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Full Length Article

Addition of Fermented Corn Juice as Bioinoculant Improved Quality of *Saccharum officinarum* Silage

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Abstract

Sugarcane had low dry matter contents (< 25%) and high content of water soluble carbohydrates which leads to an excessive presence of molds and yeast along with higher losses as effluents when used as silage. However, addition of fermented corn juice in sugarcane silage can improve its chemical composition along with decrease in effluents losses. Therefore, present study was conducted to evaluate the losses, chemical composition, microbiological profile, aerobic stability and degradability of microbial inoculated sugarcane silage with addition of varying levels of fermented corn juice. Five levels of the corn fermented juice (0, 200, 400, 800 and 1600 mL t^{-1}) were sprayed in sugarcane silage with five replicates. The highest losses of effluents and gases were verified in silages without bioinoculant ($P < 0.05$) while the highest values of dry matter (DM) recovery were observed in the silages with presence of bioinoculant ($P < 0.05$). For the DM content, the highest value was verified in the microbial inoculated silage with 200 mL t^{-1} fermented corn juice. The highest amounts of non-fibrous carbohydrates (NFC) and the highest values of acetic acid were observed in the microbial inoculated silages ($P < 0.05$). No significant amount of yeasts and molds was observed in none of the silages. In conclusion, the addition of the corn juice bioinoculant at 200 mL t^{-1} in sugarcane silage improved its fermentation profile, chemical composition, greatest aerobic stability and degradability along with significant reduction in losses of effluents.

Keywords: Aerobic stability; Biological additive; Degradability; Microorganisms; Sugarcane

Introduction

Efficiency in animal production relies largely on rational measures related to feed management (Zanine *et al*. 2016). In raising of grazing ruminants, one of the major problems faced by cattle breeders is seasonality in forage production, thus making it essential to use techniques for feed conservation (Ferreira *et al.* 2019). This strategy aims to avoid large oscillations in the feed supply to the animals throughout the year, thus maintaining the production level in both seasons (Carvalho *et al.* 2018).

The great benefit of using sugarcane (*Saccharum officinarum* L.) as feed, besides the high production of biomass per hectare, is the high content of water soluble carbohydrates. However, in practice, the management related to the daily labor demand for cutting, chopping and transport is difficult (Evangelista *et al.* 2009). Therefore, an alternative would be ensiling; however, sugarcane silage may show great loss of forage mass due to undesirable fermentations in the ensiling process.

Considering the fact that information on quality of sugarcane silages is still dubious, a possible alternate to improve its fermentation profile, nutritional value along with reduced losses in dry matter (DM) is direly needed. In this regard, use of microbial inoculants that provide fermentation beneficial to conservation is of great importance.

Thus, the use of the fermented juice of maize (*Zea mays* L.) could be a bioinoculant alternative at the time of silage since it has dominance of the lactic acid bacteria. Although yeast growth is not inhibited by low pH values during the ensiling process (McDonald *et al.* 1991). The increased initial population of lactic acid bacteria increases substrate competition among microorganisms, inhibiting the development of yeasts under anaerobic conditions, which lead to decreased losses to the detriment of alcoholic fermentation.

Another possibility is the fact that the microbial inoculated corn juice, besides the homofermentative bacteria, also shows heterofermentative bacteria (such as acetic acid bacteria) efficient to control yeasts in sugarcane

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silage (Pedroso *et al*. 2000; Kleinschmidt and Junior 2006). Several studies show that pre-Hispanic beverages derived from fermented corn juice are characterized by being rich in lactic acid bacteria (LAB) that function as probiotic for humans (Escalante *et al*. 2001; Silva *et al*. 2017).

Considering that fermented corn juice may add homo and heterofermentative LAB to silage and these bacteria reduce undesirable fermentation. Hence it was hypothesized that this bioinoculant improves the fermentative profile of sugarcane silage. Although the efficiency of industrially purified microbial additives to improve silage quality is well reported but the information to use natural bioinoculant like fermented corn juice to improve silage quality is lacking. Therefore, this study was designed to evaluate the effects of fermented corn juice used as bioinoculant on effluent losses, chemical composition, microbiological profile, aerobic stability and degradability of microbial inoculated sugarcane silage.

