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Effect of microbial inoculant on the fermentation profile, nutritional value and microbial population on corn, sorghum, and pearl millet silages

Efeito do inoculante microbiano no perfil de fermentação, valor nutricional e população microbiana em silagens de milho, sorgo e milheto

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Highlights

The inoculant improved the crude protein content of corn and sorghum silages.
The inoculant decreased the ammonia nitrogen content in pearl millet silage by 5%.
The inoculant in the ensiling process did not reduce the amount of mould (kg).

Abstract

The objective of the present study was to evaluate the use of microbial inoculant on the chemical composition, *in vitro* gas production, pH, dry matter losses, aerobic stability and microbial population on silages of corn, sorghum and pearl millet in plastic bags silos (without *vacuum*). The experiment was carried out in a randomized block design, in a 2 × 3 factorial scheme, with and without (control) inoculant consisting of *Lactobacillus plantarum* and *Propionibacterium acidipropionici* and on three crops, corn, sorghum and pearl millet, with four replicates. The use of the inoculant did not affect the chemical composition of the silages, except the crude protein (P = 0.0062) and lignin (P = 0.0567) contents. Gas production was neither affected (P > 0.05) by the inoculant nor by the crop. Regarding aerobic stability, we observed that the inoculant affected the temperature of the sorghum silage (P = 0.0123). The inoculant decreased the $N\text{-}NH_3$ (P =0.0095) content and increased (P = 0.0441) the lactic acid bacteria population in the silages. Thus, the microbial inoculant did not improve the fermentation profile or nutritional value of corn, pearl millet and

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sorghum silages in plastic bag silos (without *vacuum*).
Key words: Conservation. Fermentation capacity. Inoculant. Lactic acid bacteria.

Resumo

O objetivo do presente estudo foi avaliar o uso de inoculante bacteriano na composição química, produção de gás *in vitro*, pH, perdas de matéria seca, estabilidade aeróbia e população microbiana de silagens de milho, sorgo e milheto em silos de sacos plásticos (sem vácuo). O experimento foi conduzido em delineamento de blocos casualizados, em esquema fatorial 2 × 3, [Controle] sem inoculante e *Lactobacillus plantarum* e *Propionibacterium acidipropionici* e três culturas, milho, sorgo e milheto, com quatro repetições. O uso do inoculante não afetou a composição química das silagens, exceto proteína bruta (P = 0,0062) e lignina (P = 0,0567). A produção de gás não foi afetada (P > 0,05) pelo inoculante e nem entre as culturas. Na estabilidade aeróbia, observou-se que o inoculante afetou a temperatura da silagem de sorgo (P = 0,0123). O inoculante diminuiu o conteúdo de N-NH_3 (P = 0,0095). O inoculante aumentou (P = 0,0441) a população de bactérias ácido-láticas nas silagens. Assim, o inoculante microbiano não melhorou o perfil fermentativo e o valor nutricional das silagens de milho, sorgo e milheto em silos de sacos plásticos (sem vácuo).
Palavras-chave: Conservação. Capacidade de fermentação. Inoculante. Bactérias de ácido láctico.

Introduction

During the ensiling process, factors such as the filling, compaction, sealing and additive are critical to avoid quality losses. Microbial inoculants are recommended to reduce dry matter losses in silage of tropical grasses, since homolactic bacteria compete with epiphytic microorganisms, increasing the fermentation efficiency (Borreani et al., 2018). Lactic acid bacteria are one of the main inoculants used in ensiling, with the aim of dominating fermentation through the rapid production of lactic acid (homolactic bacteria) and consequent pH decrease, thus inhibiting the growth of undesirable microorganisms and the production of acetic and propionic acids (Muck et al., 2018). Other microorganisms, such as heterofermentative bacteria, can increase acetic and propionic acids (Bernardes & Rêgo, 2014) as heterofermentative lactic acid bacteria silage additives slowly convert lactic acid to acetic acid and 1,2-propanediol during silo storage, improving aerobic stability (Muck et al., 2018). There are several compositions of inoculants in the trade. As a rule, those produced from homolactic bacteria improve the fermentation pattern (greater efficiency in the production of lactic acid, faster acidification and lower final pH), whereas heterolactic bacteria inoculants are used to increase aerobic stability and improve fermentation (Queiroz et al., 2018). *Lactobacillus plantarum* is one of the most used heterolactic bacteria due to its vigorous growth, acid tolerance and high potential for lactic acid production (Muck et al., 2018). In the heterofermentative bacteria group, *Propionibacterium acidipropionici* uses lactic acid and glucose as substrates to produce acetic and propionic acids, which effectively control fungi under low pH (Zopollatto et al., 2009).

