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**ANIMAL SCIENCE**

# Evaluation of the type of silo associated or not with additives on the nutritional value, aerobic stability, and microbiology of pearl millet silage

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**Abstract:** The objective of this study was to evaluate the effect of the silo type with the use or not of additives on chemical composition, *in vitro* gas production, fermentative losses, aerobic stability, fermentative profile, and microbial population of the pearl millet silage. We used a randomized block design in a 2 × 3 factorial scheme, with two types of silos (plastic bags and PVC silos) and three additives ([CON] without additive; 50 g of ground corn [GC], and *Lactobacillus plantarum* and *Propionibacterium acidipropionici*, with five replicates per treatment. We evaluated the chemical analyses, *in vitro* gas production, losses, aerobic stability, pH, ammoniacal nitrogen, and microbial population of the silages. The use of GC in the ensiling process improved the chemical composition of the silages. The additives and the type of silo did not affect ($p > 0.05$) the gas production kinetics, ammoniacal nitrogen, and population of lactic acid bacteria and fungi. Thus, the use of ground corn improved the nutritional value of the pearl millet silage. In turn, the inoculant provided better aerobic stability for the pearl millet silage. The plastic bag silos without vacuum were not efficient in the ensiling process like the PVC silos, which resulted in low-quality silage.

**Key words:** Conservation, inoculant, *Pennisetum glaucum L.*, quality.

## INTRODUCTION

The storage of forage through the ensiling technique receives greater emphasis on the part of producers, as it requires simple technology and presents excellent results, and the ensiling process is not so limited by climatic factors when compared to haymaking. Among several species of forage used in silage production, pearl millet (*Pennisetum glaucum* L.) appears as an option, since it has been growing in importance in the Brazilian agribusiness scenario, especially in the milk and meat production systems. The advantage of the pearl millet lies in its adaptability to drier climates and because it has a short production cycle compared to other crops such as corn and sorghum. Although the pearl millet crop in Brazil is still expanding (Trindade et al. 2017), in some countries, such as India and Nigeria, its cultivation is intense, and therefore it is considered the sixth most planted cereal in the world (FAO 2001).

However, the low dry matter content of the pearl millet at the time of ensiling can be a limiting factor for the production of good quality silage, the high humidity in the plant can promote the growth of undesirable microorganisms such as clostridia, resulting in

high losses and production of silage with low nutritional value (Costa et al. 2018).

During the ensiling process, an important factor is the choice of additive. Although there is no official data, a small part of farmers in Brazil use additives in silage and are often influenced by lay or market information (Schmidt et al. 2014) which can lead to technical and economic frustrations. The additives used in the ensiling process must increase the recovery of nutrients and the energy of the forage, with consequent benefits on the performance of the animals. Organic or inorganic substances, biotic or abiotic, have been studied to modify the fermentation process, reduce losses, and/or improve the nutritional value of silages (Borreani et al. 2017).

Inoculants are among the main additives used in the ensiling process, aiming to dominate fermentation through the rapid production of lactic acid (homolactic bacteria) and consequent decrease in pH, inhibiting the growth of undesirable microorganisms, other bacteria such as heterofermentative ones can increase acetic and propionic acid production (Kung Jr. et al. 2003, Zopollatto et al. 2009, Bernardes & Rêgo 2014). There are several compositions of inoculants on the market, as a rule, those produced from homolytic bacteria are used to improve the fermentative pattern of ensiled material, while heterolactic bacteria inoculants are used to increase aerobic stability (Queiroz et al. 2018). Among homolactic bacteria, *Lactobacillus plantarum* is one of the most used, due to its vigorous growth, tolerance to the acid medium, and high potential for lactic acid production (Muck 2010). In the group of heterofermentative bacteria, *Propionibacterium acidipropionici* is used because it uses lactic acid and glucose as a substrate for the production of acetic and propionic acid, which are effective in controlling fungi, under low pH (Zopollatto et al. 2009).

Moisture scavenger additives are also used, which in addition to correcting the dry matter content, can also provide soluble carbohydrates and stimulate fermentation (Tavares et al. 2009, Rezende et al. 2015). The use of ground corn has been described as an important adsorbent additive in improving the fermentative and chemical quality of elephant grass silage and also in other forages (Rezende et al. 2008). Its use is justified mainly to supply some deficiency characteristics of the ensiled forage, such as facilitating the fermentation process due to the low dry matter content (Tonin et al. 2018).

The effectiveness of new additives and combinations between additives should be evaluated initially using laboratory-scale silos (Johnson et al. 2005). Experimental silos are easy to handle, as they can be weighed, processed, and analyzed with greater precision. However, for Cherney & Cherney (2003), experimental silos can only be used if the fermentation process is reasonably similar to what occurs in agricultural-scale silos. The most used experimental silos are those made with polyvinyl chloride (PVC) tubes and have a Bunsen-type valve that serves to escape gases. However, silages made in plastic bags were also examined by some authors (Jones 1970, Cai et al. 1997, Johnson et al. 2005), but all of these studies were using a suction pump, but the vacuum exerted by suction can affect the fermentative profile and nutritional value (May et al. 2001). There are few studies in the literature that used plastic bags without vacuum in the ensiling process. Thus, we hypothesized that (1) the silage's nutritional value fermentative profile, and aerobic stability will be influenced by different types of the silo, and (2) the use of additives of different nature may modify the silage's nutritional value and losses in the ensiling process.

Therefore, the aim of the present study was to evaluate the effect of the type of silo (plastic

bags (without vacuum) vs. PVC) with the use of additives (ground corn or microbial inoculant) or not on chemical composition, *in vitro* gas production, fermentative losses, aerobic stability, fermentative profile and microbial population of the pearl millet silage.

## MATERIALS AND METHODS

### Location

The experiment was carried out at the Universidade Federal Rural do Rio de Janeiro (UFRRJ) – *Campus* Campos dos Goytacazes, Rio de Janeiro State (RJ), Brazil (21º48'09 "S, 41º17'28" W, elev. 12 m a.s.l.) and at the Laboratório de Zootecnia of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) – county of Campos dos Goytacazes, RJ, Brazil (21º45'41 "S, 41º17'27" W, elev. 10 m a.s.l.). The climate in the Northern of the Rio de Janeiro State is classified as Aw, it is a humid tropical climate, with a rainy summer and a dry winter according to Köppen-Geiger's classification (Alvares et al. 2013), with annual rainfall around 1,020 mm.