Material and Methods

The experiment was performed in strict accordance with the recommendations of the National Council for the Control of Animal Experimentation (CONCEA). The protocol was approved by the Ethics committee of Animal Defense Experiments of the Federal University of Piauí, PI, Brazil (Authorization Number: 016-14).

Study Area, Silage Material and Treatments

The experiment was performed at the Federal University of Piauí, in Bom Jesus, PI, Brazil. According to the Köppen classification, the climate of the region is tropical savanna (Aw), with two seasons well defined: the dry season, from May to October; and the rainy season, from November to April. It is located at the geographic coordinates 09º 04' 28" S, 44º 21' 31" W, at 277 m altitude, with average rainfall between 900 and 1200 mm/year and average temperature of 26.2°C (Ramos *et al.* 2009).

Five levels of the corn fermented juice (0, 200, 400, 800 and 1600 mL t^{-1}) were sprayed on sugarcane silage. Experiment was laid out following completely randomized design with five replicates. The control treatment corresponded to silage without bioinoculant.

Silage Production

The sugarcane silage was performed in an annual cycle plantation already established. The cultivation of maize for bioinoculant production was carried out in March 2017.

To produce the bioinoculant, the corn plant was cut and ground when it reached the dough stage. The material (500 g of the corn plant) was collected on the chopping day, which was processed in a blender, strained and then 500 g of corn glucose and sterilized water were added until reach 2 L of the mixture. Subsequently, the solution was stored in a 2 L plastic bottle (polyethylene terephthalate) properly sterilized. The bottle cap was adapted with a Bunsen valve to release the produced gases. The bottle with the juice was stored for 35 days.

To chop the sugarcane, a stationary silage machine regulated for 2–3 cm particle cutting was used. The bioinoculant was sprayed in the chopped sugarcane with a backpack sprayer. The silages were made in silos with a capacity of 3.0 kg in polyvinyl chloride (PVC) with 100 mm diameter and 50 cm length, the latter exclusively for aerobic stability evaluation. The silages were stored at a mean density of 508 kg m^{-3}.

Determination of losses by effluent, dry matter and gases

To obtain the values of losses by gases and effluents, silos with a capacity of 3.0 kg were used. One kg of absorbent material (coarse sand) were placed in the bottom of each silo in order to absorb the possible effluents from the fermentation process. Above this absorbent layer, a piece of synthetic fibre of the nonwoven type (NWT) was used, with the function of separating the sand from the silage, thus avoiding contamination of the same. After this, about 2 kg of forage were compacted in the silos, according to the proportion of each treatment. After compaction, the silos were sealed with plastic caps that contained Bunsen valves, thus favouring the exit of gases produced during the fermentation process.

The gases (GL) and effluents losses (EL) and the dry matter recovery were obtained according to below given equations given by Zanine *et al.* (2010):

$$GL = (FWI - FWf)/(FMi * DMi) * 100$$

Where: GL: gas losses (% DM); FWI: full bucket weight at closing (kg); FWf: full bucket weight at opening (kg); FMi: forage mass at closing (kg); DMi: dry matter content of forage at closing:

$$EL = [(EWf - Tb) - (EWi - Tb)]/FMi * 100$$

Where: EL: effluent losses (kg t^{-1}NM) NM: Natural matter; EWi: empty bucket weight + sand weight at closing (kg); EWf: empty bucket weight + sand weight at opening (kg); Tb: tare of the bucket; FMi: forage mass at closing (kg):

$$DMR(gkg^{-1}DM) = [(NMfo * DMfo)/(DMi * DMsi)] * 100$$

Where: DMR (g/kg DM): DM recovery, in percentage; NMfo: Natural mass of forage (kg) at ensiling time; DMfo: DM of forage (%) at ensiling time; DMi: Mass of silage (kg) before silos opening; MSsi: DM of silage (%) at silos opening.