Therefore, we hypothesized that the microbial inoculant would improve the quality of fermentation and aerobic stability of corn, sorghum and pearl millet silages. Thus, the

objective of the present study was to evaluate the effect of microbial inoculant (*Lactobacillus plantarum* and *Propionibacterium acidipropionici*) on the chemical composition, in vitro gas production, pH, dry matter losses, aerobic stability and microbial population of corn, pearl millet and sorghum silages in plastic bag silos (without vacuum).

Material and Methods

The experiment was carried out at the Universidade Federal Rural do Rio de Janeiro (UFRRJ) - Campos dos Goytacazes *Campus*, RJ, Brazil (21º47'54"S and 41º17'34"W, 12 m a.s.l.). According to Köppen's classification (Alvares et al., 2013), the climate in the North of the Rio de Janeiro State is Aw, i.e. humid tropical with a rainy summer, dry winter and annual rainfall around 1020 mm.

The experiment was carried out in a completely randomized design with a 2 × 3 factorial scheme. The first factor was the presence (LP) or absence (control, CON) if the inoculant, *Lactobacillus plantarum* (2.5 × 1010 cfu/g) and *Propionibacterium acidipropionici* (2.5 × 1010 cfu/g; Biomax corn, Lallemand, Saint-Simon, France), and the second was the crop: corn (*Zea Mays* L.), cv. PR1150; sorghum (*Sorghum bicolor* x *Sorghum sudanense*), hybrid BRS810 or pearl millet (*Pennisetum glaucum* L.), cv. BRS 1501. We used four replicates, totalling 24 experimental units. The treatments were randomly distributed among the silos. The microbial inoculant was used according to the manufacturer's recommendations (2 g/ton of forage): diluted in tap water and sprayed on the forages to be ensiled.

The pearl millet, corn and sorghum were harvested and processed in a stationary forage chopper (JF Maxxium Model, JF Máquinas Agrícolas LTDA, Brazil) with an average particle size of ±1.5 cm. We used plastic bags (polyethylene 51 cm wide × 110 cm long and 200 microns) for ensiling the forages. The silos were closed with nylon clamps and stored at an ambient temperature of 25 ± 2.3 ºC for 90 days. The silos were packed with a density of 600 kg/m^3 (fresh forage ensiled).

After the silos were opened, the silage samples were dried in a forced-air oven at ±55 °C for 72 hours to yield the partially dried samples. Then, the samples were ground in a Wiley mill fitted with a 1-mm sieve. We determined the contents of total dry matter - DM (Association of Official Analytical Chemistry [AOAC], AOAC, 2019), crude fat – CF (AOAC, 2019), ash (AOAC, 2019), crude protein - CP [N × 6.25] (AOAC, 2019) and neutral detergent fibre using a standardized heat stable amylase solution and residual ash, acid detergent fibre (ADF) and lignin (LIG) according to the methodology described Detmann et al. (2012). The non-fibrous carbohydrate (NFC) content was estimated as follows: *NFC(g/kg)* = 1000 - *CP* - *CF* - *Ash* - *NDF.* Hemicellulose was calculated as the difference between the NDF and ADF contents, and cellulose as the difference between the ADF and lignin contents, expressed in g/kg DM.

