INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–0564/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1850
http://www.fspublishers.org

Full Length Article

Can Fermentative and Nutritional Quality of *Panicum maximum* Silage be Improved with the Use of Corn Silage Juice as a Bioinoculant?

Keuven dos Santos Nascimento[1*†], Ricardo Loiola Edvan[2†], Santos Jeovanny Aguilera Vallecillo[2†], Romilda Rodrigues do Nascimento[2†], Dhiéssica Morgana Alves Barros[2†], Mirella Almeida da Silva[3†], Marcos Jácome de Araújo[2†] and Leilson Rocha Bezerra[3†]

[1]*São Paulo State University (Unesp), São Paulo, Brazil*
[2]*Federal University of Piauí, Piauí, Brazil*
[3]*Federal University of Campina Grande, Paraíba, Brazil*
[*]For correspondence: keuvensantos03@gmail.com
[†]Contributed equally to this work and are co-first authors
Received 11 May 2021; Accepted 18 June 2021; Published 18 September 2021

Abstract

The aim of this study was to evaluate the quality of Paredão grass silage (*Panicum maximum* Jacq.) with fermented juice produced from corn silage in different fermentation periods, as bioinoculant, through the characteristics: gas and effluent losses, dry matter recovery, chemical composition, microbiological profile and aerobic stability. The treatments consisted of fermented corn silage juices obtained in different fermentation periods, which were: 0, 5, 10, 15 and 30 days of fermentation, and were used as a bioinoculant additive in Paredão grass silage. The lowest effluent losses were observed in silages containing corn silage juice fermented for 10 days, while the highest dry matter recovery values were observed in silages containing corn silage juice fermented for 30 days. The highest values of crude protein were observed in silages containing corn silage juice fermented for 10 days. The highest content of lactic acid (53 ± 2.2 g/kg DM) was observed in the Paredão grass silage containing corn silage juice fermented for 10 days. The inclusion of corn silage juice fermented for 10 days as bioinoculant improves the quality of Paredão grass silage, as it presented greater recovery of the silage dry matter, higher crude protein content and greater amount of beneficial acids.

Keywords: Additive; Fermentation; Fermented juices; Microorganisms; Paredão grass

Introduction

The *Panicum maximum* Jacq. grass presents easy cultivation, in addition to high production and good nutritional value for animals (Borjas-Ventura *et al.* 2019). Thus, the silage production with this tropical grass allows the storage of a large volume of forage mass which increases food security for the herd.

The production of silage using tropical grasses is subjected to high losses, because during the harvest despite having good nutritional value, they have a high moisture content, high buffering capacity and low concentrations of soluble carbohydrates, which results in a low-quality silage (Bureenok *et al.* 2011). In addition, plants ensiled with high humidity favor undesirable fermentations, reducing the nutritional value of the silage. The production of quality silage requires the presence of lactic acid epiphytic bacteria (LAB) and water-soluble carbohydrate (WSC) to produce sufficient lactic acid for rapid pH reduction (Kung *et al.* 2018).

The inoculants used in the silages are mainly composed of homofermentative lactic bacteria and aim to increase the initial population available in the forage to accelerate the process of organic acids production, and a consequent drop in the pH of the silage (Ludovico *et al.* 2014; Moraes *et al.* 2017; Cardoso *et al.* 2019; Costa *et al.* 2021). This also improves the aerobic stability of the silage after opening the silo and beneficial microflora (Silva *et al.* 2020). Currently the use of natural additives has been preferred to improve the fermentative quality of grass silages; however, the use of this technique is still in vogue.

The fermented juice of epiphytic lactic acid bacteria has been recommended as additive for tropical grass silage (Bureenok *et al.* 2005a, b). However, this additive can be ineffective because of the low LAB and low WSC content, especially when coming from plants that already have these characteristics in their natural composition (Bureenok *et al.* 2011). The corn silage stands out in the production of LAB, mainly due to the chemical composition of the plant that is beneficial for the action of LAB. When used as additive, the bioinoculant of this silage can improve the fermentation of the ensiled mass, reducing losses and preserving the

To cite this paper: Nascimento KDS, RL Edvan, SJA Vallecillo, RRD Nascimento, DMA Barros, MAD Silva, MJD Araújo, LR Bezerra (202x). Can fermentative and nutritional quality of *Panicum maximum* silage be improved with the use of corn silage juice as a bioinoculant?. *Intl J Agric Biol* 26:411–419

nutritional value (Silva *et al*. 2020). Thus, using fermented corn silage juice in grass silages may allow a greater conservation potential of silages inoculated with this product.

The fermented corn silage juice can be easily produced in the farm (Hang *et al*. 2003; Silva *et al*. 2020), but there is no information on its production made in silages of different storage periods, since these silages present different types of microorganisms at different fermentation stages in terms of days. It is expected that the corn silage, after fermentative stabilization may produce a fermented juice of better quality to be used as bioinoculant in grass silages. Therefore, it was hypothesized that a longer storage period of corn (*Zea mays* L.) silage for the production of fermented juice as bioinoculant, will provide better quality of Paredão grass (*P. maximum*) silage. Thus, the objective in this study was to evaluate the quality of *P. maximum* silage containing different fermented corn silage juices as bioinoculants through the analyses of gas and effluent losses, dry matter recovery, chemical composition, microbiological profile and aerobic stability of the silage.

Materials and Methods

All the methods used for the cultivation and collection of the vegetable material cultivated were carried out in compliance with relevant institutional, national and international guidelines and legislation.

Location, experimental design and treatments

The research was performed in the city of Bom Jesus, Piauí, Brazil. The city is located in the south of the state of Piauí, in the microregion of Alto-Médio Gurguéia, (09°04′S, 44°21′W, 277 m a.s.l.), with climatic classification BSh, with rainy summer and dry winter rains according to the Köppen classification of 1936, described by Alvares *et al*. (2013). The region presents an average minimum temperature of 18ºC, average maximum temperature of 36ºC and average annual rainfall of 900 mm. A completely randomized design was adopted, with five replications. The treatments consisted of five production periods of fermented corn silage juice to be used as bioinoculant, which were 0, 5, 10, 15 and 30 days of silage storage.