### Silo type, addittives, and experimental design

The pearl millet was harvested manually in each plot, after 60 days of sowing, then the material was processed in a stationary forage harvester (Model JF Maxxium, JF Máquinas Agrícolas LTDA, Brazil) with an average particle size of ± 1.5 cm.

Two different types of silo were used for the ensiling process, polyethylene plastic bags with 51 cm width × 110 cm length and 200 microns, and polyvinyl (PVC) with 150 mm diameter × 50 cm high with a Bunsen valve for exhaust gases. Approximately 600 g of dry sand separated by cotton fabric was placed in the PVC silos to determine the effluent losses. The plastic bag and PVC silos were packed with a density of 600 kg/m$^3$ (ensiled fresh material), and the plastic bag silos were closed with nylon clamps. Then, all the silos were stored at an ambient temperature of around 25 ± 2.3 ºC for 90 days.

We used a randomized block design in a 2 × 3 factorial scheme, with two types of silos (Plastic bags and PVC silos) and three additives ([Control, CON] without any additive; 50 g of ground corn [GC] per kg of ensiled material, and *Lactobacillus plantarum* [2.5 × 1010 cfu/g] and *Propionibacterium acidipropionici* [2.5 × 1010 cfu/g] Biomax corn, Lallemand, Saint-Simon, France [LP]), with five replicates per treatment. The ground corn (DM = 856.30; CP = 84.93; 31.54; Ashes = 11.01; NDF = 77.30; ADF = 24.12; 8.16; NFC = 795.23; DM expressed in g/kg as fed and the others in g/kg DM) was mixed into the ensiled material. The microbial inoculant was used according to the manufacturer's recommendations (2 g / t of forage), diluted in water, and sprayed on the ensiled material. The additives were added separately to each silo.

### Chemical composition

The forage samples before the ensiling and silage samples were dried in a forced-air oven at ± 55 ° C for 72 hours, to obtain the partially dried samples, then they were ground in a Wiley-type mill, with a 1mm sieve. We performed the analyses of total dry matter content (DM, AOAC Method 967.03, AOAC 1990), crude fat (CF, AOAC Method 2003.06; Thiex et al. 2003), ash (ASH, AOAC Method 942.05, AOAC 1990), crude protein ([N × 6.25] CP, AOAC Method 984.13 and AOAC Method 2001.11; Thiex et al. 2002), and neutral detergent fiber using a standardized heat stable amylase solution without excluding ash (aNDF, AOAC Method 2002.04; Mertens 2002). Acid detergent fiber (ADF) and lignin (sa) were determined according to the methodology described by Silva & Queiroz (2006). The non-fibrous carbohydrate (NFC) content was estimated as the difference: $NFC(g/kg) = 1000 - CP - CF - Ash - NDF$.

The hemicellulose fraction was calculated by the difference between the levels of NDF and ADF, whereas the cellulose fraction was calculated by the difference between the levels of ADF and Lignin, expressed in g/kg DM.

### Gas production kinetics

Research on animals was conducted according to the institutional committee on animal use (Protocol 419/2017).

We collected the inoculum from three sheep with permanent rumen cannulas, and body weight of 45 kg (standard deviation = 3.2 kg). These animals were kept in collective pens with feeders and drinkers. Before the collection of ruminal fluid, the sheep were adapted to a diet with Tifton 85 hay and concentrate feed (ratio 80:20, roughage-to-concentrate) to meet the maintenance requirements for 14 days. After this period, the ruminal fluid collections began and were performed moments before daytime feeding, as recommended by Yáñez-Ruiz et al. (2016). The ruminal fluid (liquid and solid) was collected at many points in the liquid-solid interface of the ruminal environment for each incubation batch. The buffer solution described by McDougall (1948) was used, and about 500 mg (standard deviation = 10 mg) of the silage samples were added in amber penicillin flasks with 50 ml of the previously prepared inoculum (ratio 1:4, ruminal fluid:buffer solution). The free space in the flasks was immediately saturated with $CO_2$, which was closed and taken to a water bath at 39 °C. During the incubation, the flasks were shaken to homogenize the entire content.

The time profiles of the accumulated gas production were obtained using a non-automated device similar to that used by Abreu et al. (2014). Pressure and volume readings were made at times 0; 1; 2; 3; 4; 6; 8; 10; 12; 16; 20; 24; 30; 36; 48; 72 and 96 hours after adding the rumen inoculum. The pressure and cumulative volume of the fermentation gases were obtained by adding the readings corrected for the onset in the times after the zero time.

To estimate the cumulative gas production profiles, we used the model proposed by Groot et al. (1996):

$$G = A/(1 + (B^c/t^c)) \quad (1)$$

$$R_M(mL\,h^{-1}) = B \times (C - 1)^{1/C} \quad (2)$$

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

### Fermentative losses and dry matter recovery

For the determination of losses and recovery of dry matter, we only used the PVC silos. The losses were calculated according to the equations proposed by Jobim et al. (2007):

$$GL = (SME - SMO)/(FME \times DME) \times 1000 \quad (3)$$

In which: GL = gas losses (% dry matter); SME = silo mass before the ensiling (kg); SMO = silo mass after the silos opening (kg); FME forage mass at the ensiling (kg); and DME = dry matter ensiled (% dry matter).

The effluent losses were calculated according to equation 4:

$$EL = \{[(MEC - ME) - (MEO - ME)]/FME \times 100\} \quad (4)$$

EL = effluent losses (kg/t fresh matter); MEC = mass of the empty silo + sand mass at closing (kg); ME = mass of the empty silo (kg); MEO = mass of the empty silo + mass of sand after opening (kg); and FME forage mass at ensiling (kg).

The dry matter recovery was calculated using equation 5:

$$DMR = (FMO \times DMO)/(FME \times DME) \times 100 \quad (5)$$

In which: DMR = dry matter recovery (% DM); FMO = forage mass opening (kg); DMO = dry matter content at opening (%); FME forage mass at ensiling (kg); and DME = dry matter ensiled (% dry matter).