We collected the ruminal fluid from three sheep, of mean weight 45 kg (standard deviation = 3.2 kg), with permanent rumen cannulas. The animals were housed in collective pens with troughs and drinkers. The sheep were adapted to a diet with Tifton 85 hay and feed concentrate (80:20, forage to concentrate ratio) for 14 days to meet their maintenance requirements. After this period, the collection of ruminal fluid began, and it took place moments before the daytime feeding, as recommended by Yáñez-Ruiz et al. (2016). The ruminal fluid (liquid and solid) was

collected at several points on the liquid-solid interface of the ruminal environment for each incubation battery. We used a silage sample of approximately 500 mg (standard deviation = 10 mg) in amber culture flasks (100 ml) together with 50 ml of an inoculum (1:4 ratio, ruminal fluid and buffer solution, respectively). The buffer solution was prepared as described by McDougall (1948). The flasks were immediately filled with CO_2, closed and placed in a water bath at 39 ºC.

The time profiles of gas production were obtained using a non-automated device, similar to that described by Abreu et al. (2014). We measured pressure and volume at times 0; 1; 2; 3; 4; 6; 8; 10;12; 16; 20; 24; 30; 36; 48; 72 and 96 hours after the addition of ruminal inoculum. The cumulative pressure and volume of fermentation gases were obtained by summing the corrected readings throughout the measurement times.

We used the model described by Groot et al. (1996) to explain the cumulative gas production profiles:

$$G = A/(1 + (B^c/t^c)) \quad \text{Eq.1}$$

$$R_M(mLh^{-1}) = B \times (C-1)^{1/C} \quad \text{Eq.2}$$

Where, the parameter *G* represents the amount of gas produced per unit of organic matter incubated at time *t* after the incubation period; the parameter *A* represents the asymptotic gas production (mg/g DM); the parameter *B* is the time (h) after incubation in which half of the asymptotic gas was produced, and it represents the gas production speed; the parameter *C* is a constant that determines the sharpness of the curve change characteristic. The R_M represents the maximum gas production rate at which the microbial population does not limit the fermentation and digestion is not reduced by chemical or structural barriers of the potentially digestible matter.

About 2.0 kg of silage was packed in plastic bags of 5.0 kg capacity, where it remained for seven days in a room with a controlled temperature (25 °C) for assessment of aerobic stability. For this, we used a data logger (Log 110 EXF Inconterm; Brazil) inserted in the ensiled mass at a depth of 10 cm in the central portion, and the temperature was recorded at intervals of 8 hours. Aerobic stability was calculated as the time, in hours, in which the temperatures of the silages were 2 °C higher than the ambient temperature after opening the silo. In addition, we collected samples from each silo during the aerobic stability assessment at 24-hour intervals to determine DM (AOAC, 2019).

After each silo was opened, the material was homogenized, a 25-g sample of fresh silage was taken, and 225 ml of saline solution (8.5 g of NaCl/L of distilled water) was added and homogenized for 1 minute in an industrial processor. The extract was filtered through a double layer of gauze, and the pH was measured with a pH meter (MPA-210, Tecnopon, Brazil). Aliquots of 2 mL of extract were transferred to test tubes with 1 mL of sulfuric acid (1N) and stored at −20 ºC. Ammoniacal nitrogen analysis was performed according to the methodology of Fenner (1965).

A 10-ml aliquot of the aqueous extract was submitted to serial dilutions (10^{-1} to 10^{-6}). Microorganisms were cultivated in sterile Petri dishes. For enumeration of enterobacteria, we used VRB (Violet Red Bile) culture medium incubated for 24 h at 37 °C; for fungi, PDA (Potato Dextrose Ágar) culture medium incubated for four days at 25 °C; for lactic acid bacteria, MRS (De Man, Rogosa, Sharpe) culture medium incubated for 48 h at 37 °C. We counted the colonies on dishes that showed

between 30 and 300 colony-forming units (CFU). The results were log-transformed (log10 cfu) for data evaluation and interpretation.

Data regarding chemical composition, dry matter losses, ammoniacal nitrogen, pH, temperature, the microbial population and cumulative gas production were compared through the Tukey test with a 0.05 significance level using the MIXED procedure of SAS (SAS University Edition, SAS Institute Inc., Cary, NC, USA).

We used the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$$

Where, Y_{ijk} is the value observed for the variable under study referring to the *k-th* replicate of the combination of the *i-th* level of factor α with the *j-th* level of factor β; μ is the mean of all experimental units for the variable under study; α_i is the use or not of inoculant in the silage with i = 1.2; β_j is the crop effect, with j = 1,2,3; $\alpha\beta_{ij}$ is the interaction between the use or not of inoculant and crops and e_{ijk} is the error associated with observation Y_{ijkl}.