Cultivation and ensiling

In the experimental field of corn crop (BAS hybrid) and Paredão grass, the soil was representatively sampled at a depth of 0-20 cm. After sampling, the samples were sent to the Soil Analysis Center Laboratory of the Professora Cinobelina Elvas Campus (CPCE) of the Federal University of Piauí (UFPI).

The soil was classified as Dystrophic Yellow Latosol, with a clay texture (clay, 257 g/kg; mud, 34 g/kg, sand, 709 g/kg) and had the following characteristics: 5.3 pH in water;, 15.7 mg/dm^3 of phosphorus; 116.9 mg/dm^3 of potassium; 1.7 cmol c/dm^3 of calcium; 0.4 cmol c/dm^3 of magnesium; 0.0 cmol c/dm^3 of aluminum; 0.1 cmol c/dm^3 of hydrogen + aluminum; 4.3 cmol c/ dm^3 of sum of bases; 7.5 cmol c/dm^3 of cation exchange capacity at pH 7.0; 660 g/kg of base saturation; and 0.0 g/kg of aluminum saturation.

The Paredão grass plants were sown in January 2016, under irrigated system. The cultivation of corn was performed in March 2017 in the same experimental field. In both crops, 40 kg/ha of phosphorus (simple superphosphate, 180 g/kg of P_2O_5, 110 g/kg of S and 180 g/kg of Ca) were applied and 30 days after planting (corn) and right after uniform cutting of Paredão grass, 120 kg/ha of nitrogen (urea, 450 g/kg of N) were applied. It was not necessary to correct the soil or fertilize with potassium, because according to the soil analysis, the chemical values were sufficient for both species (Martha Júnior *et al*. 2007).

A stationary silage machine regulated to cut the material into particles of 2 to 3 cm was used to chop the material for the production of the Paredão grass silage. After chopping, the bioinoculant was added in the material through spraying, applying the equivalent of 500 mL/ton of fresh forage.

Two types of experimental silos were produced, silos in plastic buckets with a storage capacity of 3.0 kg of silage and experimental PVC silos (100 mm in diameter) with a length of 50 cm and a capacity of 2.5 kg of silage. Both were filled with silage from the same processing, and at the same time and place of filling of the silos, to allow homogeneity of the ensiled material. The aerobic stability was analyzed in the silages from the experimental PVC silos, while the analyses of chemical composition, losses through effluents and gas, organic acids content (lactic acid, acetic acid, propionic acid and butyric acid) and microbial population count were performed in the silage from the 3 kg experimental silos. The Paredão grass silages already inoculated were stored with an average density of 500 kg m^{-3}. In order to characterize the silage, both the fresh forage and the product of the silage were analyzed 120 days after ensiling. The analyses were performed at the Microbiology and Animal Nutrition Laboratories of CPCE/UFPI, in the city of Bom Jesus, Piauí, Brazil.

Production of fermented corn silage juice

For the production of fermented corn silage juice, the plants of corn crop were cut and chopped when they reached R4 stage (dough). The silage was produced following all procedures to obtain quality silage. Samples for the production of fermented corn silage juice were obtained according to periods of 0, 5, 10, 15 and 30 days of fermentation, always removing from the same silo. All ensiling procedures were also performed in the silage stored for 0 days (Fig. 1).

For the production of fermented corn silage juice, at

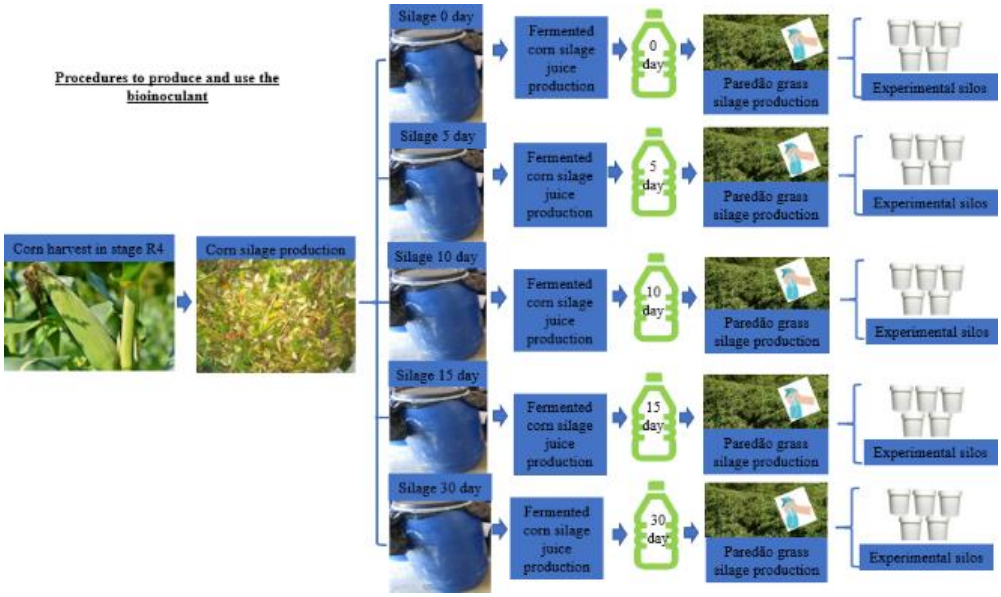

Fig 1: Procedures to produce and use the bioinoculant

the end of each fermentative period (0, 5, 10, 15 and 30 days) 500 g of silage were collected and processed in a blender, then the collected liquid was strained through a 1.0-mm sieve, and added 500 g of corn glucose and distilled water until filling to 2 L of mixture (Silva *et al*. 2020). The resulting mixture was stored in plastic bottles for 30 days.

Determination of losses through effluents, dry matter and gas

To obtain the values of losses through gas and effluents, 1.0 kg of sand was deposited at the bottom of each experimental silo of 3 kg of capacity, separated from the forage by a layer of cotton fabric, making it possible to measure the amount of effluent retained. The losses through gas, effluents and the dry matter recovery were obtained according to equations described by Zanine *et al*. (2010). Based on the weight difference of the dry forage mass, the gas losses were obtained using the following equation: G = (iFW-fFW) / (iFM × iDM) × 100, where: G: gas losses (% DM); iFW: weight of the full silo after closing (kg); fFW: weight of the full silo after opening (kg); iFM: forage mass after closing (kg); iDM: forage dry matter content after closing.

