in sugarcane favor alcoholic fermentation, which is performed by yeasts, and entails the loss of dry matter and the concomitant loss in nutritive value of the ensiled material (Bernardes et al., 2007).

The presence of sucrose, the main carbohydrate found in sugarcane, favors the proliferation of the yeast population during fermentation, which converts sugars to ethanol and CO_2. The ethanol decreases the amount of sugar available to lactic acid bacteria and, thus, under aerobic conditions, many yeast species degrade lactic acid, causing an increase in silage pH (McDonald, 1991).

To circumvent these problems, the use of biological additives has been studied. Heterolactic bacteria have been gaining prominence because they are producers of acetic and propionic acids, as well as lactic acid, which together have the ability to reduce yeast and fungal activity during the fermentation phase of silage. The *Lactobacillus buchneri* species has shown promising results in sugarcane silages by inhibiting yeast growth and increasing aerobic stability (Schmidt et al., 2014). Another group of microorganisms that has been studied is the genus *Propionibacterium*, which characteristically produces propionic acid. However, according to Pedroso et al. (2011), the results with the application of these additives have been inconsistent and with little information on the resulting nutritional value of sugarcane silages. Therefore, in addition to evaluating these bacteria, it is important to monitor the action of inoculants during the fermentation process (Carvalho et al., 2014). Thus, the objective of this study was to evaluate the nutritive value of sugarcane silages with or without the addition of bacterial inoculants over different fermentation periods.

MATERIAL AND METHODS

The experiment was carried out in the Forage Industry Sector of the Center for Agricultural and Environmental Sciences of the Federal University of Maranhão (CCAA / UFMA), in Chapadinha, MA, region of Baixo Parnaíba, Brazil (03°44′33″ S, 43°21′21″ W). The climate, according to the Köppen climate classification, is hot, humid and tropical , with an annual average temperature of over 27 °C and cumulative annual rainfall of 1,835 mm, with periods of rain between January and June and dry from July to December.

Two bacterial inoculants were evaluated: *Propionibacterium acidipropionici* and *Lactobacillus buchneri* in different fermentation periods of 10, 60 and 90 days in a completely randomized design (CRD), in a 3 x 3 factorial arrangement (uninoculated silage, inoculated with *Propionibacterium acidipropionici,* inoculated with *Lactobacillus buchneri*; over three fermentation periods), with five replications per treatment. *L.*

buchneri was applied to the sugarcane to achieve an inoculation dose of 5 x 10^4 cfu / g of forage and *P. acidipropionici* at a dose of 1 x 10^5 cfu / g of forage. These doses were recommended by the manufacturer to ensure optimal fermentation of the silage, in this particular case, for sugarcane.

The sugarcane variety used was SP 813250, at 12 months of growth, harvested manually and without burning. The material was ground to 1.0 to 2.0 mm particles in a forage machine on the same day of harvest. The material was separated into 3 parts, one for silage without additives (control) and the other two for the application of additives.

For ensilation, PVC mini silos were used (0.10 m in diameter and 0.25 m high), with Busen-type valves to allow the escape of fermentation gases. The compaction was performed with the aid of PVC-coated concrete sockets, thus providing a specific mass with density of 750 kg green material / m³. After compaction, the silos were sealed and weighed.

The mini silos were opened after the prescribed days of fermentation (10, 60 and 90 days). The silage was homogenized, and two samples of the ensiled material were collected. One sample was used to determine the pH, which was performed by diluting nine grams of fresh silage in 60 mL of distilled water and reading after 30 minutes of rest, as described by Silva & Queiroz (2002). The second 300 g sample was dried in a 55 °C forced-air oven until the sample maintained a constant weight, then milled in a 1 mm sieve (Willey mill) for further chemical analysis and for the assay of *in situ* degradability of dry matter (DM).

Nutrient content of the silage was determined by the following methods: dry matter (DM) content for 24 hours in a drying oven at 105 ºC, crude protein (CP) (method 988.05; AOAC procedures, 1998), neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to the methodology of Van Soest et al. (1991), and acid detergent lignin was determined by solubilization of cellulose with 72% sulfuric acid (method 973,18; AOAC, 1998).

Data on the chemical composition of sugarcane at the time of ensiling are described in Table 1.