We analysed pH and temperature data (aerobic stability) as repeated measures over time, and we applied regression analysis with a significance level of 0.05 using the SAS MIXED procedure of SAS (SAS University Edition, SAS Institute Inc., Cary, NC, USA).

We used the following statistical model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \tau_k + \alpha\beta_{ij} + \alpha\tau_{ik} + \beta\tau_{jk} + \alpha\beta\tau_{ijk} + e_{ijkl}$$

Where, Y_{ijk} is the value observed for the variable under study referring to the *l-th* replicate of the combination of the *i-th* level of factor α with the *j-th* level of factor β in the *k-th* hour; μ is the mean of all experimental units for the variable under study; α_i is the use or not of inoculant in the silage with i = 1.2; β_j is the crop effect, with j = 1,2,3; τ_k as the random effect of the evaluation hours with k = 0.24,..., 144 for pH and 0.8, 16,..., 162 for temperature; $\alpha\beta_{ij}$ is the interaction between the use or not of inoculant and crops; $\alpha\tau_{ik}$ is the interaction between the use of inoculant or not and the evaluation hours; $\beta\tau_{jk}$ is the interaction between crops and evaluation hours; $\alpha\beta\tau_{ijk}$ is the interaction between the use of inoculant or not, crops, and evaluation hours; and e_{ijkl} is the error associated with observation Y_{ijkl}.

The slice option was used to separate means when the crops × inoculant interaction was different (P < 0.05) using the SLICE option of SAS.

Results and Discussion

We observed the chemical composition of the silages and noted a difference between the crops, except for the CF (P = 0.7695), NDF (P = 0.0607) and NCF (P = 0.1429) contents (Table 1). The lower DM content observed in pearl millet and sorghum silages can be explained by their higher resistance to drought due to an adaptive process that prevents excessive dehydration, including smaller stomata, early stomatal closure, low stomatal density, and increased leaf serosity, all of which act to increase water retention in the plant (Levitt, 1980). There was a crop × inoculant interaction (P = 0.0357; Table 1) for CP content. Analysing the unfolding of the interaction, we observed that corn and sorghum silages without inoculant had lower CP contents. In addition to the difference between crops, we also observed that the inoculant increased the CP contents (P = 0.0062) (Table 1) but could not reduce losses caused by proteolysis.

Table 1
Effects of the use of inoculants on the chemical composition of corn, sorghum, and pearl millet silage

Variables	Pearl millet	Corn	Sorghum	SEM	P-values		
					Inoculant	Culture	Interaction
DM					0.4603	<0.0001	0.1995
W/add	241.28b	333.77a	224.75b	12.93			
Inoculant	274.97b	322.92a	224.77b	10.49			
CP					0.0062	0.0003	0.0357
W/add	59.45Aa	42.06Bc	50.15Bb	2.20			
Inoculant	57.83Ba	52.35Ab	58.61Aa	1.11			
CF					0.7001	0.7695	0.9305
W/add	10.77	10.59	10.70	0.44			
Inoculant	10.75	10.70	10.77	0.07			
Ashes					0.0859	<0.0001	0.2428
W/add	10.54a	6.66b	10.55a	0.50			
Inoculant	10.45a	5.77b	10.40a	0.60			
NDF					0.8496	0.0607	0.0919
W/add	438.28	448.13	492	9.24			
Inoculant	431.21	485.22	454.42	7.99			
ADF					0.087	<0.0001	0.753
W/add	381.2b	362.16b	427.77a	7.51			
Inoculant	373.53b	350.65b	406.73a	6.44			
NFC					0.8328	0.1429	0.0834
W/add	480.94	492.54	436.59	9.64			
Inoculant	489.74	445.95	465.78	6.96			
LIG					0.0567	<0.0001	0.1591
W/add	51.8 Aa	38.14Ab	56.52 Aa	2.05			
Inoculant	46.07Ba	37.25Bb	55.98Ba	1.91			
Hemic					0.4639	0.0098	0.1944
W/add	57.08b	85.97a	64.22b	7.75			
Inoculant	57.67b	134.57a	47.68b	11.63			
Cell					0.1672	0.0002	0.6684
W/add	329.4b	324.01b	371.25a	5.94			
Inoculant	327.46b	313.39b	354.02a	4.97			