Determination of Chemical Composition

Samples of sugarcane and maize in *natura* (Table 1) and silages (Table 2) were packed in plastic bags and frozen for further analysis. Samples were placed in paper bags,

Table 1: Chemical composition of sugarcane used for silage and corn used for the extraction of fermented juice

Chemical composition (g kg^{-1} DM)	Ingredients	
	Sugarcane	Maize
Dry matter (g kg^{-1})	257	332
Crude protein	39.8	79.4
Neutral detergent fiber	490	562
Acid detergent fiber	408	278
Mineral matter	1.49	4.59
$N\text{-}NH_3$(%TN)[1]	3.10	2.78
pH	5.64	5.20
Ether extract	6.36	31.6
Non-fibrous carbohydrates	462.35	322.41

[1]$N\text{-}NH_3$= Ammonical nitrogen in relation to the percentage of total nitrogen

Table 2: Effluent losses (EL), gas losses (GL) and dry matter recovery (DMR) of sugarcane silage with addition of fermented corn juice

Variables	Fermented corn juice (ml t^{-1} silage)					Average	SEM**	P-value
	0	200	400	800	1600			
EL (kg/t NM)	70.6a	29.0c	51.6b	54.7b	54.7b	52.1	2.70	<0.01*
GL (% DM)	2.2a	1.5b	1.5b	1.7ab	2.0ab	1.80	0.14	<0.01*
DMR (% DM)	76.0b	90.9a	92.9a	90.4a	90.8a	88.2	2.87	<0.01*

Averages followed by different letters on the same row do not differ among themselves by Tukey's test ($P < 0.05$)
*Significant at $P < 0.01$; [ns] Not significant at $P > 0.05$; **SEM = standard error of the mean

tagged, weighed and pre-dried in a convection oven at 55ºC for 72 h and then ground in a Thomas Wiley mill with a 1.0 mm sieve.

The laboratory analyses were performed in triplicate to determine the chemical composition according to the AOAC (1990) recommendations for dry matter (DM, method 967.03), ash (method 942.05), crude protein (CP, method 981.10) and ether extract (EE, method 920.29). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the methods of Soest *et al.* (1991) with alteration proposed by Senger *et al.* (2008) to use an autoclave. Non-fibrous carbohydrates (NFC) were calculated according to Weiss (1999), as follows:

$$NFC(gkg^{-1}) = 1000 - (NDF + CP + EE + ash)$$

Determination of Volatile Fatty Acids (VFAs)

Organic acids (lactic, acetic, propionic and butyric) and ethanol were determined using the methodology described by Junior and Ranjit (2001). The analysis was performed on high-performance liquid chromatography (HPLC), SHIMADZU brand, model SPD-10A VP coupled to the ultraviolet (UV) using a wavelength of 210 nm.

Fermentation Characteristics and Microbial Population

The pH in distilled water was determined in duplicate, collecting approximately 25 g sample of the ensiled material from each treatment and adding 100 mL water A.O.A.C. (1990). The reading was performed after 1 h, according to the methodology described by Bolsen *et al.* (1992) with a microprocessor-based benchtop pH meter.

To determine the $N\text{-}NH_3$ of the samples, a methodology according to Bolsen *et al.* (1992) was used. In 200 g of fresh sample, 200 mL of 0.2 N H_2SO_4 solution was added. After standing for 48 h under refrigeration, the mixture was filtered using filter paper for estimation based on the dry matter content of the silage, according to Detmann *et al.* (2012).

To enumerate the microbial groups, the samples were obtained from the homogenization of all replicates of each treatment, with 90 mL distilled water added in the samples and homogenized in an industrial blender for 1 min, obtaining a 10^{-1} dilution. Then, successive dilutions were performed aiming to obtain dilutions ranging from 10^{-1} to 10^{-9} (Santos *et al.* 2014).

Plating was performed in duplicate on sterile Petri dishes. Microbial populations were quantified using selective culture media for each microbial group listed below: MRS (Difco) for enumeration of lactic acid bacteria (LAB) after incubation for 48 h in a BOD oven at 37°C; Brillant Green Agar (Difco) for enumeration of enterobacteria (ENT) after incubation for 24 h in a BOD oven at 35°C; and potato dextrose agar added with 1 dag kg^{-1} 1% tartaric acid after sterilization for mold and yeast counts (M and Y) after incubation for 3–7 days at room temperature.