### pH and ammoniacal nitrogen

After opening each silo, the material was homogenized and a sample of 25 g 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 the aid of a pHmeter (MPA-210, Tecnopon, Brazil) (Kung Jr. 1996). Aliquots of 2 mL of extract were transferred to test tubes containing 1 mL of sulfuric acid (1N) and stored at –20◦C. Ammoniacal nitrogen analysis was performed according to the methodology of Fenner (1965).

### Microbial population

A 10 ml aliquot of the aqueous extract was subjected to serial dilutions ($10^{-1}$ to $10^{-6}$). The cultivation of the microorganisms was performed in sterile Petri dishes, for the counting of enterobacteria we used the culture medium VRB (Violet Red Bile) with an incubation period of 24 h at 37 °C; for the fungi count we used the culture medium PDA (Potato Dextrose Ágar) with an incubation period of four days at 25 °C and for the count of lactic acid bacteria we used the culture medium MRS (De Man, Rogosa, Sharpe) for 48 h, at 37 °C. We counted the dishes that showed between 30 and 300 colony-forming units (CFU). For the evaluation and interpretation of the data, the results obtained were transformed into a logarithmic basis (log10 cfu).

### Aerobic stability test

For the evaluation of aerobic stability, we used 2.0 kg of silage, which was packed in plastic bags capacity of approximately 5.0 kg, where it remained for seven days in a controlled temperature room (25 °C). The temperature was measured using a data logger (Log 110 EXF Inconterm; Brazil) inserted in the central portion of the ensiled mass in each bag, the temperature was recorded every 8 hours. The aerobic stability was calculated as time, in hours, until the silages had a temperature of 2 ° C above ambient temperature (Kung Jr. et al. 2000). During this period a sample of 25 g of fresh silage was taken and 225 ml of saline solution 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 the aid of a pHmeter (MPA-210, Tecnopon, Brazil) (Kung Jr. 1996).

Besides, during the aerobic stability assessment, samples were collected from each silo every 24 hours to determine DM (AOAC Method 967.03, AOAC 1990).

### Statistical analysis

The data of chemical composition, losses, aerobic stability, microbial population analysis, and cumulative gas production were analyzed in randomized blocks in a 3 × 2 factorial scheme with five replicates. The data were compared using the Tukey test with a significance level of 0.05, using the SAS MIXED package (version SAS University Edition, SAS Institute Inc., Cary, NC, USA).

The following statistical model was used: $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + b_k + e_{ijk}$ ; in which, $Y_{ijk}$ is the observed value 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; $\mu$ is the mean of all experimental units for the variable under study; $\alpha_i$ is the addition or not of additives in the pearl millet silage with i = 1,2,3; $\beta_j$ is the effect of the silo type with j = 1,2; $\alpha\beta_{ij}$ is the interaction between the addition or not of additives and the types of silo; $b_k$ is the effect of the *k-th* block on observation; $e_{ijk}$ is the error associated with observation $Y_{ijk}$.

The data of dry matter recovery and pH were analyzed as a repeated measure over time by regression analysis with a significance level of 0.05, using the SAS MIXED package (version SAS University Edition, SAS Institute Inc., Cary, NC, USA).

The following statistical model was used: $Y_{ijkl} = \mu + \alpha_i + \beta_j + \tau_k + \alpha\beta_{ij} + \alpha\tau_{ik} + \beta\tau_{jk} + \alpha\beta\tau_{ijk} + b_l + e_{ijkl}$; in which, $Y_{ijkl}$ is the observed value for the variable under study regarding 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; $\mu$ is the mean of all experimental units for the variable under study; $\alpha_i$ is the addition or not of additives in the pearl millet silage with i = 1,2,3; $\beta_j$ is the effect of silo types with j = 1,2; $\tau_k$ is the random effect of the hours of evaluation with k = 0, 24, ..., 144 for pH and 0, 8, 16, ..., 162 for temperature; $\alpha\beta_{ij}$ is the interaction between the addition or not of additives and the type of silo; $\alpha\tau_{ik}$ is the interaction between the addition or not of additives and the hours of evaluation; $\beta\tau_{jk}$ is the interaction between the ensiling methods and the hours of evaluation; $\alpha\beta\tau_{ijk}$ is the interaction between adding or not adding additives, ensiling methods, and the hours of evaluation; $b_k$ is the effect of the *l-th* block on observation; $e_{ijkl}$ is the error associated with observation $Y_{ijkl}$.

## RESULTS

### Chemical composition and gas production kinetics

There was no interaction effect (p > 0.05) between the additives and the type of silo (Table I). The use of ground corn in the ensiling process increased the CP (p = 0.0384), NFC (p = 0.0004), and CF (p = 0.0004) contents, in addition to reducing the Ashes (p = 0.0021), NDF (p < 0.0001), ADF (p = 0.014), Lignin (p = 0.0007), Hemicellulose (p = 0.007), and Cellulose (p = 0.0039) (Tables I and II). However, the additives did not affect the DM content (p = 0.6502) (Tables I and II).

We analyzed the type of silo and observed that in the PVC silos, there was an increase in the content of CF (p = 0.0.384) and a decrease in the content of Ashes (p = 0.0042), NDF (p = 0.0004), NFC (p < 0.0001) Hemicellulose (p = 0.0038), and Cellulose (p < 0.001).

The gas production kinetics was not affected (p > 0.05) by the additives or by the type of silo (Tables I and III and Figure 1).

### Fermentative losses and dry matter recovery

There was no interaction effect (p > 0.05) between additives and the type of silo (Table I). There was no effect of additives on losses by gases (p = 0.3871), effluents (p = 0.8371), and the recovery of dry matter (p = 0.8579) (Tables I and IV). However, silos containing ground corn produced 59.56% more gas than silos without additives and 60.69% more than silos containing LP additives (Table IV). The dry matter recovery of silos containing ground corn was 4.81% less than silos without additives and 3.64% less than silos containing LP additives (Table IV).