SEM = Standard error of the mean; W/add = Without inoculant; Culture = pearl millet, corn, and sorghum silages; DM = Dry matter (expressed as g/kg as fed); CP = Crude protein; CF = Crude fat; NDF = Neutral detegent fiber; ADF = Acid detergent fiber; NFC = Non-fibrous carbohydrate; LIG = Lignin Hemic = Hemicellulose; and Cell = Cellulose. All expressed as g/kg.
*Means followed by the different capital letters in a column and lower case letters on the lines differ significantly by the Tukey test ($P < 0.05$).

Gas production was not affected (P > 0.05) by the inoculant and did not differ between crops (Table 2). According to Bach et al. (2005), crude protein concentrations below 70 g/kg may restrict microbial activity by limiting nitrogen. In this study (Table 2), these concentrations were from 40 to 60 g/kg. However, we observed that, although there was no difference (P > 0.05), the time taken for half of the asymptotic gas to be produced (Parameter B) in the corn silage with or without inoculant was shorter than that in other silages (Table 2). Gas production rates peaked in the first hours of incubation, being longer in the corn silage without inoculant, but all silages had a final rate below 0.1 ml/h.

Table 2
Effects of inoculants on gas production parameters of pearl millet, corn, and sorghum silage

Variables	A, mg/g DM	SE	C	SE	B, h	SE	P-values		
							Inoculant	Culture	Interaction
		Pearl millet					0.4783	0.2458	0.7513
W/add	26.194	1.612	0.907	0.044	31.34	3.76			
Inoculant	20.492	2.903	0.969	0.054	28.43	3.344			
		Corn					0.3201	0.8814	0.2588
W/add	18.424	2.79	1.25	0.089	18.53	1.625			
Inoculant	30.808	1.214	1.013	0.031	27.68	1.643			
		Sorghum					0.2713	0.6245	0.7584
W/add	19.722	1.466	0.881	0.056	22.9	3.525			
Inoculant	17.921	2.067	1.015	0.072	20.44	2.739			

SEM = Standard error of the mean; W/add = Without inoculant; Culture = pearl millet, corn, and sorghum silages; DM = Dry matter (expressed as g/kg as fed); CP = Crude protein; CF = Crude fat; NDF = Neutral detegent fiber; ADF = Acid detergent fiber; NFC = Non-fibrous carbohydrate; LIG = Lignin Hemic = Hemicellulose; and Cell = Cellulose. All expressed as g/kg.
*Means followed by the different capital letters in a column and lower case letters on the lines differ significantly by the Tukey test (P < 0.05).

Analysing the ammoniacal nitrogen (Table 3), we observed that the CP degradation was between 25% and 30%. For Kung et al. (2018), the rapid pH decline is crucial to reduce protein degradation during ensiling, which probably occurred in corn silage without inoculant, as it had a low degradation rate (21.11% [8.88/42.06]). Another factor that may have influenced protein degradation is the material of the silo (polyethylene) in terms of oxygen permeability, i.e. gas exchange between the silage and the external environment, with oxygen entering even without any physical damage to the plastic bags (Amaral et al., 2014), which may have allowed the increased growth of undesirable microorganisms such as mould (Table 4). The activity of these microorganisms is intensified in the presence of soluble carbohydrates, acids and proteins, increasing (P < 0.05) the silage pH (Table 3).