The plaques with values between 30 and 300 CFU (colony-forming unit) in a Petri dish were considered susceptible to counting. The plaque averages of the selected dilution were then considered. The differentiation between yeasts and molds was given by the physical structure of the colonies, which was visually perceptible, since yeasts are unicellular and molds multicellular (González and Rodríguez 2003).

Aerobic Stability

The aerobic stability (AS) of the silages (expressed in hours) was evaluated by monitoring the temperatures (superficial and internal) of the silages exposed to air. The silages in

PVC silos were taken to a climate-controlled room. The room temperature was measured with thermometers located near the silages. In the evaluation of the aerobic stability of the silage surface layer, after the periods determined for each experiment, the silage was opened by withdrawing the package with full opening. The material was exposed to air from 0 to 96 h.

After opening the silo at the given time, the surface temperature and silage mass were verified hourly over a period of 12 h. The silage surface temperature was measured using a non-contact digital infrared thermometer, while the temperature of the forage mass required a digital immersion thermometer inserted at 10 cm in the center of the silage. The ambient temperature was measured by a thermometer suspended in the air. The aerobic stability was calculated as the observed time for the silage to increase by 2°C in relation to the ambient temperature after exposure to air, according to Taylor and Kung (2002).

Determination of *in situ* Degradability

In order to evaluate the dry matter (DM) degradability of the microbial inoculated silages, the *in situ* technique was used by means of 5 × 6 cm synthetic fiber bags (100 mm hemp) nonwoven type (NWT) in an amount of approximately 1.3 g DM bag^{-1} to maintain a ratio of about 20 mg DM cm^{-2} bag surface area, according to Nocek (1988). A total of 1.5 g of pre-dried samples were weighed at each bag, then sealed, placed in porous synthetic fabric bags (mesh bag) and attached to a string throughout the incubation period.

For the incubation, two crossbred sheep averaging two years and 45±2.5 kg live weight with rumen fistula were used. The animals were housed in separate pens, 1.10 m wide and 2.10 m long, with cement floor and provided with drinking fountain and feeder. Animals had access to sugarcane, corn silage and watered *libitum* in two periods day^{-1} (8 am and 5 pm).

The incubation periods corresponded to the times of 0, 6, 12, 24, 48, 72, and 96 h. The bags were placed in reverse order and in quadruplicate to be withdrawn simultaneously, promoting uniform washing of the material at the time of rumen removal. After the total incubation period of 96 h, all the bags were removed from the rumen, washed in running water and then oven dried at 55°C for 72 h.

After pre-drying, the bags were taken to the oven at 105°C for 2 h and then weighed to obtain the non-degraded DM. The degradation rate of the DM at each time was determined according to AOAC (1990) and the *in situ* degradability data were obtained by the weight difference found for each component between the weighing before and after the rumen incubation and expressed as a percentage.

Statistical Analysis

Collected data were subjected to analysis of variance (ANOVA) followed to check the overall significance of data by statistical software SAS 9.1 (SAS Institute, Cary, NC, USA). Moreover, treatments means were separated using Tukey's test at 5% probability level.

Results

Effluent Losses, Gas Losses and Dry Matter Recovery of the Silages

Different levels of fermented corn juice used as bioinoculant had significant effect on effluent losses (EL), gas losses (GL) and dry matter recovery (DMR) of sugarcane silage (Table 2). The highest EL in sugarcane silage was recorded where no bioinoculant was used. In contrast, the microbial inoculated silage with 200 mL t^{-1} fermented juice showed the lowest EL (29 kg/t FM) in sugarcane silage (Table 2). The microbial inoculated silage with 200 mL t^{-1} and 400 mL t^{-1} fermented juice showed the lowest GL (1.5 % DM) in sugarcane silage (Table 2). The bioinoculant levels showed highest values of dry matter recovery when compared no bioinoculant, however, there was no statistical difference among the levels (Table 2).

Chemical Composition, Volatile Fatty Acids and Ethanol

Different levels of fermented corn juice used as bioinoculant had significant effect on dry matter (DM), acid detergent fiber (ADF), mineral matter (MM), non-fibrous carbohydrates (NFC) and ether extract (Table 3). The highest dry matter content (246.3 g kg^{-1} DM) was found in the microbial inoculated silage with 200 mL t^{-1} fermented corn juice, which did not differ from the level of 400 mL t^{-1} (Table 3). For the values of crude protein, neutral detergent fiber, ammoniacal nitrogen ($N\text{-}NH_3$) and pH, was no significant difference verified (Table 3).

