

Fermentation Profile, Nutritional Value, and Microbial Population of C4 Grasses Silages with or Without Bacterial Additive

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1 **Fermentation profile, nutritional value, and microbial population of C4 grasses**
2 **silages with or without bacterial additive**

3

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6

7 **Abstract** - The objective of the present study was to evaluate the use of bacterial additive
8 (*Lactobacillus plantarum* and *Propionibacterium acidipropionici*) on chemical
9 composition, *in vitro* gas production, pH, losses, aerobic stability, and microbial
10 population of corn, pearl millet, and sorghum silages in plastic bags silos (without
11 *vacuum*). The experiment was carried out in a randomized block design, in a 2 × 3
12 factorial scheme, with or without additive ([Control] without additive and *Lactobacillus*
13 *plantarum* [2.5 × 10¹⁰ cfu/g] and *Propionibacterium acidipropionici* [2.5 × 10¹⁰ cfu/g]
14 Biomax corn, Lallemand, Saint-Simon, France [LP]) and three crops of agricultural
15 interest; pearl millet, sorghum, and corn, with four replicates per treatment. We
16 performed chemical analyses and *in vitro* gas production to determine the nutritional
17 value of the silages. We also evaluated the aerobic stability, ammoniacal nitrogen (NH₃),
18 pH, and microbial population of the silages. The additive increased the crude protein
19 content ($P = 0.0062$) in corn and sorghum and decreased the LIG content ($P = 0.0567$).
20 The gas production was not affected ($P > 0.05$) by the additive and neither between crops.
21 In aerobic stability, we observed that the additive affected the temperature of the
22 sorghum silage ($P = 0.0123$). The additive decreased NH₃ ($P = 0.0095$) content. The
23 additive increased ($P = 0.0441$) the lactic acid bacteria population in the pearl millet,
24 corn, and sorghum silages. Thus, the bacterial additive did not improve the fermentation
25 profile and nutritional value of corn, pearl millet, and sorghum silages in plastic bag
26 silos.

27

28 **Keywords:** Conservation. Fermentation capacity. Inoculant. Lactic acid bacteria

29

30 **Introduction**

31 In Brazil, milk and beef productions are based on pastures, as they are a less expensive
32 feed source for the farmers. However, the seasonality of forage production is a critical

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33 point for planning livestock activity. Under such conditions, it is necessary to establish
34 feeding strategies for the herds in which the production and conservation of
35 supplementary forage must be considered (Chaudhry, 2008). The most widespread
36 methods for preserving feed are haymaking and ensiling.

37 The storage of forage through the ensiling technique receives a greater emphasis on
38 the part of the farmers for presenting excellent results. Still, some factors must be
39 considered, and the main one is respecting the characteristics of each crop, as they can
40 directly influence the silage quality. Specifically for the ensiling, the final quality of the
41 feed is directly related to the material that gave rise to it and the conditions in which it
42 was ensiled (Jobim et al., 2007).

43 During the ensiling process, another critical factor is the additive. The microbial
44 inoculants are recommended to reduce losses in silage of tropical grasses since
45 homolactic bacteria compete with epiphytic microorganisms, increasing the fermentation
46 efficiency (Kung Jr et al., 2003; Borreani et al., 2018). Inoculants are one of the main
47 additives in the ensiling, aiming to dominate fermentation through the rapid production
48 of lactic acid (homolactic bacteria) and consequent pH decrease, inhibiting the growth of
49 undesirable microorganisms and production of acetic and propionic acid (Kung Jr. et al.,
50 2003). Other bacteria, such as heterofermentative, can increase acetic and propionic acid
51 (Kung Jr. et al., 2003; Zopollatto et al., 2009; Bernardes and Rêgo, 2014). There are
52 several compositions of inoculants on the market. As a rule, those produced from
53 homolactic bacteria improve the fermentation pattern, whereas heterolactic bacteria
54 inoculants are used to increase aerobic stability (Queiroz et al., 2018). *Lactobacillus*
55 *plantarum* is one of the most used among homolactic bacteria due to its vigorous growth,
56 acid tolerance, and a high potential for lactic acid production (Muck, 2010). In the
57 heterofermentative bacteria group, the *Propionibacterium acidipropionici* uses lactic acid

58 and glucose as substrates to produce acetic and propionic acid, which effectively control
59 fungi under low pH (Zopollatto et al., 2009).