Table 3
Effects of inoculant in the fermentative parameters of pearl millet, corn, and sorghum silages

Variables	Pearl millet	Corn	Sorghum	SEM	P-values		
					Inoculant	Culture	Interaction
T, °C					0.8911	0.3196	0.9576
W/add	24.37	27.23	27.3	0.43			
Inoculant	24.83	27.1	27.63	1.59			
pH					0.0013	<0.0001	0.0672
W/add	3.56Aa	3.68Aa	3.75Aa	0.02			
Inoculant	3.44Bb	3.64Ba	3.35Bb	0.044			
DMlos, g/kg					0.9487	0.2986	0.3778
W/add	95.48	43.62	70.23	8.62			
Inoculant	67.77	63.08	75.78	5.19			
NH_3-N, g/kg CP					0.0040	0.0095	0.2200
W/add	17.38Aa	8.88Bb	12.61Ba	1.53			
Inoculant	16.51Ba	15.90Ab	17.02Aa	1.12			

SEM = Standard error of the mean; W/add = Without inoculant; Culture = pearl millet, corn, and sorghum silages; T = Temperature (after opening the silo); DMlos = Dry matter losses; NH3-N = Ammoniacal nitrogen CP = crude protein; pH after opening the silo.
*Means followed by the different capital letters in a column and lower case letters on the lines differ significantly by the Tukey test (P < 0.05).

Hemicellulose degradation has been neglected for many years (Ning et al., 2017), but some studies have shown this degradation to occur during ensiling (Chen et al., 2015). Ning et al. (2017) reported that the degradation products of hemicellulose (xylose) and starch (glucose) could be substrates for microorganisms to produce acids during ensiling; this was in fact observed in our study (Table 1). LIG and CEL contents were lower in corn silage (Table 1), and the LIG content reduced (P = 0.0567) in corn (19.65% [42.06/52.35]) and sorghum (14.43% [50.15/58.61]) silages. These values can be explained by the translocation of carbon from the leaf to the formation and filling of grains, which increases the starch content. In our study, the NFC contents were not different (P = 0.1429) between crops, but that of corn silage was 2.35% higher than that of pearl millet silage and 11.36% higher than that of sorghum silage without inoculant (Table 1). Corn silage presented the lowest ash content (Table 1). According to Borreani et al. (2018), the low ash content indicates excellent forage conservation, as the occurrence of inadequate fermentation results in losses of organic matter, increasing the share of ash in DM.

Regarding the aerobic stability of the silage, we observed that the silage temperature at the opening was not affected by the inoculant (P = 0.8911) and did not differ between crops (P = 0.3196). However, pH was affected by both the inoculant (P = 0.0013) and the crops (P < 0.0001) (Table 3).

Analysing the day-to-day temperature, we observed that in the first 36 hours, there was a peak temperature in sorghum silage regardless of the inoculant. However, the temperature of silage with an inoculant decreased (P = 0.0123) more quickly (Figure 1C). According to K. Wang et al. (2016), the initial increase in temperature (up to three days of exposure to air) is caused by the growth of enterobacteria and yeasts, and according to McDonald et al. (1991) and Muck et al. (2018), the second occurs three to four days after the first and can be attributed to filamentous fungi, as observed in our study (Figures 1 A, B and C). Plastic bag (polyethylene) silos can present oxygen permeability at a temperature of 25 °C. Gas exchange between the interior of the silo and the environment is close to 1 L/ m^2, which is the value for an intact bag without any physical damage (Greenhill, 1964). This exchange can make the silage more prone to aerobic deterioration due to the increase in the permeability of the bags, as the aeration of this mass allows the action of yeasts that oxidize the silage's preservative organic acids, triggering aerobic degradation and increasing pH. In our study, the pH of pearl millet, corn and sorghum silages without inoculant increased by 2.0 points 24, 96 and 96 hours, respectively, whereas that of silages with inoculant increased by 2.0 points in pH in 48 hours (Figures 1D, E, and F). The loss of aerobic stability of silages is usually manifested by an increase in temperature and a change in pH.