The effluent losses were calculated through the following equation, based on the difference in weight of the sand and related to the fresh forage mass after closing. E = [(fEW-St)-(iEW-St)]/iFM×100, where: E: effluent production (kg/ton of silage); iEW: weight of empty bucket + weight of sand after closing (kg); fEW: weight of empty silo + weight of sand after opening (kg); St: silo tare; iFM: forage mass after closing (kg). The following equation was used to estimate the dry matter recovery: DMR (g kg^{-1} of DM) = [(foGM×foDM)/(iSM×siDM)]×100, where: DMR (g kg^{-1} of DM): DM recovery as percentage; foGM: forage green mass (kg) during ensiling; foDM: forage DM (%) during ensiling; iSM: silage mass (kg) before opening the silos; siDM: Silage DM (%) after opening of the silos.

Determination of chemical composition and fatty acids

Fresh samples of Paredão grass and corn (Table 1) and silages were properly stored and frozen in a freezer (-20°C) for further analysis. The samples were stored in paper bags, identified, weighed and pre-dried in a forced air circulation oven at 55ºC for 72 h and later, ground in a Willey knife mill with a 1.0-mm sieve.

To determine the chemical composition the analyses were performed in triplicate, following the AOAC recommendations (1990) regarding the contents of dry matter (method 967.03), crude protein (method 981.10), ether extract (method 920.29) and ash (method 942.05). The determination of neutral detergent fiber (NDF) and acid detergent fiber (FDA) was carried out according to the methods described by Van Soest *et al*. (1991) with the modification proposed by Senger *et al*. (2008). The temperature of the autoclave was adjusted to 110°C for 40 min. Determination of non-fibrous carbohydrates (NFC) was done according to Weiss (1999): NFC (g/kg)=1000-(NDF+PB+EE+ash).

To determine volatile fatty acids (lactic, acetic, propionic and butyric), 10g of each silage was weighed in triplicate and added to 90 mL of distilled water, this material was later homogenized and filtered through a fine mesh sieve covered with gauze. From the solution, a 10 mL sample of the filtrate was taken and placed in tubes for centrifugation, with 2.0 mL of metaphosphoric acid (3M) being added, and then the solution was centrifuged for 15 min at 13,000 × g. After this process, the supernatant was transferred to Eppendorf tubes sealed, identified and frozen

Table 1: Chemical composition of Paredão grass and corn before ensiling

Chemical composition	Ingredients	
	Paredão grass	Corn
Dry matter (g kg^{-1} of feed)	262	332
Crude protein (g kg^{-1} of DM)	879	794
Neutral detergent fiber (g kg^{-1} of DM)	621	562
Acid detergent fiber (g kg^{-1} of DM)	-	278
Ashes (g kg^{-1} of DM)	61.0	45.0
pH	6.10	5.20
Ether extract (g kg^{-1} of DM)	-	316
Nin-fiber carbohydrates (g kg^{-1} of DM)	-	322

for analysis of organic acid by high performance liquid chromatography (HPLC) (Vrátný and Mudřík 1985).

Microbial population count

For the enumeration of microbial groups of lactic acid bacteria (LAB), enterobacteria, molds and yeasts, homogenization of replicates of each treatment was performed with 90 mL of distilled water added to the samples, in order to obtain a dilution of 10^{-1}. Dilutions were performed in order to obtain variations from 10^{-1} to 10^{-9}.

Plating was performed in sterile Petri dishes (duplicate). For the quantification of microbial populations selective culture media were used for each microbial group, as follows: Agar Rogosa (Difco), for enumeration of LABs after 48 h of incubation at 37°C; Bright Green Agar (Disc), for enumeration of enterobacteria (ENT) after 24 h of incubation at 35°C; and Potato Dextrose Agar, which was added with 1 kg of 0.01 g/kg tartaric acid, after sterilization, to count molds and yeasts (M and Y) after incubation for 3–7 days at room temperature. The incubations were performed in a B.O.D. oven. The plates petri dish considered susceptible to counting were those in which there were values between 30 and 300 CFU (colony forming units). 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). The enumeration of the microbial groups present in the fermented juices of corn silage in each fermentative period was carried out as described above (Fig. 2).

Aerobic stability and pH

To determine aerobic stability, the silages were taken to a closed room with temperature control, where a suspended digital thermometer measured the temperature constantly. The aerobic stability of the silage surface layer was determined after opening and unpacking the top layer of the silo. The material was exposed to air for a period of 0 to 48 h.

After opening the silo in the determined time, the surface temperature and the mass of the silage were checked every hour, over a period of 12 h (0, 12, 24, 36 and 48 h)

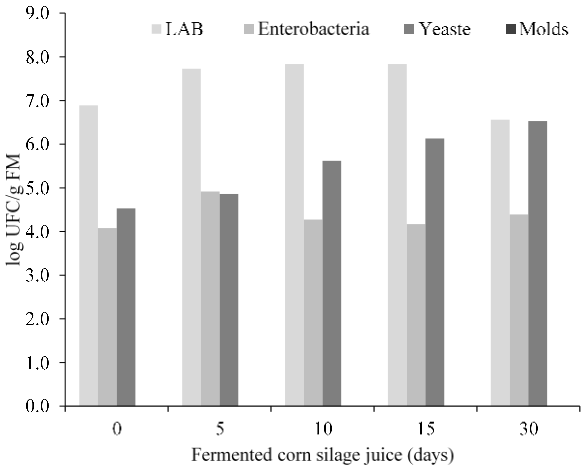

Fig 2: Microorganisms count values in fermented corn silage juice of different fermentation periods
LAB: lactic acid bacteria ¥FM: fresh material

(Silva *et al*. 2020). The surface temperature of the silage was measured by a digital infrared thermometer and the internal temperature of the forage mass by a digital immersion thermometer, inserted in the center of the silo (at a depth of 10 cm). Aerobic stability was determined according to Taylor and Kung Jr. (2002), to considers the time necessary for the silage, after exposure to air, an increase of 2°C above room temperature, thus considering the break in stability aerobic use of the material.