### pH and ammoniacal nitrogen

There was no interaction effect (p > 0.05) between additives and the type of silo (Table I). There was no effect of additives on pH (p = 0.7677). However,

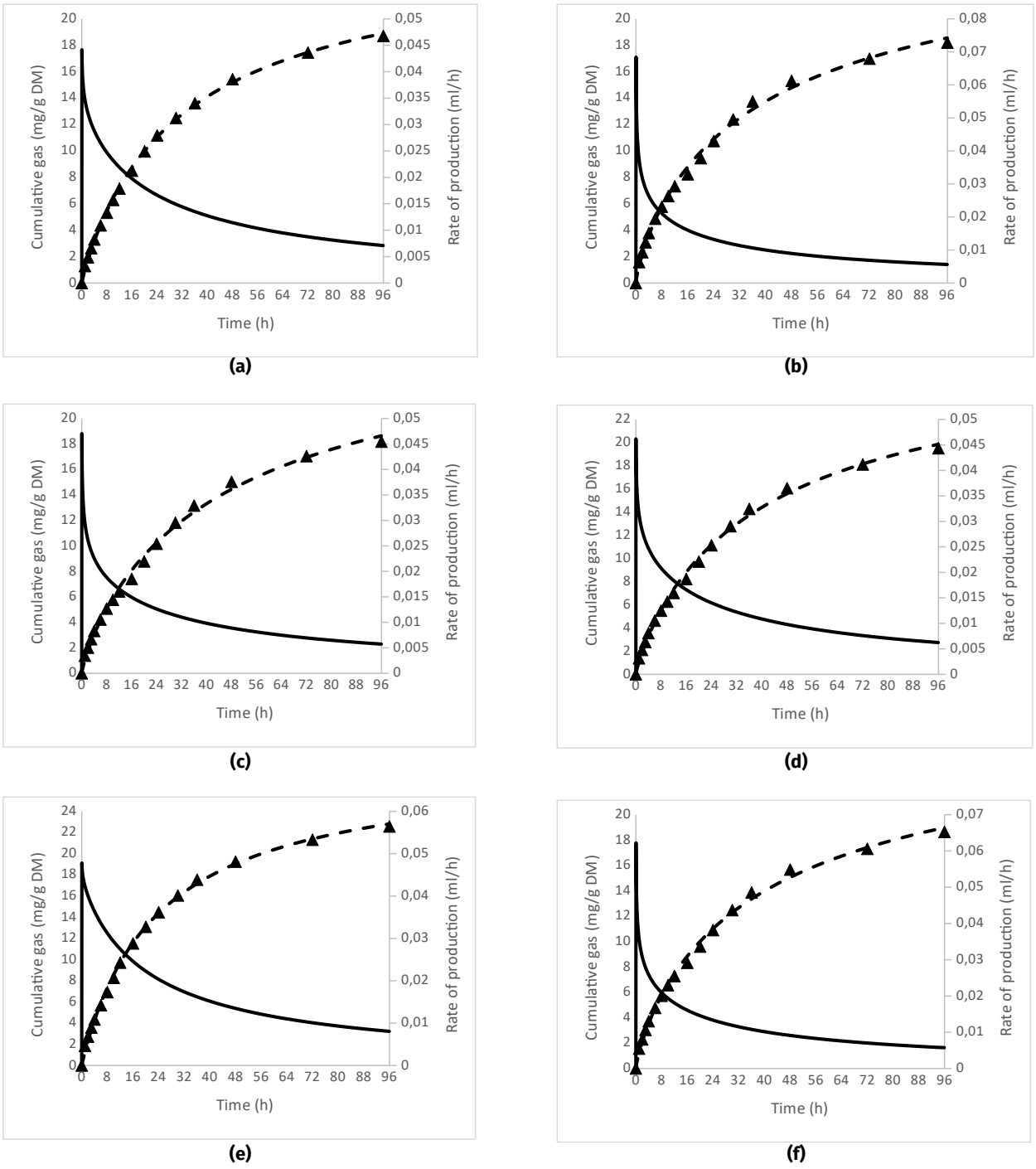


**Figure 1.** Cumulative gas production and rate of gas production profiles from pearl millet silage with different additives and ensiling methods. On panel (1a) Bag/CON; (1b) PVC/CON; (1c) Bag/Ground corn; (1d) PVC/Ground corn; (1e) Bag/*Lactobacillus plantarum* (2.5 × $10^{10}$ cfu/g) and *Propionibacterium acidipropionici* (2.5 × $10^{10}$ cfu/g); and (1f) PVC/*Lactobacillus plantarum* (2.5 × $10^{10}$ cfu/g) and *Propionibacterium acidipropionici* (2.5 × $10^{10}$ cfu/g).

**Table I.** ***P-values*** **related to the measured variables analyzed for the effects of the additives, silo type, and interaction between these.**

| Variables | Additives | Silo Type | Interaction |
|---|---|---|---|
| Dry matter, g/kg of as fed | 0.6502 | 0.8644 | 0.3864 |
| Crude protein, g/kg | 0.0384 | 0.5053 | 0.7284 |
| Crude fat, g/kg | 0.0004 | 0.0391 | 0.5212 |
| Ashes, g/kg | 0.0021 | 0.0042 | 0.3999 |
| Neutral detergent fiber (NDF), g/kg | <0.0001 | 0.0004 | 0.0810 |
| Acid detergent fiber, g/kg | 0.014 | 0.3525 | 0.9632 |
| Non-fibrous carbohydrate, g/kg | <0.0001 | 0.0004 | 0.0717 |
| Lignin, g/kg | 0.0007 | 0.6600 | 0.4455 |
| Hemicellulose, g/kg | 0.0007 | 0.0038 | 0.3286 |
| Cellulose, g/kg | 0.0039 | <0.0001 | 0.1377 |
| Parameter A (Gas production), mg/g OM | 0.5944 | 0.7991 | 0.9322 |
| Parameter B (Gas production), h | 0.1960 | 0.9280 | 0.3943 |
| Parameter C (Gas production) | 0.2603 | 0.9875 | 0.7434 |
| Gas losses, % DM | 0.3871 | - | - |
| Effluent losses, kg/t fresh matter | 0.8371 | - | - |
| Dry matter recovery, % DM | 0.8579 | - | - |
| Temperature, °C | 0.5363 | 0.7002 | 0.4652 |
| pH | 0.7677 | 0.0002 | 0.7836 |
| $NH_3$-N, (mg/dL) | 0.4907 | 0.5424 | 0.8699 |
| Dry matter losses, g/kg | 0.6231 | 0.3902 | 0.5591 |
| Mold, kg of ensiled mass | 0.1194 | 0.0003 | 0.0755 |
| Mold, % of ensiled mass | 0.1814 | 0.0613 | 0.0884 |
| Lactic Acid Bacteria, $log_{10}$/g fresh silage | 0.8178 | 0.3598 | 0.8871 |
| Fungi, $log_{10}$/g fresh silage | 0.5400 | 0.2462 | 0.1130 |