The lowest amount of acid detergent fiber was observed in the silage microbial inoculated with 200 mL t^{-1} (493 g kg^{-1} DM), and highest content (569 g kg^{-1} DM) was observed in 400 mL t^{-1} level (Table 3). For the mineral matter contents, the lowest value (27.6 g kg^{-1} DM) was verified with the addition of 400 mL t^{-1} of the bioinoculant. The highest amounts of non-fibrous carbohydrates (NFC) were observed in the microbial inoculated silages with 200 mL t^{-1} (273.6 g kg^{-1} DM) and 400 mL t^{-1} (280.3 g kg^{-1} DM) levels (Table 3). The lowest amount of ether extract was observed in the silage microbial inoculated with 400 mL t^{-1} (5.89 g kg^{-1} DM), and highest content (6.25 g kg^{-1} DM) was observed in 200 mL t^{-1} level (Table 3). There were no statistical differences for lactic acid and ethanol contents (Table 4). The lowest values of acetic acid were verified at no bioinoculant (11.4 g kg^{-1} DM) and 200 mL t^{-1} (9.60 g kg^{-1} DM; Table 4). The highest levels of butyric acid (4.92 g kg^{-1} DM) were verified in the silages without addition of fermented corn juice (Table 4).

Table 3: Chemical composition of sugarcane silage with addition of fermented corn juice expressed in g kg^{-1} DM

Variables	Fermented corn juice (mL t^{-1} silage)					Average	SEM**	*P*-value
	0	200	400	800	1600			
Dry matter	183.4c	246.3a	227.9ab	219.2b	212.7b	217.9	5.77	<0.01*
Crude Protein	35.3	43.3	40.5	36.5	34.6	38.0	43.99	0.58^{ns}
NDF[1]	687.8	630.7	645.7	674.0	665.7	661	23.7	0.47^{ns}
ADF[2]	550ab	493b	569a	532ab	548ab	539	16.4	0.05*
Ash	53.0a	46.2ab	27.6b	44.9ab	30.8ab	40.3	5.29	0.02*
N-NH_3(%TN)[3]	2.8	2.0	2.3	2.4	3.7	2.6	0.78	0.58^{ns}
NFC[4]	217.8b	273.5a	280.3a	238.5ab	263.0ab	21.97	1.24	<0.02*
Ether extract	6.03b	6.25a	5.89c	6.03b	5.95bc	6.03	0.03	<0.01*
pH	4.3	4.3	4.3	4.4	4.3	4.3	0.37	0.89^{ns}

Averages followed by different letters on the same row do not differ among themselves by Tukey's test ($P < 0.05$)
*Significant; ^{ns} Not significant; **SEM= standard error of the mean; [1]NDF= neutral detergent fiber; [2]ADF= acid detergent fiber; [3]N-NH_3 (%TN) = ammoniacal nitrogen in relation to the percentage of total nitrogen; [4]NFC= non-fibrous carbohydrates

Table 4: Volatile fatty acids and ethanol of sugarcane silage with the addition of fermented corn juice

Variables g kg^{-1} DM	Fermented corn juice (mL t^{-1} silage)					Average	SEM**	P-value
	0	200	400	800	1600			
Lactic acid	62.8	61.0	59.5	59.9	49.7	58.6	5.50	0.50^{ns}
Acetic acid	11.4b	9.6b	16.3a	19.5a	18.3a	15.1	1.62	<0.01*
Propionic acid	0.36b	0.36b	0.35b	1.01a	0.72a	0.56	0.11	<0.01*
Butyric acid	4.92a	1.07bc	1.94b	0.64bc	0.46c	1.81	0.33	<0.01*
Ethanol	33.1	24.5	21.2	32.0	27.0	27.5	4.58	0.35^{ns}

Averages followed by different letters on the same row do not differ among themselves by Tukey's test ($P < 0.05$). *significant; ^{ns} not significant; **SEM= standard error of the mean

Microbial Population

The microorganism population of the fermented corn juice was maintained in approximately the same amount verified in the fresh plant after being chopped (before ensiling) (Fig. 1A).