Table 1. Chemical composition of sugarcane before ensiling

Parameters	**Sugarcane**
Dry matter (% of FM)	26,72
Crupe protein (% of DM)	2,13
Neutral detergent fiber (% of DM)	65,11
Hemicellulose (% of DM)	29,17
Acid detergent fiber (% of DM)	35,94
Lignin (% of DM)	20,28

Dry matter degradability (DMD) was estimated by the *in situ* technique using a cross-bred sheep with a live weight of 60 kg, approved by the Ethics and

Biosafety Committee under the number: (23115.011059 / 2015-26). This procedure was suggested by Tomich and Sampaio (2004). 4 g of the ground sample was placed in nylon bags measuring 12 x 8 cm with pores of 50 µm in diameter (NOCEK, 1988). For the degradability test, a completely randomized design with repeated measures was used. Repeated measures were the incubation periods of 6, 24, 72 and 96 hours.

To determine the disappearance of the material at time zero, the bags were kept in a water bath for 1 hour at a temperature of 39 °C (Brito et al., 2007). After this time, the bags were removed from the water bath and were washed along with the ruminal incubation bags and were kept in a forced-air oven at 55 °C for 48 hours.

The percentage of dry matter disappearance (DMS) for each time was calculated by the proportion of food that disappeared from the bags after ruminal incubation. To evaluate the DMS parameters, the model of Orskov & McDonald (1979), subsequently adapted by Sampaio (1988), was used:

$$Deg = A - B * (- ct),$$

Where A - corresponds to the potential degradation of the incubated material when time is not a limiting factor; B - parameter without biological value, that is, if there was no time of colonization, it would correspond to the total to be degraded by microbial action; c - rate of degradation by fermentative action of B; t = rumen incubation time in hours.

Once the coefficients A, B and c were calculated, they were applied to the equation proposed by Ørskov and McDonald (1979) to calculate the effective degradability:

$$DE = a' + (b' * C) / (C + k),$$

Where a' = % disappearance at time zero (Average); b'= A - a'; C = constant rate of degradation; K = food passage rate, a digesta to duodenum passage rate of 2, 5 and 8 % per hour was assumed.

The data were subjected to the comparison of means by the SNK test at 5 % probability by the PROC GLM procedure of the SAS statistical software (2002). The degradation parameters *a*, *b* and *c* and the *in situ* degradation curves were determined according to the Gauss-Newton method by the SAS PROC NLIN procedure (2002).

RESULTS AND DISCUSSION

There was no interaction between the inoculants tested and fermentation periods ($P > 0.05$) on silage pH. There was significant difference ($P < 0.05$) only between fermentation periods (Table 2). Lower pH values were measured in the 90 day fermentation for all silages, with an average of 3.29. Properly fermented silages have a pH of 3.8 to 4.2, a range that restricts the action of proteolytic enzymes on the ensiled mass, inhibiting the development of bacteria of the genus *Clostridium* (Muck, 1988). However, many yeasts are able to grow at pH 3.5, which is below the pH of most silages (Muck, 2010), but is more frequent in sugarcane silage.

Table 2. Average pH values of sugarcane silage with different bacterial inoculants and fermentation periods

pH							
Inoculant	Fermentation period			Average	p-value		
	10	60	90		FP[3]	I[4]	FP*I[5]
Control	3,56	3,55	3,27	3,46A	<0,0001	0,2963	0,0523
Buch[1]	3,45	3,51	3,33	3,43A			
Prop[2]	3,48	3,54	3,27	3,43A			
Average	3,50a	3,53a	3,29b				
CV (%)	1,55						

Averages followed by similar capital letters in columns and lowercase letters in rows do not differ significantly from each other by the SNK test at P < 0.05. 1 *L. buchneri*; 2 *P. acidipropionici;* 3 Fermentation periods; 4 Inoculant; 5 Interaction effect between fermentation period and inoculant.

Valeriano et al. (2009) observed a pH value of 3.53 in sugarcane silages inoculated with *L. buchneri* after 90 days of fermentation. The pH of 3.29 in this study and that of Valeriano et al. (2009) is characteristic of alcoholic fermentation, which occurs in sugarcane because of the high levels of soluble carbohydrates.

An interaction effect (P < 0.05) was observed for DM content as a function of fermentation periods and additives (Table 3). The silage without inoculant produced a higher DM content over the fermentation periods of 10 and 30 days, while after 90 days, it was observed that the DM content of silage with *P. acidipropionici* and the control silage did not differ significantly. Between fermentation periods, it was observed that there was a reduction in dry matter content for the 90-day fermentation period in control silage, which was not observed in inoculated silages. The reduction in DM content of the control silage can probably be attributed to the loss of soluble carbohydrates during the fermentation process (Fortaleza et al., 2012), caused by excessive yeast overgrowth. This contribute to a significant loss of DM, because the metabolic pathway in the production of alcohols is very inefficient (McDonald et al., 1991).