**DM = Dry matter.**

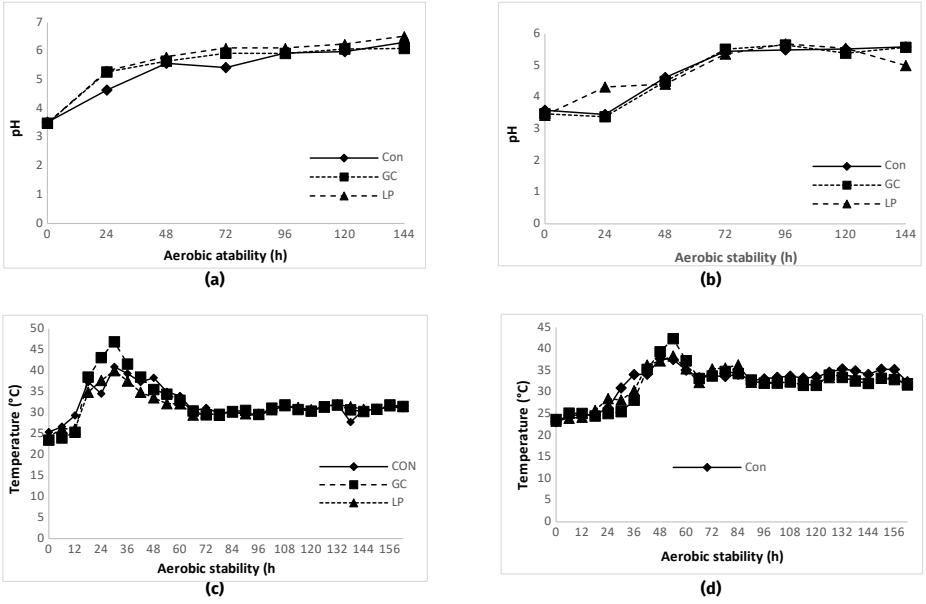


**Figure 2. pH and temperature values of pearl millet silage with different additives and ensiling methods for seven-days. CON = Control; GC = Ground corn; LP =** ***Lactobacillus plantarum*** **(2.5 × $10^{10}$ cfu/g) and** ***Propionibacterium acidipropionici*** **(2.5 × $10^{10}$ cfu/g); Bag = Silo Bag (51 × 110 cm and 200 micras); PVC = Polyvinyl chloride. On panel (2a) Bag; (2b) PVC; (2c) Bag; and (2d) PVC.**

**Table II.** Effects of additives and silo type on the chemical composition of pearl millet silage.

| Variables | CON | GC | LP | SEM |
|---|---|---|---|---|
| **DM** | | | | |
| Bag | 272.9 | 259.7 | 269.71 | 3.938 |
| PVC | 256.68 | 271.74 | 278.52 | 4.298 |
| **CP** | | | | |
| Bag | 63.38 b | 71.64 a | 62.76 b | 2.274 |
| PVC | 68.38 b | 74.78 a | 61.39 b | 2.224 |
| **CF** | | | | |
| Bag | 18.21 Bb | 23.02 Ba | 18.80 Bb | 0.802 |
| PVC | 20.47 Ab | 27.26 Aa | 19.83 Ab | 0.944 |
| **Ashes** | | | | |
| Bag | 10.86 Aa | 9.9 Ab | 10.95 Aa | 0.223 |
| PVC | 9.92 Ba | 8.15 Bb | 10.32 Ba | 0.251 |
| **NDF** | | | | |
| Bag | 623.56 Aa | 553.18 Ab | 639.19 Aa | 10.940 |
| PVC | 589.67 Ba | 459.87 Bb | 610.3 Ba | 13.817 |
| **ADF** | | | | |
| Bag | 390.91 a | 343.89 b | 404.37 a | 7.793 |
| PVC | 381.72 a | 325.69 b | 385.33 a | 9.177 |
| **NFC** | | | | |
| Bag | 283.99 Bb | 342.26 Ba | 268.32 Bb | 9.340 |
| PVC | 311.57 Ab | 429.94 Aa | 298.16 Ab | 11.859 |
| **LIG** | | | | |
| Bag | 41.98 a | 37.23 b | 42.77 a | 0.781 |
| PVC | 42.25 a | 33.94 b | 43.84 a | 1.125 |
| **Hemic** | | | | |
| Bag | 232.65 Aa | 209.29 Ab | 234.82 Aa | 5.038 |
| PVC | 207.95 Ba | 171.12 Bb | 224.97 Ba | 5.439 |
| **Cell** | | | | |
| Bag | 348.93 Aa | 306.66 Ab | 361.23 Aa | 7.086 |
| PVC | 339.46 Ba | 256.87 Bb | 341.49 Ba | 9.594 |

**CON = Control; GC = Ground corn; LP = *Lactobacillus plantarum* (2.5 × $10^{10}$ cfu/g) and *Propionibacterium acidipropionici* (2.5 × $10^{10}$ cfu/g); Bag = Plastic bags (51 × 110 cm and 200 micras); PVC = Polyvinyl chloride; SEM = Standard error of the mean; DM = Dry matter; 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, except DM expressed as as-fed.**
***Means followed by the different letters capital letters in a column and rows letters on the lines differ significantly by the Tukey test ($p < 0.05$).**
**†Chemical composition of forage before ensiling: DM (263.83); CP (6.41); CF (10.93); NDF (575.86); ADF (397.09); LIG (60.07); Cell (178.77); Hemic (337.02), all expressed as g/kg, except DM expressed as as-fed.**

the type of silo affected the pH (p = 0.0002), PVC silos had lower values than plastic bag silos (Tables I and V). The ammoniacal nitrogen was not affected (p = 0.4907) by the additives or by the type of silo (p = 0.5424) (Tables I and V).