The determination of pH in distilled water was carried out in duplicate. Being determined from the addition of 100 mL of water in samples of 25 g of ensiled material for each treatment. After 1 h, a reading was taken with a bench microprocessor pH meter (Bolsen *et al.* 1992); Marconi – Piracicaba, São Paulo, Brasil). The data were collected during the periods of exposure of the silage to air at 0, 12, 24, 36 and 48 h.

Statistical Analysis

The data were subjected to an analysis of variance for determine the level of $P < 0.05$. The data related to the losses through effluents, gases and dry matter, chemical composition and volatile fatty acids of the Paredão grass with fermented corn silage juices of different fermentation periods, were analyzed by the Tukey's Test, through the iterative process of the SAS® PROC NLIN implementation of the Marquardt algorithm. The statistical model used was: $Zij=\mu+Fi+\varepsilon i$, where Z represents the observed value, and Fi the fixed effect of the fermentation period i (i = 0, 5, 10, 15, 30 days).

The aerobic stability data of Paredão grass silage was performed for the analysis of interaction between the fermented juices from corn silage of different fermentation periods (0, 5, 10, 15 and 30 days) × air exposure times of the Paredão grass silage (0, 12, 24, 36 and 48 h). The

Table 2: Losses of Paredão grass silage with the inclusion of fermented corn silage juice of different fermentation periods

Variables	Fermented corn silage juice (days)					Mean	SEM[£]	*P-value*
	0	5	10	15	30			
E[†] (kg t^{-1} AF)	141b	67a	11b	59a	66a	437	54	< 001*
G[‡] (% DM)	130	162	096	111	066	113	023	014[ns]
DMR[§] (% DM)	721ab	604b	594b	599b	85a	674	346	< 001*

Means followed by different letters in the row are statistically different by the Tukey's test with $P < 0.05$; *significant at $P < 0.05$; [ns]non significant at $P > 0.05$; [†]E: Loss through effluents kg/t on as fed basis (AF); [‡]G: Loss through gas, percentage (%) on dry matter basis (DM); [§]DMR: Dry Matter Recovery in percentage (%); [£]SEM: standard error of the mean

statistical model $Z_{ij}=\mu+F_i+E_j+(F\times E)_{ij}+\varepsilon_{ij}$ was used, where Z represents the observed value, Fi the fixed effect of the fermentation period i (i = 0, 5, 10, 15, 30 days), Ej the fixed effect of the air exposure times j (j = 0, 12, 24, 36, 48 h), and (F × E)ij the interaction between fermentation periods and the air exposure times. A regression analysis (linear and quadratic) was performed with the data of air exposure time of the Paredão grass silage using the MIXED and REG procedures implemented in the statistical software SAS® (version 9.1. Cary, NC, USA).

Results

Losses through effluents, dry matter and gas

There was a significant effect of the treatments ($P < 0.01$) on effluent losses and dry matter recovery (DMR). The highest losses through effluents were observed in silages containing corn silage juice fermented for 5, 15 and 30 days (Table 2) showing mean values of 67, 59 and 66 kg/t AF, respectively. However, the silage containing the bioinoculant fermented for 10 days presented the lowest effluent loss values (11±5.4 kg/t AF). No effect ($P = 0.14$) was observed on losses through gas of Paredão grass silage, averaging 1.13±0.23 g/kg DM. The highest DMR value was observed in the silages containing corn silage juice fermented for 30 days, presenting 85±3.46 g/kg DM, followed by the silages treated with corn silage juice fermented for 0 days, which presented 72±3.46 g/kg DM. The silages inoculated with corn silage juice fermented for 5, 10 and 15 days presented DMR values statistically not different.

Chemical composition and volatile fatty acids

Regarding the chemical composition of Paredão grass silage containing fermented corn silage juice, only the crude protein content were affected (P = 0.03). No effect (P > 0.05) of the inclusion of fermented corn silage juices was observed on the dry matter (DM), neutral detergent fiber (NDF), Ashes, and pH values (Table 3). The highest CP content value was observed in the silage containing corn silage juice fermented for 10 days, showing 82.1±3.82 g/kg of CP; while the lowest CP value was observed in the silage containing corn silage juice fermented for 30 days, with 61.2±3.82 g/kg. The other treatments were not statistically different, presenting values of 79±3.82, 73.1±3.82 and 71.7±3.82 g/kg of CP for the silages containing corn silage juice fermented for 0, 5 and 15 days, respectively.

Table 3: Chemical composition of Paredão grass silage with the inclusion of fermented corn silage juice of different fermentation periods

Variables	Fermented corn silage juice (days)					Mean	SEM[¤]	*P-value*
	0	5	10	15	30			
DM[†],	188	174	193	183	202	188	86	0.27[ns]
CP[‡]	79ab	731ab	821a	717ab	612b	734	382	0.03*
NDF§	554	557	547	570	611	568	166	0.14[ns]
Ashes	455	532	492	524	492	499	237	0.25[ns]
pH	7.00	7.10	7.00	7.20	6.40	7.01	063	0.88[ns]

Means followed by different letters in the row are statistically different by the Tukey's test with $P < 0.05$; *significant at $P < 0.05$; [ns]non-significant at $P > 0.05$; [†], DM: dry matter, expressed as g/kg; [‡]CP: crude protein, expressed as g/kg DM; [§]NDF: Neutral Detergent Insoluble Fiber, expressed as g/kg DM;, expressed as g/kg DM; [¤]SEM: standard error of the mean

Table 4: Volatile fatty acids (VFA) of Paredão grass silage with the inclusion of fermented corn silage juice of different fermentation periods

VFA	Fermented corn silage juice (days)					Mean	SEM[†]	*P-value*
(g kg^{-1} DM)	0	5	10	15	30			
Lactic acid	88c	00d	53a	128b	00d	149	22	< 001*
Acetic acid	00c	133b	83bc	9bc	354a	133	17	< 001*
Propionic acid	03b	34b	16b	1b	91a	31	06	< 001*
Butyric acid	69	107	71	91	84	76	25	033[ns]