There was no inoculant × crop interaction effect (P > 0.05) (Tables 3). The temperature immediately after the opening of the silos and dry matter losses were not affected (P > 0.05) by the inoculant (P > 0.05) and did not differ between crops (P > 0.05) (Table 3). The inoculant decreased the pH of corn, sorghum and pearl millet silage by 1.09%, 10.67% and 3.37%, respectively. There was a statistical difference (P < 0.0001) between crops (Table 3). Ammoniacal nitrogen followed a similar behaviour to pH. The inoculant increased the ammonia nitrogen content in corn silage, sorghum silage and pearl millet silage by 44.15%, 25.91% and 5%, respectively. The crops differed significantly (P = 0.0095) (Table 3). Ammoniacal nitrogen (NH_3-N) indicates the amount of protein degraded during fermentation. It is an indicator of the extent of clostridial activity, since it is produced in small amounts by other microorganisms in the silage and by plant enzymes (Borreani et al., 2018). According to Blajman et al. (2020), the combination of hetero- and homofermentative bacteria favours reduction of pH values, NH_3-N and fermentative losses in silages. The NH_3-N levels were affected by the inoculant (0.0040) and differed between crops (P = 0.0095) (Table 3). According to Gomes et al. (2017), NH_3-N values below 10 g/kg CP in silage indicate good fermentation, and those above 15 g/kg CP indicate a significant amount of proteolysis. In the present study, only corn silage without inoculant (8.88 g/kg CP) presented NH_3-N values below 10 g/kg CP. This fact may indicate a higher intensity of proteolysis, especially by amino acid degradation by bacteria of the genus Clostridium (Diether & Willing, 2018).