### Aerobic stability test

There was no interaction effect (p > 0.05) between additives and the type of silo (Table I). There was no effect of additives on temperature (p = 0.5363), and dry matter losses (p = 0.6231) (Tables I and VI). However, there was no effect of the silo type on temperature (p = 0.7002) and dry matter losses (p = 0.3902) (Tables I and VI). In the plastic bag silos that received additives, the pH increased by 2 points (3.5 to 5.5) in the first 24 hours (Figure 2a), different behavior from the PVC silo, which increased by only 1 point (3.5 to 4.5) with the addition of LP (Figure 2b). The temperature in the plastic bag silos increased to 2 °C in approximately 18 hours regardless of additives (Figure 2c), in the PVC silos this increase was in 36 hours in the ones that we used additives (Figure 2d).

### Microbial populations

There was no interaction effect (p > 0.05) between additives and the type of silo (Table I). In the plastic bag silos without additive, we detected enterobacteria 5.27 $\log_{10}$/g of fresh silage, in the other silos there was no presence. There was no effect of the additives on the population of lactic acid bacteria (p = 0.8178) and fungi (p = 0.54). The type of silo did not affect the population of lactic acid bacteria (p = 0.3598) and fungi (p = 0.2462) (Tables I and VII). However, the plastic bag silos showed a higher amount of mold (kg) per silage mass compared to the PVC silo (Tables I and VII).

## DISCUSSION

The ensiling is one of the most critical moments in the entire silage-making process, as it refers to good silo conditioning, storage, and sealing practices to ensure proper fermentation, conservation, and maintenance of good-quality biomass brought from the field. At this stage, the quality potential of silage from good crops can be lost due to errors in the process. Another important factor about the use or not of additives depends on previous knowledge about the challenge that the forage presents to be ensiled, and how the additives work and interfere in the process.

During the evaluation of the chemical composition of the silages, we observed that the DM content in the silage was not affected (p = 0.6502) with the use of additives, possibly the amount we used was not enough to change the silage DM content. Although ground corn has a high DM content and a great capacity to absorb moisture from silage (Andrade & Melotti 2004). But, the chemical composition of pearl millet silage improved, with an increase in the levels of CP, CF, NFC, and a decrease in the indigestible fraction (LIG) (Table II). This change in the nutritional value may be associated with the additive. This improvement in the nutritional quality of silage provided by ground corn as an additive was also observed by Rezende et al. (2015) and Bezerra et al. (2019). The type of silo also influenced the quality of the pearl millet silage (Table II). However, the increase in the CF content is not clear. The decrease in the NDF content in the PVC silo can be justified as it kept the most appropriate anaerobic condition than plastic bag silos. A problem with polyethylene is the oxygen permeability, that is, gas exchange occurs between the silage and the external environment, with oxygen entering even without the plastic bags showing any physical

**Table III.** Effects of additives on gas production parameters of pearl millet silage.

| Variables | CON | SE | GC | SE | LP | SE |
|---|---|---|---|---|---|---|
| Parameter A, mg/g OM | | | | | | |
| Bag | 29.039 | 2.011 | 28.830 | 3.236 | 29.352 | 1.377 |
| PVC | 27.953 | 2.424 | 28.69 | 1.98 | 28.543 | 2.716 |
| Parameter C | | | | | | |
| Bag | 0.857 | 0.0390 | 0.932 | 0.1039 | 0.884 | 0.0310 |
| PVC | 0.813 | 0.0513 | 0.974 | 0.0745 | 0.829 | 0.0574 |
| Parameter B, h | | | | | | |
| Bag | 48.592 | 8.174 | 32.728 | 9.835 | 41.650 | 4.737 |
| PVC | 41.609 | 9.395 | 23.867 | 3.977 | 41.978 | 10.215 |

**CON = Control; GC = Ground corn; LP = *Lactobacillus plantarum* (2.5 × $10^{10}$ cfu/g) and *Propionibacterium acidipropionici* (2.5 × $10^{10}$ cfu/g); Bag = Plastic bags (51 × 110 cm and 200 micras); PVC = Polyvinyl chloride; OM = Organic matter; SE = Standard error.**
***Means followed by the different letters capital letters in a column and rows letters on the lines differ significantly by the Tukey test ($p < 0.05$).**

**Table IV.** Effects of additives on losses, and dry matter recovery of pearl millet silage.

| Variables | CON | GC | LP | SEM |
|---|---|---|---|---|
| Gas losses, % DM | 5.73 | 14.17 | 5.57 | 1.572 |
| Effluent losses, kg/t fresh matter | 1.17 | 1.25 | 1.13 | 0.049 |
| Dry matter recovery, % DM | 93.12 | 88.64 | 91.99 | 2.552 |

**CON = Control; GC = Ground corn; LP = *Lactobacillus plantarum* (2.5 × $10^{10}$ cfu/g) and *Propionibacterium acidipropionici* (2.5 × $10^{10}$ cfu/g); SEM = Standard error of the mean; DM = Dry matter.**
***Means followed by the different letters capital letters in a column and rows letters on the lines differ significantly by the Tukey test ($p < 0.05$).**

**Table V.** Effects of additives and silo type on pH and ammoniacal nitrogen ($NH_3$-N, mg/dL) of pearl millet silage.

| Variables | CON | GC | LP | SEM |
|---|---|---|---|---|
| pH | | | | |
| Bag | 5.34 A | 5.49 A | 5.66 A | 0.098 |
| PVC | 4.81 B | 4.78 B | 4.82 B | 0.118 |
| $NH_3$-N | | | | |
| Bag | 9.77 | 10.41 | 10.82 | 1.538 |
| PVC | 9.30 | 9.26 | 10.78 | 1.487 |

**CON = Control; GC = Ground corn; LP = *Lactobacillus plantarum* (2.5 × $10^{10}$ cfu/g) and *Propionibacterium acidipropionici* (2.5 × $10^{10}$ cfu/g); Bag = Plastic bags (51 × 110 cm and 200 micras); PVC = Polyvinyl chloride; SEM = Standard error of the mean.**
***Means followed by the different letters capital letters in a column and rows letters on the lines differ significantly by the Tukey test ($p < 0.05$).**