Table 3. Average contents of dry matter (DM) and crude protein (CP) of sugarcane silage with different bacterial inoculants and fermentation periods

Inoculant	Fermentation periods			Average	p-value		
	10	60	90		FP[3]	I[4]	FP x I[5]
Dry matter (DM)							
Control	28,06Aa	28,33Aa	25,03Ab	27,14	0,0061	0,0001	0,0005
Buch[1]	25,79Ba	25,55Ba	23,47Ba	24,84			
Prob[2]	25,69Ba	23,75Ba	26,18Aa	25,21			
Average	26,51	25,77	24,9				
CV (%)				4,38			
Crude protein (CP)							
Control	2,32	2,11	2,70	2,38A	0,0682	0,3521	0,076
Buch	2,50	2,17	2,20	2,29A			
Prob	2,23	2,20	2,28	2,23A			
Average	2,35a	2,16a	2,39a				
CV (%)				9,72			

Averages followed by similar capital letters in columns and lowercase letters in rows do not differ significantly from each other by the SNK test at $P < 0.05$. 1 *L. buchneri*; 2 *P. acidipropionici;* 3 Fermentation periods; 4 Inoculant; 5 Interaction effect between fermentation period and inoculant.

The average DM content for *L. buchneri* containing silages (24.84%) was close to the 23.35 % DM value observed by Balieiro et al. (2009). Siqueira et al. (2011) found values of 24.8 % and 25.9 % DM for silage containing *L. buchneri* and silages without inoculants, respectively. There was no significant difference ($P > 0.05$) for crude protein contents. The CP content of inoculated silages was similar to that observed in sugarcane before ensiling (2.13%) and produced no significant differences ($P > 0.05$) between the treatment groups.

Among the evaluated structural components (Table 4), only NDF was different between the evaluated silages ($P > 0.05$). *P. acidipropionici* silages showed a lower NDF content at 10 days of fermentation. The applied dose of *P. acidipropionici* may not have been sufficient to prevent alcoholic fermentation over long periods, because the NDF content increased over the longer periods of fermentation (60 and 90 days).

Table 4. Average contents of neutral detergent fiber (NDF), hemicellulose (HEM), acid detergent fiber (ADF) of sugarcane silage with different bacterial inoculants and fermentation periods

Inoculant	Fermentation periods			Average	p-value		
	10	60	90		PF[3]	I[4]	PF x I[5]
Neutral detergent fiber							
Control	74,05Aa	75,67Aa	76,7Aa	75,47	<0,0001	0,0263	0,01
Buch[1]	73,6Aa	75,91Aa	76,32Aa	75,28			
Prob[2]	69,8Bb	76,22Aa	75,99Aa	74,00			
Average	72,48	75,93	76,34				
CV (%)				1,80			
Hemicellulose							
Controle	31,74	31,74	30,00	31,16A	0,2195	0,0588	0,2184
Buch	27,45	32,13	34,15	31,74A			
Prob	28,95	30,53	28,63	28,87A			
Average	29,38a	31,47a	30,93a				
CV (%)				9,70			
Acid detergent fiber							
Control	42,32	43,93	46,69	44,31A	0,2709	0,5256	0,2219
Buch	44,65	43,78	42,17	43,53A			
Prob	42,35	45,70	47,36	45,14A			
Average	43,11a	44,47a	45,41a				
CV (%)				7,72			
Lignin							
Control	16,18	20,94	21,32	19,48A	0,0026	0,4805	0,5716
Buch	17,71	18,82	24,03	20,19A			
Prob	18,19	22,57	22,66	22,67A			
Average	17,36b	20,78a	22,57a				
CV (%)				16,47			

Averages followed by similar capital letters in columns and lowercase letters in rows do not differ significantly from each other by the SNK test at P < 0.05. 1 *L. buchneri*; 2 *P. acidipropionici;* 3 Fermentation periods; 4 Inoculant; 5 Interaction effect between fermentation period and inoculant.

This may have been due to the low pH of silages (Table 1), as *P. acidipropionici* bacteria are intolerant to acidic conditions (at a value close to or below 4). Therefore, a silage pH below 4.2 may have inhibited bacterial growth and propionic acid production (Michel et al., 2017).

Siqueira et al. (2011) evaluated fresh sugarcane silage (without burning) and burned with calcium oxide (CaO) and / or *L. buchneri*, and observed an NDF content of 75.9 % for sugarcane silage with added *L. buchneri* at 60 days of fermentation, which was higher than the content of the material before ensiling. This anomaly was also observed in the

present study, where sugarcane had a lower NDF content before ensiling (Table 1). The aforementioned authors state that fermentation performed by yeast in an anaerobic environment will produce ethanol, carbon dioxide, water and ATP, generating losses of DM and consequently, proportionally increasing fibrous fractions.