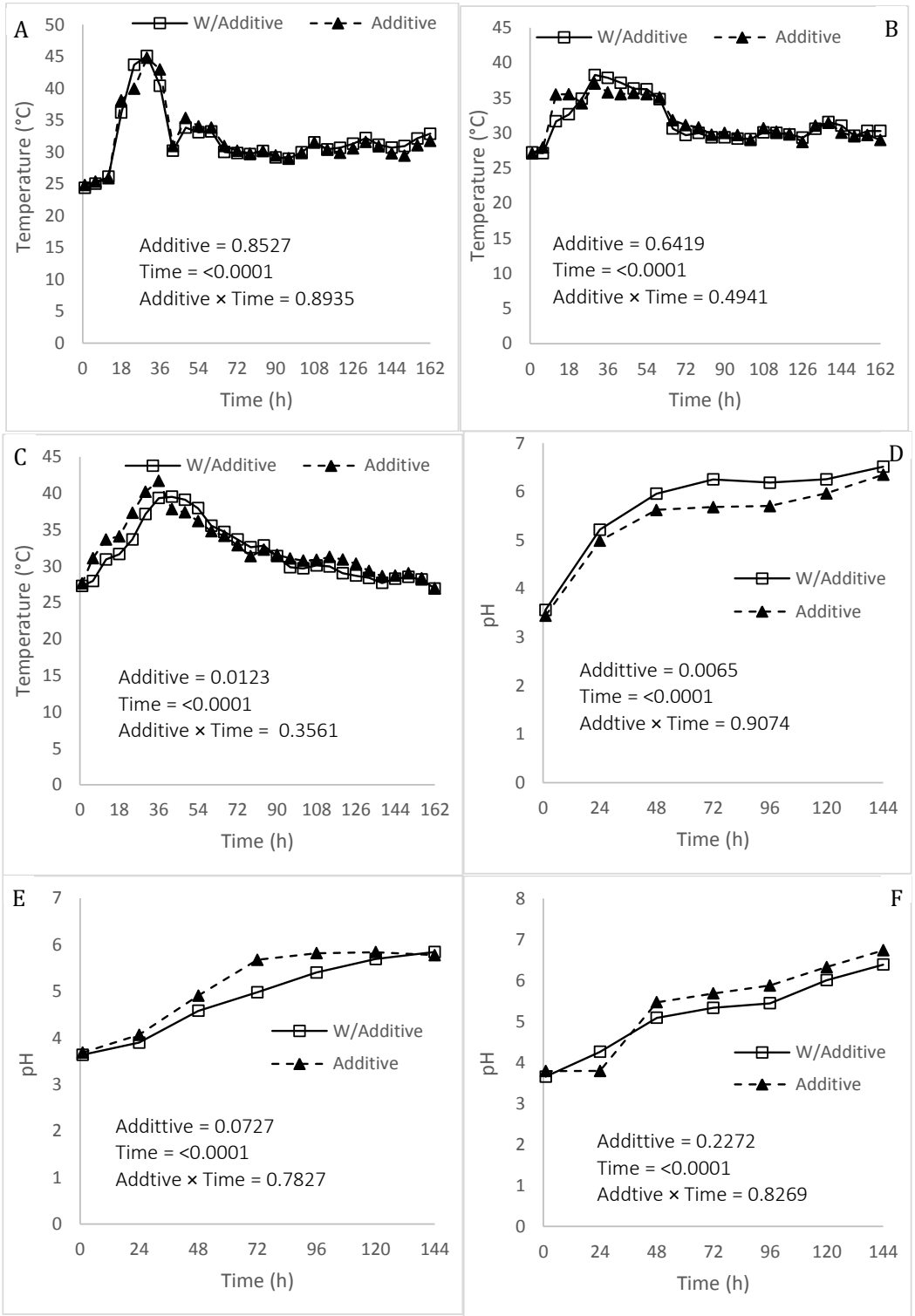

Figure 1. Aerobic stability of pearl millet, corn, and sorghum silage with or not inoculant. On panel (1A) temperature of pearl millet silage; (1B) temperature of corn silage; (1C) temperature of sorghum silage; (1D) pH of pearl millet silage; (1E) pH of corn silage; and (1F) pH of sorghum silage.

It is also essential to understand the microbial population, as ensiling will preserve the forage and inhibit undesired microorganisms (Clostridium sp., enterobacteria, yeasts and fungi), influencing silage quality (Muck et al., 2018). In this study, there were no counts for enterobacteria in the silos. This fact is related to the active growth of lactic acid bacteria (LAB) during the fermentation process; as the pH decreases to values between 3.35 and 3.75 (Table 3), it favours the rapid population decline of enterobacteria, turning LAB into the main microorganisms in silage (Kung et al., 2018). The inoculant increased the LAB population and decreased the pH in the silos. The inoculant increased (P = 0.0441) the population of lactic acid bacteria in pearl millet, corn and sorghum silages by 9.63%, 19.46% and 31.27%, respectively (Table 4).

Corn silage presented a more significant amount of fungi (P < 0.0001) than pearl millet and sorghum silages, regardless of the use of inoculant (Table 4). The activity of Lactobacillus plantarum (one of the microorganisms in the inoculant of this study) increases lactic acid production, consequently reducing the pH of the ensiled mass and inhibiting the growth of unwanted microorganisms (Muck et al., 2018). Analysing the crops, we observed that corn silage presented the highest (P < 0.001) fungal population, about 37.75% more than pearl millet silage and 29.27% more than sorghum silage (Table 4). This fact is probably because plastic bag (polyethylene) silos can present oxygen permeability, and most fungi are strictly aerobic. They can use sugars (glucose, sucrose and maltose) and more complex carbohydrates (starch, cellulose and hemicellulose) as substrates for their growth (B. Wang et al., 2020).

Table 4
Effects of inoculants on microbial populations of pearl millet, corn, and sorghum silages

Variables	Pearl millet	Corn	Sorghum	SEM	P-values		
					Inoculant	Culture	Interaction
Lactic acid bacteria, log10 g fresh silage					0.0441	0.7518	0.6054
W/add	6.19B	6.12B	5.12B	0.36			
Inoculant	6.85[a]	7.60A	7.45A	0.12			
Fungi, log10 g fresh silage					0.4341	<0.0001	0.667
W/add	4.74b	7.45a	5.0b	0.4			
Inoculant	4.67b	7.64a	5.69b	0.4			
Mold, kg of ensiled mass					0.2241	0.4262	0.4639
W/add	1.48	4.18	0.56	0.62			
Inoculant	1.64	5.65	6.81	1.24			
Mold, % of the ensiled mass					0.2241	0.4262	0.4638
W/add	7.82	22.02	2.95	3.28			
Inoculant	8.62	29.74	35.85	6.55			

SEM = Standard error of the mean, W/add = Without aditive; Culture = pearl millet, corn, and sorghum silages.
*Means followed by the different capital letters in a column and lower case letters on the lines differ significantly by the Tukey test (P < 0.05).

Conclusion

The microbial inoculant did not improve the fermentation profile and nutritional value of corn, sorghum and pearl millet silages in plastic bag silos (without vacuum).

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