Means followed by different letters in the row are statistically different by the Tukey's test with $P < 0.05$; *significant at $P < 0.05$; [ns]non-significant at $P > 0.05$; [†]SEM: standard error of the mean

Regarding the volatile fatty acids (Table 4), the contents of lactic, acetic, and propionic acids were affected (P < 0.01) by the inclusion of the bioinoculant. Non-significant effects were observed only on the butyric acid content. The highest content of lactic acid was observed in the treatment containing corn silage juice fermented for 10 days, which presented 53±2.2 g/kg. The treatments containing corn silage juice fermented for 0 and 15 days presented values of 8.8 and 12.8 g/kg, respectively. The acids acetic and propionic were found in greater concentration in silages inoculated with the fermented corn silage juice of the longest fermentation period, 30 days, showing 35.4±1.7 and 9.1±0.6 g/kg, respectively. The other treatments did not present significant differences in the content of propionic acid with the addition of fermented corn silage juice in the silage of Paredão grass.

Microbial population

All treatments showed similar results for the development of

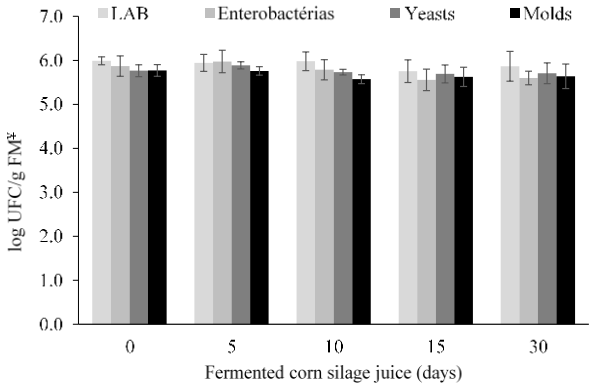

Fig 3: Microorganisms count values in Paredão grass silage with inclusion of fermented corn silage juice of different fermentation periods
LAB: lactic acid bacteria ¥FM: fresh material

lactic acid bacteria, enterobacteria, yeasts and molds in the Paredão grass silages (Fig. 3). Regarding the presence of microorganisms in the corn plant (6.43, 3.47, 7.15 and 7.13 log CFU/g AF, of LAB, enterobacteria, yeasts and molds, respectively) when compared to the silages of Paredão grass containing fermented corn silage juice of different fermentation periods, there was a reduction in the amount of LAB, yeasts and molds, presenting averages of 5.84, 5.75 and 5.67 CFU/g AF, respectively, in the silages of Paredão grass. An increase of 2.82 log CFU/g AF was also observed for enterobacteria in the Paredão grass silages when compared to the corn plant, with mean values of 5.75 and 3.47 log CFU/g AF, for the silages and corn plant, respectively.

Aerobic stability and pH

There was no interaction ($P > 0.05$) of silage fermentation period for the production of the bioinoculant×hours of air exposure of the Paredão grass silage (Table 5). The surface and internal temperatures of the Paredão grass silage were affected ($P < 0.01$) only by the hours of air exposure. There was no effect on the pH values, which averaged 6.96. There was an increasing linear effect of hours of air exposure on the surface temperature of the silage, which increased by 4ºC after 48 h. However, even with the increase in the surface temperature, it remained on average 4.8°C below the room temperature. There was a linear decreasing effect on the internal temperature of the silage, where the highest temperature was obtained in the silage of Paredão grass with 0 h of air exposure. There was a reduction of 2.1ºC after 48 h of air exposure in the Paredão grass silage.

Discussion

In general, our results do not support our initial hypothesis that a longer storage period of corn silage for the production of fermented juice as a bioinoculant would provide better quality of Paredão grass silage. However, an issue of

Table 5: Analysis of aerobic stability of Paredão grass silage with inclusion of fermented corn silage juice of different fermentation periods

FP† (days)	Air exposure of silage (h)					Mean	*P-value*	
	0	12	24	36	48		x	x^2
Room temperature (°C)								
	252	248	258	252	248			
Silage surface temperature (°C)								
0	158	222	21	211	202	200	-	-
5	153	209	205	21	203	196	-	-
10	165	221	205	206	195	198	-	-
15	165	215	205	206	194	197	-	-
30	16	143	205	207	205	184	-	-
Mean	16	202	206	208	20	-	< 001*	< 001*
Silage internal temperature (°C)								
0	268	231	24	23	24	242	-	-
5	235	225	226	23	24	231	-	-
10	258	224	233	223	23	233	-	-
15	271	216	233	24	246	241	-	-
30	261	208	226	223	233	23	-	-
Mean	259	221	232	229	238	-	< 001*	< 001*
Silage pH								
0	7.90	7.10	7.00	6.40	6.90	7.00	-	-
5	7.00	7.10	7.30	6.70	7.50	7.10	-	-
10	7.40	7.00	7.00	7.70	7.00	7.00		-
15	7.70	7.20	7.60	5.80	7.90	7.20	-	-
30	5.50	6.20	7.00	5.60	7.70	6.40	-	-
Mean	7.10	6.90	7.20	6.20	7.40	-	-	-

Analysis of variance	*P-value*			
	FP¥	H	FP† × h	‡SEM
Silage surface temperature	046ns	< 001*	042ns	067
Silage internal temperature	009ns	< 001*	059ns	037
Silage pH	031ns	009ns	086ns	029

†FP: corn silage fermentation period for the production of fermented juice ‡SEM: standard error of the mean *significant at $P < 0.05$; nsnon-significant at $P > 0.05$; x: linear effect; x^2: quadratic effect

tropical grass silage is inadequate fermentation due to the low content of soluble carbohydrates and reduced presence of bacteria that actively reduce pH (McDonald *et al.* 1991; Kung *et al.* 2018). In our study, we observed that the inclusion of fermented corn juice with 10 days of fermentation acts as an enhancer for the presence of BAL in Paredão grass silages. These effects opposite to the ones we hypothesized (that is, they are not the best in the longest fermentative periods, but in the smallest ones) are positive because they indicate that the production of fermented corn silage juice can enter the strategic planning of rural properties with a period shorter fermentation, allowing the procedure to be performed with a better window for collecting the material to be ensiled.