HEM and ADF contents increased in *L. buchneri* silages by 8.09 % and 17.43 %, respectively, in relation to pre-ensiled sugarcane (Table 1). This was also observed by Mendes et al. (2008) when working with *L. buchneri* silages, which presented 46.3 % of ADF and 23.1 % of HEM, while the fresh material contained 28.9 % and 21.0 % of ADF and HEM, respectively. As with NDF, the increased concentration of ADF and HEM is caused by the loss of soluble carbohydrates during alcoholic fermentation, producing increased levels of cell wall constituents.

Lignin content was not influenced by inoculants ($P > 0.05$). There was variation only between fermentation periods ($P < 0.05$), where, at 10 days of fermentation there was a lower lignin content. The high levels of lignin observed in this study were due to the accumulation of dead material that the sugarcane presented at the time of ensiling, since no shredding was performed. Siqueira et al. (2011) evaluated fresh and burnt sugarcane silage and observed a 28% reduction in lignin content for burnt sugarcane before ensiling, since bagasse is a fraction with high concentrations of NDF, ADF and lignin.

At 10 days of fermentation, silages inoculated with *P. acidipropionici* bacteria presented higher water soluble fractions (Table 5), with 27.50 %, which was 34.18 % and 35.65 % higher than silage without additives and with *L. buchneri*. respectively. The dry matter fraction "*a*" represents the portion of the food that is readily available to rumen microorganisms (Bezerra et al., 2015). Access of ruminal microbial enzymes to the cell wall results in the reduction of the insoluble fraction, and an increased digestibility (Rocha et al., 2015).

Although silage without inoculant had higher value of fraction *b* in 10 days, *P. acidipropionici* silages had higher potential degradation and, consequently, higher effective degradability at all passage rates. The highest percentage of ruminal degradation of *P. acidipropionici* silages is due to the lower amount of NDF at 10 days of fermentation (Table 4).

Table 5. Ruminal degradation parameters (*a*, *b* and *c*), potential degradability (A) and effective degradation (ED) of dry matter at passage rates 2, 5 and 8 % per hour of sugarcane silage with different bacterial inoculants and fermentation periods

Fermentation Days	Additive	*a (%)*	*b(%)*	*c(%/h)*	A	R^2	Effective degradation (%)		
							2 %/h	5 %/h	8 %/h
10	Control	18,10	67,50	0,50	85,60	96,18	31,69	24,28	22,10
	Buch[1]	17,70	38,84	1,58	56,54	80,59	34,84	27,03	24,11
	Prob[2]	27,50	63,58	0,52	91,08	98,61	40,56	33,46	31,36
60	Control	15,60	32,58	2,79	48,18	96,84	34,58	27,27	24,02
	Buch	15,60	30,11	2,77	45,71	95,24	33,09	26,33	23,34
	Prob	15,20	27,61	3,16	42,81	79,04	32,11	25,89	23,02
90	Control	16,20	62,28	0,69	78,48	99,57	32,18	23,75	21,15
	Buch	21,33	40,86	1,36	62,19	97,14	37,87	30,07	27,27
	Prob	14,80	46,65	0,96	61,45	99,31	29,89	22,29	19,78

a = water soluble fraction; b = water insoluble fraction, but potentially degradable; c = fraction degradation rate b; R^2 = coefficient of determination, A = potential degradability [1] *L. buchneri;* [2]*P. acidipropionici*

At 60 days of fermentation there was no difference in soluble fraction between silages. Silage without additives obtained a higher *b* fraction, higher potential degradability and higher effective degradation for all passage rates. The soluble and insoluble fraction percentages of *L. buchneri* silages were higher than those found by Rocha et al. (2015), who found in sugarcane silage with *L. buchneri* at 60 days of fermentation, an *a* fraction of 17.73% and 57.43 % potential degradation.

At 90 days of fermentation, *L. buchneri* silages presented a higher *a* fraction, A, and ED at all passage rates, while silage without additives obtained higher values of fraction *b* and A. Filya et al. (2003) worked with corn, wheat and sorghum silages with added *L. buchneri* bacteria and homofermentative bacteria and did not observe significant differences in DM degradability values among the inoculated silages.

The low degradation values are related to the high lignin content that the silages presented at 60 and 90 days, given that lignin is negatively related to ruminal degradation. According to Jung & Deetz (1993), cell wall lignification may limit polysaccharide digestion through the physical impediment caused by lignin polysaccharide binding, which limits the access of fibrolytic enzymes to the reaction center of a specific carbohydrate. Inoculants are efficient in maintaining the silage DM content during prolonged fermentation periods and maintaining the nutritional value of the silage material.

ACKNOWLEDGEMENTS

To FAPEMA for the granting of the scholarship and to the FOPAMA forage research group.

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