60 Therefore, we hypothesized that the bacterial additive would not affect the
61 fermentation of C4 grasses silages. Thus, the objective of the present study was to
62 evaluate the bacterial additive (*Lactobacillus plantarum* and *Propionibacterium*
63 *acidipropionici*) on chemical composition, in vitro gas production, pH, losses, aerobic
64 stability, and microbial population of corn, pearl millet, and sorghum silages in plastic
65 bag silos (without *vacuum*).

66

67 **Material and Methods**

68 **Location**

69 The experiment was carried out at the Universidade Federal Rural do Rio de Janeiro (UFRRJ)
70 - Campos dos Goytacazes *Campus*, RJ, Brazil (21°47'54"S and 41°17'34"W, 12 m a.s.l.) and
71 at the Animal Science Laboratory of the Universidade Estadual do Norte Fluminense Darcy
72 Ribeiro (UENF) - Campos dos Goytacazes, RJ, Brazil (21°45'41"S and 41°17'27"W, 10 m
73 a.s.l.). According to Köppen's classification (Alvares et al., 2013), the climate in the North of
74 the Rio de Janeiro State is classified as Aw, i.e., humid tropical with a rainy summer, dry
75 winter, and annual rainfall around 1,020 mm. The soil of the experimental area is classified as
76 Cambisol.

77

78 **Ensiling, additive, and experimental design**

79 The pearl millet, corn, and sorghum were harvested and processed in a stationary forage
80 chopper (JF Maxxium Model, JF Máquinas Agrícolas LTDA, Brazil) with an average
81 particle size of ± 1.5 cm. We used plastic bags (polyethylene 51 cm wide \times 110 cm long and
82 200 microns) for ensiling the forages. The silos were closed with nylon clamps and stored at

83 an ambient temperature of 25 ± 2.3 °C for 90 days. The silos were packed with a density of
84 600 kg/m³ (fresh forage ensiled).

85 The experiment was carried out in randomized blocks in a 2 × 3 factorial scheme, with or
86 without the additive ([Control, CON] without additive and *Lactobacillus plantarum* [$2.5 \times$
87 10^{10} cfu/g] and *Propionibacterium acidipropionici* [2.5×10^{10} cfu/g] Biomax corn,
88 Lallemand, Saint-Simon, France [LP]) and three crops of agricultural interest: pearl millet
89 (*Pennisetum glaucum* L.), cv. BRS 1501; sorghum (*Sorghum bicolor* x *Sorghum sudanense*),
90 hybrid BRS810; and corn (*Zea Mays* L.), cv. PR1150. We used four replicates per treatment,
91 totaling 48 experimental units. The treatments were randomly distributed in the silos. The
92 microbial inoculant was used according to the manufacturer's recommendations (2 g/ton of
93 forage), it was diluted in water and sprayed on the forages to be ensiled.

94

95 **Chemical composition**

96 After opening the silos, the silage samples were dried in a forced-air oven at ± 55 °C for 72
97 hours to yield the partially dried samples. Then, the samples were ground in a Wiley mill
98 fitted with a 1-mm-sieve. We determined the contents of total dry matter (DM, AOAC
99 Method 967.03, AOAC, 1990), crude fat (CF, AOAC Method 2003.06; Thiex et al., 2003),
100 ash (ASH, AOAC Method 942.05, AOAC, 1990), crude protein ([N×6.25]CP, AOAC
101 Method 984.13 and AOAC Method 2001.11; Thiex et al., 2002), neutral detergent fiber
102 using a standardized heat stable amylase solution and the expressed results with residual ash
103 (aNDF, AOAC Method 2002.04; Mertens, 2002) and lignin (LIG) (Möller, 2009). The non-
104 fibrous carbohydrate (NFC) content was estimated as: $NFC(g/kg) = 1000 - CP - CF -$
105 $Ash - NDF$.