**Table VI. Effects of additives and silo type on seven-day aerobic stability of pearl millet silage.**

| Variables | CON | GC | LP | SEM |
|---|---|---|---|---|
| Temperature, h | | | | |
| Bag | 32.05 | 32.36 | 31.47 | 0.222 |
| PVC | 32.52 | 31.81 | 32.01 | 0.193 |
| Dry matter losses, g/kg | | | | |
| Bag | 79.06 | 54.64 | 86.91 | 9.141 |
| PVC | 128.37 | 90.94 | 71.51 | 15.901 |

**CON = Control; GC = Ground corn; LP = *Lactobacillus plantarum* (2.5 × $10^{10}$ cfu/g) and *Propionibacterium acidipropionici* (2.5 × $10^{10}$ cfu/g); Bag = Plastic bags (51 × 110 cm and 200 micras); PVC = Polyvinyl chloride; SEM = Standard error of the mean.**
***Means followed by the different letters capital letters in a column and rows letters on the lines differ significantly by the Tukey test (p < 0.05).**

**Table VII. Effects of additives and silo type on microbial populations of pearl millet silage.**

| Variables | CON | GC | LP | SEM |
|---|---|---|---|---|
| Lactic acid bactéria, $log_{10}$/g fresh silage | | | | |
| Bag | 6.66 | 7.12 | 6.89 | 0.191 |
| PVC | 6.15 | 6.30 | 6.71 | 0.347 |
| Fungi, $log_{10}$/g fresh silage | | | | |
| Bag | 7.32 | 5.56 | 5.64 | 0.286 |
| PVC | 5.01 | 7.46 | 7.66 | 0.409 |
| Mold, kg of ensiled mass | | | | |
| Bag | 1.41 A | 0.33 A | 0.58 A | 0.084 |
| PVC | 0.05 B | 0.11 B | 0.12 B | 0.016 |
| Mold, % of the ensiled mass | | | | |
| Bag | 9.75 | 2.57 | 3.97 | 0.293 |
| PVC | 1.6 | 1.48 | 4.01 | 0.528 |

**CON = Control; GC = Ground corn; LP = *Lactobacillus plantarum* (2.5 × $10^{10}$ cfu/g) and *Propionibacterium acidipropionici* (2.5 × $10^{10}$ cfu/g); Bag = Plastic bags (51 × 110 cm and 200 micras); PVC = Polyvinyl chloride; SEM = Standard error of the mean.**
***Means followed by the different letters capital letters in a column and rows letters on the lines differ significantly by the Tukey test (p < 0.05).**

damage (Amaral et al. 2014). As for the NFC, for Bernardes et al. (2017) the greater exposure of silage to oxygen in the plastic bag silos allowed the increase of undesirable microorganisms such as mold (Table VII), these microorganisms have their activity intensified in the presence of soluble carbohydrates, acids, and proteins which increases the pH of the silage (Table V). The degradation of hemicellulose and starch for many years was neglected (Ning et al. 2017), however, some studies have shown that degradation occurs in the ensiling process (Muck 1990, Chen et al. 2015). Melvin (1965) and Yahaya et al. (2001) indicated that hemicellulose (xylose) and starch (glucose) degradation products can serve as substrates for microorganisms to produce acids during the ensiling. In this study, we observed a decrease in the levels of hemicellulose and cellulose when adding the GC, this finding corroborates with the studies made by Melvin (1965), Yahaya et al. (2001), and Ning et al. (2017) in which, structural carbohydrates can

serve as microbial substrates for the production of acids during the ensiling.

The *in vitro* gas production technique can accurately reflect the *in vivo* digestibility of feed in ruminants, being fast and cheap (Prasad et al. 1994, Kitessa et al. 1999, Niderkorn & Baumont 2009). For Nsahlai et al. (1994) the chemical composition of the incubated substrates can influence the production of gas, however, in this study despite observing the change in chemical composition concerning the factors analyzed, we did not find any effect of different additives and type of silo on gas production (Table III, Figure 1). Another fact to be noted would be the crude protein contents, as Bach et al. (2005) observed that below 70 g/kg can restrict microbial activity due to a lack of nitrogen. The high levels of the indigestible fraction of the fiber (LIG) may have contributed to the reduction of the cumulative gas volume of the silages due to the lower availability of fermentable carbohydrates in the rumen. Nevertheless, Getachew et al. (2004) call attention to the fact that the effect of NDF fermentation becomes less important as its levels decrease, due to the increase in NFC, so, the profile of cumulative gas production changes, and the degradation rate is greater causing a fermentation peak. We observed that although there was no difference ($p = 0.1960$), the time taken for half of the asymptotic gas to be formed was less (8,864 ~ 9 h) when we used ground corn (Table III). The gas production rates peaked in the first hours of incubation, being higher in plastic bag silos, but the final rate was lower (Figure 1a, 1c, and 1e), unlike PVC silos, which had the initial rate lower, but a higher final production rate (Figure 1b, 1d, and 1f).

The production of high-quality silage and loss reduction is a challenge, but some of these losses are inevitable (Borreani et al. 2017). In this study, we evaluated only the losses of the PVC silos and observed that the additives did not affect the losses by gases, effluents, and the recovery of DM (Tables I and IV). However, when we inoculated the LP in the silage, we observed less gas loss concerning the silage without additive (2.79% [5.57/5.73]) and the silage with ground corn (60.69% [5.57/14.17]). Gas losses are associated with the type of fermentation that occurs during the ensiling process. When fermentation occurs by homofermentative bacteria (*Lactobacillus plantarum* and *Propionibacterium acidipropionici*), glucose is used as a substrate to produce lactic acid, promoting fewer losses. However, when fermentation is carried out by heterofermentative bacteria, it is produced in addition to lactic acid, carbon dioxide ($CO_2$), and ethanol, culminating in significant gas losses (1 glucose → 1 lactic acid + 1 ethanol + 1 $CO_2$). The high production of gases is associated with the presence of enterobacteria (1 glucose → 1 lactic acid + 1 ethanol + 2 $CO_2$ + $H_2$), highlighting that butyric fermentation is caused by bacteria of the genus *Clostridium* (McDonald et al. 1991, Muck 2010). In this study, the use of ground corn generated a very large gas loss, which is probably due to the greater amount of soluble carbohydrates available for fermentation. The losses by effluents had a behavior similar to the losses by gases (Table IV). According to McDonald et al. (1991), effluent production represents losses in the nutritional value of silage, crops with high moisture content (above 75%) in the ensiling show greater losses due to effluents. Despite producing about 6.4% more effluents than silage without additives, the use of ground corn provided an improvement in the nutritional quality of the silage (Table II). The recovery of DM was not influenced by the use of additives in this study, however, we observed that the LP decreased with greater efficiency (3.64% [88.64/91.99]) the fermentative losses concerning ground corn. For Borreani et al. (2017), homofermentative inoculants are efficient in