The Paredão grass silages without fermented corn silage juice and with corn silage juice fermented for 10 days provided lesser effluent losses. The silage with corn silage juice fermented for 30 days provided 178.9 g/kg more dry matter recovery (DMR) than the silage without fermented corn silage juice. The losses by effluents observed in the silages of Paredão grass containing fermented corn silage juice were lower than those observed by Moraes *et al.* (2017), who added different mixtures microbial inoculants (*Lactobacillus buchneri*; *Propionibacterium acidipropionici*; *P. acidipropionici* + *L. plantarum*; *L. buchneri* + *P. acidipropionici*; *L. buchneri* + *P. acidipropionici* + *L. plantarum*). This shows that the use of

fermented corn silage juice may be viable for the conservation of Paredão grass silage, as they reduce the loss of nutritional components and green matter.

The low values of effluent losses observed in silages containing corn silage juice fermented for 10 days may have been due to the microbiological composition of the corn silage during the juice production (Fig. 2), since the active fermentation phase of the silage occurs from 7 to 30 days (Muck and Pitt 1993). The presence of lactic fermenting microorganisms that act as pH-reducing acid producers, allows the conservation of the material and the stabilization of losses by inactivating microorganisms such as enterobacteria and yeasts. Oliveira *et al*. (2010) observed in their studies that the volume of effluent produced in a silo is mainly influenced by the DM content of the ensiled species, and that the effluent losses are minimized when the DM content in the silage reaches 300 g/kg. In fact that was not observed in the Paredão grass (Table 1).

The silage goes through 5 phases of different durations and intensities of proliferation of microorganisms, which vary according to the native fauna, processing quality and fermentation period (McDonald *et al.* 1991; Tao *et al*. 2020). This shows that the fermented corn silage juice with 0 and 5 days of fermentation have reduced quality, since this period may be insufficient for the proliferation of anaerobic microorganisms that conserve the silage. Different fermented corn silage juices used as additives in the Paredão grass silage had no effect on the chemical composition of these silages, as well as the silage without fermented corn silage juice, except for the crude protein (CP) content. This probably occurred due to the little influence in the change promoted by the fermented juices in the silage on the components of dry matter, neutral detergent fiber and ashes, resulting in less alteration of the chemical composition due to the treatments applied.

The Paredão grass silage containing corn silage juice fermented for 10 days showed the highest CP content. This is due to the microbial activity related to this fermentation period, when it presents consumption of soluble carbohydrates and consequent increase of CP (Siqueira *et al*. 2011). Adequate levels of CP serve as indicative of lower intensities of proteolysis during the fermentation of ensiled material. This fact can be due to lower activity of Clostridiums and, consequently, the lowest concentration of butyric acid (McDonald *et al*. 1991; Drahokoupil and Patáková 2020). The CP content of the Paredão grass silages inoculated with fermented corn silage juice were larger than those observed by Bezerra *et al*. (2019), who found silages of grasses with contents ranging from 64.6 to 72.7 g/kg. In addition, treatments with lower CP values presented greater losses, which indicate greater proteolysis activity.

A greater amount of lactic acid was observed in the silages containing corn silage juice fermented for 10 days (Table 4). The greater amount of lactic acid reduces the proliferation of bacteria of the genus *Clostridium* (Muck *et al*. 2018) and the quality of the silage is maintained for a longer period, as well as the better palatability and forage intake (Muck and Bolsen 1991; Sofyan *et al*. 2017). According to Bureenok *et al*. (2005b) increase in the population of lactic acid bacteria and decrease in other microorganisms occurs in the first days of fermentation, showing that microbial succession occurs very quickly and very definitively. However, these microorganisms were still present in the silage. This corroborates to the present study that showed an increase in the lactic acid content in silages inoculated with corn silage bioinoculant fermented for 10 days (Table 4), with no reduction in the microorganism population (Fig. 3).

There was a greater amount of acetic acid in the Paredão grass silage containing corn silage juice fermented for 30 days, showing levels higher than those recommended as ideal for silages, which is < 20 g/kg DM, a level that defines a good quality silage (Rego *et al*. 2013). The increase in the acetic acid values of the Paredão grass silages inoculated with corn silage juice fermented for more than 5 days is likely owing to the peak of enterobacterial development that occurred from the third day of fermentation, as observed by Pinho *et al.* (2013), since the final product of these microorganisms from the consumption of glucose is one molecule of lactate and one molecule of acetate or ethanol (Mcdonald *et al.* 1991; Yang *et al*. 2020). According to Luis and Ramírez (1988), enterobacteria usually multiply until approximately the seventh day of fermentation, when they are replaced by lactic acid groups. At the beginning of fermentation, enterobacteria compete with LABs for the available soluble carbohydrates, and the greater competitive and antagonistic capacity arising from the bacteriocins produced by LABs is crucial, an effect not observed in the present experiment possibly due to the resistance in decreasing pH, which made it difficult the development of lactic acid bacteria.

The results obtained on the production of acids in the Paredão grass silage inoculated with corn silage juice fermented for 10 days demonstrate that this fermentation period was more efficient, probably for conserving and cultivating the beneficial epiphytic micro fauna. Silages with 10 days of storage enhance the actions of beneficial microorganisms, in addition to keeping the proliferation of microorganisms that deteriorate the silage quality stable (Bureenok *et al*. 2005a). Likewise, Pinho *et al.* (2013) observed peak of the development of LAB populations from the seventh day of fermentation. The highest concentration of lactic acid in the silage inoculated with corn silage juice fermented for 10 days helps to control the proliferation of bacteria of the genus Clostridium, and contributes to better values of silage dry matter recovery and acceptability of the silage by the animals (Denek *et al.* 2011; He *et al*. 2020).

The presence of butyric acid in the Paredão grass silage was probably due to high moisture content (Table 1), combined with the high buffering capacity intrinsic to grasses; the conditions that favor butyric fermentation instead of lactic fermentation (Meeske *et al.* 1999). The

fermentation periods of 0, 5, 10, 15 and 30 days of the corn silage for the production of fermented juice had no influence on the number of microorganisms (LAB, enterobacteria, yeasts and molds) in the Paredão grass silages. These data indicated that there was stabilization of microorganisms in the Paredão grass silage 120 days after ensiling, regardless of the use of corn silage bioinoculant.