All silages showed similar results for the development of lactic bacteria as well as for enterobacteria, with a light increase only for the enterobacteria population in the silages with 200 mL t^{-1} of bioinoculant but no difference among treatments (Fig. 1B). No significant amount of yeasts and molds was observed in the silages of all treatments (Fig. 1B).

Aerobic Stability

Different levels of fermented corn juice used as bioinoculant had significant effect on the silage surface temperature, the silage internal temperature and the pH value of the silages, and exposure time to air also showed statistical differences (Table 5).

In general, the surface temperature, and especially the internal temperature of the sugarcane silages, presented higher values in the silages without bioinoculant, 22.4°C and 26.4°C, respectively (Table 5). For pH values, intermediate levels of bioinoculants (200 and 400 mL t^{-1} silage) showed more acidic silages with lower pH values 4.61 and 4.65, respectively (Table 5). There was interaction effect between the factors (bioinoculant levels × exposure time of silages to air) only for the internal temperature of the silage (Table 5).

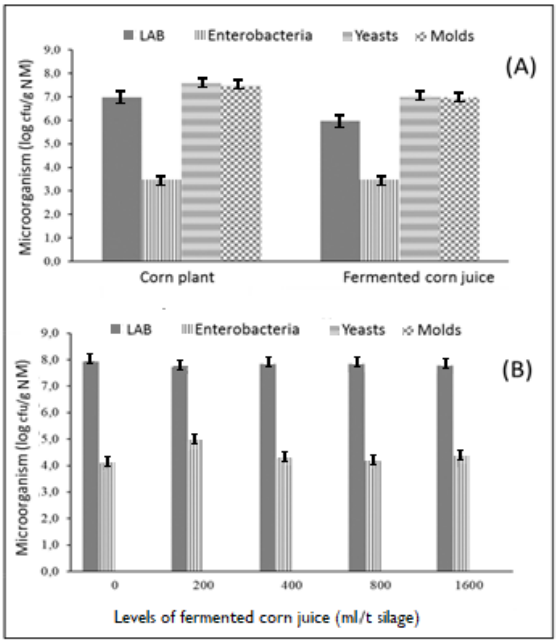

Fig. 1: (**A**) Values of the microorganism counts in the corn plant, in the fermented juice of the corn silage, and (**B**) sugarcane silage without and with addition levels of fermented corn juice. LAB: lactic acid bacteria; NM: natural matter

Degradability

In general, it was verified that sugarcane silages with 200 mL fermented corn juice had higher *in situ* degradability values of DM ($P < 0.05$) up 48 h, mainly in relation to silage without bioinoculant (Table 6).

Table 5: Average values of the temperatures and ph for the aerobic stability of sugarcane silage

FJS[1] (ml t^{-1} silage)	Hours					Average
	0	24	48	72	96	
			Ambient temperature (°C)			
	25.2	24.8	25.8	25.2	24.8	
			Silage surface temperature (°C)			
0	20.0	21.9	23.1	23.6	22.7	22.3A
200	18.8	20.3	23.2	23.4	21.8	21.5AB
400	19.2	20.1	23.1	23.4	22.7	21.7AB
800	19.6	18.5	23.4	22.7	21.9	21.2B
1600	19.2	18.6	23.2	22.5	23.8	21.5AB
Average	19.4c	19.9b	23.2a	23.1a	22.6a	
			Silage internal temperature (°C)			
0	26.1Ab	23.1Ac	32.2Aa	26.9Ab	23.8Ac	26.4A
200	24.9Bb	20.7Bd	26.3Ba	25.0Bb	23.1Ac	24.0B
400	25.9Aab	22.3Ad	26.6Ba	25.7ABb	23.7Ac	24.8B
800	26.1Ab	22.2Ad	27.1Ba	25.5Bb	23.5Ac	24.9B
1600	25.3ABb	21.9Ad	26.6Ba	23.7Cc	22.7Ad	24.0B
Average	25.7b	22.0d	27.8a	25.4b	23.4c	
			pH silage			
0	4.36	4.22	4.35	5.17	5.36	4.69AB
200	4.36	4.39	4.38	4.85	5.26	4.65B
400	4.35	4.28	4.35	4.94	5.12	4.61B
800	4.40	4.37	4.65	5.58	5.34	4.87A
1600	4.34	4.30	4.35	5.11	5.24	4.67AB
Average	4.36b	4.31b	4.42b	5.13a	5.26a	