reducing DM losses. Besides, the recovery of DM is highly affected by gas and effluent losses in the ensiled material, being inversely proportional, that is, in treatments with greater losses due to gas and effluent production, the recovery of dry matter is smaller (Pacheco et al. 2014).

When the silage process is carried out, its main objective is to maintain the original quality of the harvested forage. The occurrence of good fermentation helps to maintain aerobic stability and ensures that the silage keeps its quality for a longer time (Muck 2010). The aerobic stability of the silage can be conceptualized as the resistance of the forage mass to deterioration after opening the silo, that is, the speed at that the mass deteriorates after being exposed to air (Jobim et al. 2007). Thus, we observed that the temperature of the silage was not affected by the inoculants ($p = 0.5363$) and neither by the type of silo ($p = 0.7002$). However, over 168 hours, there was an effect of additives ($p < 0.05$) between the type of silos (Figures 2c and 2d). We observed that in the first 36 hours there was a peak temperature in the silage made in the plastic bag silos, whereas, in the silage made in the PVC silos, the peak temperature was in 54 hours. For Woolford (1990), the initial increase in temperature is caused by the growth of yeasts and filamentous fungi, but after some time, according to Muck & Pitt (1992), the increase in pH (above 5.0) may promote the growth of bacilli which can cause a second temperature rise of the material. This fact was observed in this study (Figures 2a and b). The silage produced in the PVC silos showed a lower pH than in the plastic bag silos ($p = 0.0002$) (Table I). Plastic bag (polyethylene) silos may present permeability to oxygen at a temperature of 25 °C, the gas exchange between the interior of the silo and the environment is around 1 liter/$m^2$, this value corresponds to an intact bag without any physical damage (Greenhill 1964). This can make the silage more prone to aerobic deterioration due to the increased permeability of the bags, as the gas exchange in this mass allows the action of yeasts that oxidize the organic acids that preserve the silage, which can trigger aerobic degradation and elevation of the pH. In this study, the silage in the plastic bag silos increased from 3.5 to 5.5 (2.0 points) in 24 hours, whereas the silage in the PVC silos had an increase of 2.0 in the pH in 72 hours (Figures 2a and 2b). Regarding DM losses, this variable was not affected by the use of additives ($p = 0.6231$) or type of silo ($p = 0.3902$), despite that, the silage in PVC silos had higher losses (CON = 38.41%; GC = 39.91%). But, the silage in PVC silos with LP had lower losses (17.71%). *Lactobacillus plantarum*, one of the inoculants used in this study, aims to increase lactic acid production, consequently, it reduces ammonia N and DM loss during fermentation (McDonald et al. 1991, Muck 2010). The decrease in the concentration of $NH_3$-N in the silage is a sign of low proteolytic activity. However, increased ammonia concentrations are related to the slow drop in pH, this fact can be observed in this study (Table V).

The microbiological composition of the ensiled material has a great influence on the quality of the silage. For the ensiling to be carried out successfully, in addition to having lactic fermentation, some undesirable microorganisms must be inhibited, they are *Clostridium* sp., enterobacteria, yeasts, and fungi (Muck 2010). In this study, we observed counts for enterobacteria only in the plastic bag siloS without any additive, this fact is related to the active growth of lactic acid bacteria (BALs) during the fermentation, as the pH decreases to values between 3.8 to 5.0, which favors the decline of the enterobacterial population quickly, and the BALs becomes the main microorganisms in the silage (McDonald et al. 1991). In this study, we did not observe any effect of additives or type of

silo ($p > 0.05$) on the microbiology of the silage, except for the amount of mold (g/kg) concerning the type of silo (Tables I and VII). The presence of oxygen favors the growth of yeasts, fungi, and bacteria that produce acetic acid in silage from fermentation products and silage sugars, which results in the production of $CO_2$, water, and heat (Muck 2010). As the fermentation products are used, the pH increases (Table V, Figure 2a). The pH above 5.0 favors a wide variety of other aerobic microorganisms to proliferate, causing heating (Figure 2c) and reducing the quality of silage (McDonald et al. 1991, Muck 2010). Thus, we observed that the use of the plastic bag silos in making the silage caused greater exposure of the silage to oxygen, resulting in greater deterioration of the silage. For Kung Jr. et al. (2018), the quantification of yeasts and molds in silage is very useful, since large amounts of yeasts in silage are usually associated with high concentrations of ethanol and can be inversely related to aerobic stability, this fact was also observed in this study (Figure 2c). Although they were not statistically different ($p = 0.2462$), the plastic bag silos presented 31.55% more fungi than PVC silos without any additives (Table VII). In this study, there was no effect of the additives ($p = 0.8178$) or the silo type ($p = 0.3598$), but the use of additives increased the population of BALs in the silages. BALs play a fundamental role during the ensiling since, in addition to inhibiting the growth of spoilage microorganisms, they enable greater recovery of energy from fermented carbohydrates through the production of lactic acid. It highlights the importance of using some type of additive in the ensiling, as they are tools that help to ensure that the process is within acceptable limits (Muck 2010, Borreani et al. 2017).

## CONCLUSIONS

The use of ground corn (50 g per kg of silage material) improved the nutritional value of the pearl millet silage, on the other hand, maximized the losses by gases, in addition to promoting heat in the silage. In turn, the inoculant provided better aerobic stability for the pearl millet silage.

The plastic bag silos without vacuum were not efficient in the ensiling process like the PVC silos, which resulted in low-quality silage.

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