A difference was observed between the population of microorganisms of the Paredão grass plant and its silage, and this occurred because the microbial population of the plant at the time of harvest is different from the one found during the fermentation process as well as in the silage (Bureenok *et al*. 2011). The development of these microbial populations in the ensiled material is related to the conditions of the environment, which will naturally select the microbial groups that may develop (Bezerra *et al.* 2019), mainly due to the chemical characteristics of the plant. It can also be stated that the concentration of lactic acid in all treatments, was possibly not sufficient to prevent the development of molds and yeasts. However, the population of lactic acid bacteria and enterobacteria observed in the present study were similar to those found in previous studies that used corn silage as a bioinoculant (Silva *et al*. 2018; 2020).

Regarding the aerobic stability of the Paredão grass silage, an increase in the surface temperature was observed in all treatments, but none exceeded the room temperature, with no break in the aerobic stability, thus deducing that this increase in the surface temperature occurred due to exogenous factors and not by intrinsic factors of the silage itself (Silva *et al.* 2020). Increase in temperature observed after opening the silo may be the result of reactions promoted by undesirable microorganisms, such as yeasts, filamentous fungi and aerobic bacteria (Amaral *et al.* 2008), however, the exposure of the Paredão grass silages, regardless of the treatment, did not cause increase of the temperature in relation to the environment. This may have occurred mainly due to the low amount of soluble carbohydrates and low number of endogenous bacteria present in the grass (Bureenok *et al*. 2005a), which keep the activity of microorganisms at low levels even after opening the silo.

Higher pH levels found in the Paredão grass silage, which obtained an average of 7.0 regardless of treatment, can be explained by high buffering capacity that grasses have and low content of soluble carbohydrates in the plant. The consumption of carbohydrates by microorganisms is vital for the reduction of pH (Pinho *et al*. 2013). The authors found decreasing carbohydrates content from values close to 3% (%DM), up to values of 0.5%, for the pH decrease from 5.4 to 4.4.

Conclusion

The inclusion of fermented corn silage juice influences the quality of Paredão grass silage. The addition of corn silage juice fermented for 10 days is recommended because it promotes greater recovery of the silage dry matter, higher crude protein content and greater amount of beneficial acids. However, it is important to carry out further studies regarding the storage of fermented juices and their use as a bioinoculant, especially regarding the aerobic stability of silages.

Acknowledgements

This work was financially supported by Federal University of Piauí.

Author Contributions

RLE, MJA and LRB conceived and designed the experiments, KSN, MAS and RRN carried out the experiments and analyzed the data, KSN and SJAV wrote the manuscript with the help of DMAB. All authors read, edited, and approved the manuscript.

Conflicts of Interest

The authors reported no potential conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

References

Alvares CA, JL Stape, PC Sentelhas, JLM Gonçalves, G Sparovek (2013). Köppen's climate classification map for Brazil. *Meteorol Z* 22:711–728

Amaral RC, TF Bernardes, GR Siqueira, RA Reis (2008). Aerobic stability of marandu grass silages submitted to different intensities of compaction in silage. *Rev Bras Zootec* 37:977–983

Association of Official Analytical Chemists (AOAC) (1990). *Official Methods of Analysis*, 15th edn,. AOAC, Arlington, Texas, USA

Bezerra HFC, EM Santos, JS Oliveira, GGP Carvalho, RMA Pinho, TC Silva, AM Zanine (2019). Fermentation characteristics and chemical composition of elephant grass silage with ground maize and fermented juice of epiphytic lactic acid bacteria. *S Afr J Anim Sci* 49:522–533

Bolsen KK, DG Tiemann, RN Sonon, RA Hart, B Dalke, JT Dickerson, C Lin (1992). Evaluation of inoculant-treated corn silages. *Kansas Agric Exp Stn Res Rep* 1:104–107

Borjas-Ventura R, LR Alves, R Oliveira, CA Martínez, PL Gratão (2019). Impacts of warming and water deficit on antioxidant responses in *Panicum maximum*. *Physiol Plantarum* 165:413–426

Bureenok S, T Namihira, Y Kawamoto, T Nakada (2005a). Additive effects of fermented juice of epiphytic lactic acid bacteria on the fermentative quality of guineagrass (*Panicum maximum* Jacq) silage. *Grassl Sci* 51:243–248

Bureenok S, T Namihira, M Tamaki, M Mizumachi, Y Kawamoto, T Nakada (2005b). Fermentative quality of guineagrass silage by using fermented juice of the epiphytic lactic acid bacteria (FJLB) as a silage additive. *Asian-Aust J Anim Sci* 18:807–811

Bureenok S, W Suksombat, Y Kawamoto (2011). Effects of the fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses on digestibility and rumen fermentation characteristics of ruzigrass (*Brachiaria ruziziensis*) silages. *Livest Sci* 138:266–271

Cardoso LL, KG Ribeiro, MI Marcondes, OG Pereira, K Weiβ (2019). Chemical composition and production of ethanol and other volatile organic compounds in sugarcane silage treated with chemical and microbial additives. *Anim Prod Sci* 59:721–728

Costa DM, BF Carvalho, TF Bernardes, RF Schwan, CLA Silva (2021). New epiphytic strains of lactic acid bacteria improve the conservation of corn silage harvested at late maturity. *Anim Feed Sci Technol* 274:48–52

Denek N, A Can, M Avci, M Aksu (2011). The effect of fresh and frozen pre-fermented juice on the fermentation quality of first-cut lucerne silage. *Sci Commun Fac Anim Sci* 54:785–790

Drahokoupil M, P Patáková (2020). Production of butyric acid at constant pH by a solventogenic strain of *Clostridium beijerinckii*. *Czech J Food Sci* 38:185–191

González G, AA Rodríguez (2003). Effect of storage method on fermentation characteristics, aerobic stability, and forage intake of tropical grasses ensiled in round bales. *J Dairy Sci* 86:926–933

Hang YD, EE Woodams, LE Hang (2003). Use of corn juice by *Kluyveromyces marxianus*. *Bioresour Technol* 3:305–307