Averages followed by different lowercase letters in the same row and uppercase letters in the same column are differ by Tukey's test ($P < 0.05$)
[1]FJS = fermented juice of silage; [**]SEM= standard error of the mean

Table 6: Potential degradability of microbial inoculated sugarcane silage with fermented corn juice as a function of rumen residence time (h)

Incubation time	Fermented corn juice added to sugarcane silage (mL)				
	0	200	400	800	1600
0 hours	8.41b	10.9a	8.25b	11.8a	3.05c
6 hours	8.87b	16.9a	10.1ab	13.4a	7.98b
12 hours	9.94b	13.4a	14.6a	15.2a	9.09b
24 hours	17.4b	20.5a	16.1b	20.3a	12.1c
48 hours	22.5c	37.0a	24.4c	29.3b	24.5c
72 hours	31.4b	30.3b	41.8a	35.0ab	39.8ab
96 hours	42.5b	39.2bc	49.6a	38.0c	42.1b

Averages followed by different lowercase letters on the same row are differ by Tukey's test ($P < 0.05$)

Discussion

The bioinoculant of fermented corn juice was efficient in reducing silage effluent losses, preventing 59% of effluent losses when comparing the silages inoculated with 200 mL t^{-1} with the silages without bioinoculants (Table 2). Similar results were described by Queiroz *et al.* (2015), Rigueira *et al.* (2017) and Moraes *et al.* (2017) in experiments with several microbial inoculants in sugarcane silages, thus showing to be efficient in reducing effluent losses and hence losses of nutritional components and natural matter by leachate.

The main causes to the effluent volume produced are dry matter content, particle size and compaction density (McDonald *et al*. 1991) that related). As all treatments had the same characteristics mentioned above, it is inferred that the difference in the observed effluent production may be related to fermentations undesirables. Effluent loss was greatest in the first three to four days when proteolytic bacterial proliferation may also occur. These bacteria promote increased butyric acid production during this process which causes increase in water, CO_2 production and increasing losses of dry matter and energy (McDonald *et al.* 1991). Likewise, higher or lower gas production may be related to dry matter losses during fermentation process, probably caused by undesirable fermentations originating from the metabolism of microorganisms such as clostridia, enterobacteria and yeasts that develop at higher pH and are responsible for the production of gases (Melo *et al.* 2016). Nonetheless, the use of fermented corn juice inhibited secondary fermentations, reducing gas and effluent losses as well as favoring dry matter recovery (Table 2). These findings associated with lower butyric acid concentration (Table 4) with the inclusion of bioinoculant confirm that there was a lower incidence of undesirable fermentation.

Addition of inoculants could improve silage fermentation by favoring degradation of forage structural carbohydrates since it provides additional sugar for fermentation. Moreover, the microbial inoculated silages had higher amount of non-fibrous carbohydrates (NFC) in relation to silages without inoculants (Junior and Muck (1997), being inversely proportional to the ADF values

(Table 3). In general, the amount of ash was shown as ideal values close to 3% for sugarcane silage (Table 3). The content of $N\text{-}NH_3$ observed in the present study is considered adequate in the evaluated silages, being less than 10% of the total nitrogen. Low concentrations of ammonical nitrogen indicate a lower proteolysis intensity during the fermentation process, resulting from the lower activity of clostridia and hence lower production of butyric acid (McDonald *et al.* 1991). This can be confirmed when the lowest amounts of butyric acid are observed in the same treatments (Table 4), which had a higher amount of acetic and propionic acid. These acids make it difficult to grow *Clostridium* bacteria, thus maintaining the quality of the silage, since the presence of butyric acid is indicative of the proliferation of this genus of bacteria and is positively correlated with the reduction of the acceptability and the forage intake (Muck and Bolsen 1991). Furthermore, the highest contents of acetic acid recorded is silage (Table 4) might have occurred due to the presence of heterolactic bacteria in the ensiled mass, which besides producing lactic acid also produces acetic acid (McDonald *et al.* 1991).