He L, C Wang, Y Xing, W Zhou, R Pian, X Chen, Q Zhang (2020). Ensiling characteristics, proteolysis and bacterial community of high-moisture corn stalk and stylo silage prepared with *Bauhinia variegata* flower. *Bioresour Technol* 296; Article 122336

Kung Jr L, RD Shaver, RJ Grant, RJ Schmidt (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *J Dairy Sci* 101:4020–4033

Ludovico A, EH Hoshi, LC Silva, FCAR Grecco, LFV Cunha Filho, MR Baran (2014). Chemical composition and losses of nutrients involved in sugarcane ensiling with microbial and chemical additives. *Arch Latinoamer Prod Anim* 22:33–42

Luis L, M Ramírez (1988). Evolución de la flora microbiana en un ensilaje de king grass. *Pastos For* 11:3–12

Martha Júnior GB, LA Vilela, DM Sousa (2007). *Cerrado: Uso eficiente de corretivos e fertilizantes em cerrados*. Embrapa Cerrados, Planaltina, Brazil

McDonald P, AR Henderson, SJ Heron (1991). *The Biochemistry of Silage*, 2nd edn. Chalcombe Publications, Marlow, Buckinghamshire, England

Meeske R, HM Basson, CW Cruywagen (1999). The effect of a lactic acid bacterial inoculant with enzymes on the fermentation dynamics, intake and digestibility of *Digitaria eriantha* silage. *Anim Feed Sci Technol* 81:237–248

Moraes RLD, KG Ribeiro, OG Pereira, MI Marcondes, LL Cardoso (2017). Silage from sugarcane treated with microbial inoculants and mixtures thereof. *Rev Bras Agropec Sustentável* 7:76–83

Muck RE, KK Bolsen (1991). Silage preservation and silage additive products. *In: Field Guide for Hay and Silage Management in North America*, pp:105–126 National Feed Ingredients Association Des Moines, Iowa, USA

Muck RE, RE Pitt (1993). Ensiling and its effect on crop quality. *In: Proceedings of the National Silage Production Conference*, pp:57–66. Syracuse, New York NRAES-67 Northeast Reg Agric Ext Service, Ithaca, New York, USA

Muck RE, EMG Nadeau, TA McAllister, FE Contreras-Govea, MC Santos, L Kung Jr (2018) Silage review: Recent advances and future uses of silage additives. *J Dairy Sci* 101:3980–4000

Oliveira LB, AJV Pires, GGP Carvalho, LSO Ribeiro, VV Almeida, CAM Peixoto (2010). Perdas e valor nutritivo de silagens de milho, sorgo-sudão, sorgo forrageiro e girassol. *Rev Bras Zootec* 39:61–67

Pinho RMA, EM Santos, GGPD Carvalho, APGD Silva, TCD Silva, FS Campos, CHO Macedo (2013). Microbial and fermentation profiles, losses and chemical composition of silages of buffel grass harvested at different cutting Heights. *Rev Bras Zootec* 42:850–856

Rego FCA, A Ludovico, LC Silva, LD Lima, EW Santana, MC Françozo (2013). Fermentation profile, chemical composition and dry matter losses of orange pulp silage with different microbial inoculants. *Semina: Ciências Agrárias* 33:3411–3420

Senger CC, GV Kozloski, LMB Sanchez, FR Mesquita, TP Alves, DS Castanino (2008). Evaluation of autoclave procedures for fibre analysis in forage and concentrate feedstuffs. *Anim Feed Sci Technol* 146:169–174

Silva LD, OG Pereira, TC Silva, ES Leandro, RA Paula, SA Santos, KG Ribeiro, SCV Filho (2018) Effects of *Lactobacillus buchneri* isolated from tropical maize silage on fermentation and aerobic stability of maize and sugarcane silages. *Grass Forage Sci* 73:660–670

Silva MA, RL Edvan, HN Parente, AM Zanine, JM Pereira Filho, EM Santos, LR Bezerra (2020). Addition of fermented corn juice as bioinoculant improved quality of *Saccharum officinarum* silage. *Intl J Agric Biol* 23:349–356

Siqueira GR, RA Reis, RP Schocken-Iturrino, APDTP Roth, MDTP Roth, FD Resende (2011). Perfil fermentativo de silagens de cana-de-açúcar in natura ou queimada e tratadas ou não com *Lactobacillus buchneri*. *Rev Bras Zootec* 40:1651–1661

Sofyan A, Y Widyastuti, R Utomo, LM Yusiati (2017). Improving physico-chemical characteristic and palatability of king grass (pennisetum hybrid) silage by inoculation of *Lactobacillus plantarum-Saccharomyces cerevisiae* consortia and addition of rice bran. *Buletin Peternakan* 41:61–71

Tao Y, D Niu, F Li, S Zuo, Q Sun, C Xu (2020). Effects of ensiling *Oxytropis glabra* with whole-plant corn at different proportions on fermentation quality, alkaloid swainsonine content, and lactic acid bacteria populations. *Animals* 10:1733–1741

Taylor CC, L Kung Jr (2002). The Effect of *Lactobacillus buchneri* 40788 on the fermentation and aerobic stability of high moisture corn in laboratory silos. *J Dairy Sci* 85:1526–1532

Van Soest PJ, JB Robertson, BA Lewis (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74:3583–3597

Vrátný P, Z Mudřík (1985). Liquid chromatography of organic acids in silage extracts using dual detection. *J Chromatogr A* 322:352–357

Weiss WP (1999). Energy prediction equations for ruminant feeds *In: Proceedings from the Cornell Nutrition Conference for Feed Manufacturers*, pp:176–185 Cornell University, Ithaca, New York, USA

Yang Y, Z Zhang, X Lu, J Gu, Y Wang, Y Yao, J Hao (2020). Production of 2, 3-dihydroxyisovalerate by *Enterobacter cloacae*. *Enz Microb Technol* 140; Article 109650

Zanine AM, EM Santos, JRR Dórea, PAS Dantas, TCS Silva, OG Pereira (2010) Evaluation of elephant grass silage with the addition of cassava scrapings. *Rev Bras Zootec* 39:2611–2616