The silage without fermented corn juice showed higher concentration of butyric acid (Table 4), demonstrating that this additive from the epiphyte microflora was able to hinder the proliferation of clostridia, unlike the results obtained by Valle *et al.* (2018), who evaluated the effect of LAB homofermentative on the response of sugarcane silage and observed that the silage with these inoculants did not affect the concentrations of butyric acid and reduced $N\text{-}NH_3$ content.

According to the classification criteria of quality silage proposed by Kaiser *et al*. (2006) when the silage has butyric acid content of up to 3 g kg^{-1} characterizes very good quality silages as occurred with the inclusion of bioinoculant. The silage without fermented corn juice promoted silages of satisfactory quality but it needs improvement. The production of lactic acid leads to a rapid reduction in the pH value of silages, which aided to control the bacterial proliferation of the genus *Clostridium*, also contributing to the highest DM content observed in treatments with bioinoculants and the acceptability of silage by the animals (McDonald *et al.* 1991).

Fermented corn juice showed to be a good bioinoculant in maintaining the same amount of microorganisms contained in the plant *in natura* even after being stored for 35 days (Fig. 1). This assures to the producer that the silage is microbial inoculated with a material similar to the corn plant itself, which is one of the best forages for silage (Nussio *et al.* 2001), thus enriching sugarcane silage.

The high concentration of lactic acid and mainly acetic acid (Table 4) in all treatments possibly prevented the development of molds and yeasts, a favorable characteristic for silage. Both the lactic acid bacteria and enterobacteria populations were larger than those observed by Silva *et al.* (2018) when sorted *Lactobacillus buchneri* strains from corn silage and used them as a bioinoculant.

All silages showed increase in surface temperature, but none exceeded the ambient temperature, so that this increase in surface temperature was influenced by the environment and not by the action of microorganisms, *i.e*. by exogenous factors and not by factors intrinsic to the silage itself.

The accumulation of temperature after silo opening reflects the intensity of reactions promoted by filamentous fungi, yeasts and aerobic bacteria (Amaral *et al.* 2008). According to Guim *et al.* (2002), the respiration of aerobic microorganisms can be considered as one of the main agents that influence the quality of the silages. Therefore, silage without bioinoculant is certainly of inferior quality to those microbial inoculated with fermented corn juice.

Inhibition of molds and yeasts through fermented corn juice results in lower silage internal temperature as well as lower silage pH during aerobic stability, so it can be inferred that the use of this bioinoculant favors the preservation of silage quality when exposed aerobic environment (Junior *et al.* 2018). The longevity of the lowest pH of silage when it is exposed to aerobiosis favors the maintenance of bromatological quality of this feed. Proteolytic bacteria such as *Clostridium*, proliferate at pH above 4.8 and decrease protein and non-fibrous carbohydrate contents in silage, may produce an unpleasant odor that inhibits silage intake and may cause pathologies in animals (McDonald *et al.* 1991; Junior *et al.* 2018).

The higher efficiency of the degradability of sugarcane silages inoculated with 200 mL fermented corn juice can be explained by the lowest effluent loss (Table 2). It is inferred that there was less leaching of non-fibrous carbohydrates (Table 3) associated with lower incidence of secondary fermentations, lower contents of butyric acid (Table 4). The sum of these factors above resulted in greatest dry mater content and higher dry matter recovery (Table 2), consequently, greater nutrient conservation in dry matter that provide greater replication of ruminal microorganisms. Thus, increase the degradability rate mainly in the first 48 h (Table 6). These results favor the use of fermented corn juice in sugarcane silage in 200 and 400 mL t^{-1}, will enabling the silage to have better nutritional values, being optimized its utilization when used in the nutrition of ruminants.

Conclusion

The addition of the fermented corn juice as bioinoculant at 200 mL t^{-1} in sugarcane silage improved the fermentation profile and chemical characteristics, decreased losses and promoted greatest aerobic stability and degradability.

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