106 The hemicellulose was calculated by the difference between the NDF and ADF contents,
107 and the cellulose by the difference between ADF and Lignin contents, expressed in g/kg

108 DM.

109 **Gas production kinetics**

110 The Ethics Committee on the Use of Experimental Animals approved all experimental
111 procedures, 419 protocol. We collected the ruminal fluid from three sheep, 45 kg (standard
112 deviation = 3.2 kg), with permanent rumen cannulas. The animals were housed in collective
113 pens with troughs and drinkers. The sheep were adapted to a diet with Tifton 85 hay and
114 concentrate feed for 14 days to meet the maintenance requirements. After this period, the
115 collection of ruminal fluid began, and it took place moments before daytime feeding, as
116 recommended by Yáñez-Ruiz et al. (2016). The ruminal fluid (liquid and solid) was collected
117 at several points of the liquid-solid interface of the ruminal environment for each incubation
118 battery. We used about 500 mg (standard deviation = 10 mg) of silage sample in amber
119 penicillin flasks together with 50 ml of an inoculum (1:4 ratio, ruminal fluid, and buffer
120 solution, respectively). The buffer solution was prepared as described by McDougall (1948).
121 The flasks were immediately filled with CO₂, closed, and placed in a water bath.

122 The time profiles of gas production were obtained using a non-automated device, similar to
123 Abreu et al. (2014). We measured pressure and volume at times 0; 1; 2; 3; 4; 6; 8; 10;12; 16;
124 20; 24; 30; 36; 48; 72 and 96 hours after the addition of ruminal inoculum. The cumulative
125 pressure and volume of fermentation gases were obtained by summing the corrected readings
126 throughout the measurement times.

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

$$129 \quad G = A/(1 + (B^c / t^c)) \quad \text{Eq.1}$$

$$130 \quad R_M(mLh^{-1}) = B \times (C - 1)^{1/C} \quad \text{Eq.2}$$

131 Where, the parameter G represents the amount of gas produced per unit of organic matter
132 incubated at time t after the incubation period; the parameter A represents the asymptotic gas

133 production (mg/g OM); the parameter B is the time (h) after incubation in which half of the
134 asymptotic gas was produced, it represents the gas production speed; the parameter C is a
135 constant that determines the sharpness of the curve change characteristic. The R_M represents
136 the maximum gas production rate when the microbial population does not limit the
137 fermentation and digestion is not reduced by chemical or structural barriers of the potentially
138 digestible matter.

139

140 **Aerobic stability test**

141 About 2.0 kg of silage was packed in plastic bags of 5.0 kg capacity, where it remained for
142 seven days in a room with a controlled temperature (25°C) to assess aerobic stability. For this,
143 we used a data logger (Log 110 EXF Inconterm; Brazil) inserted in the ensiled mass at a
144 depth of 10 cm in the central portion, and the temperature recording was carried out at
145 intervals of 8 hours. Aerobic stability was calculated as the time, in hours, in which the
146 silages had a temperature 2 °C higher than the ambient temperature after opening the silo. In
147 addition, we collected samples from each silo during the aerobic stability assessment at 24-
148 hour intervals to determine DM (AOAC Method 967.03, AOAC, 1990) (Kung Jr. et al.,
149 2003).

150

151 **Fermentative parameters**

152 After opening each silo, the material was homogenized, a sample of 25 g of fresh silage was
153 taken, and 225 ml of saline solution (8.5 g of NaCl/L of distilled water) was added and
154 homogenized for 1 minute in an industrial processor. The extract was filtered through a
155 double layer of gauze, and pH was measured with a pH meter (MPA-210, Tecnoyon, Brazil)
156 (Kung Jr., 1996). Aliquots of 2 mL of extract were transferred to test tubes with 1 mL of

157 sulfuric acid (1N) and stored at -20°C . Ammoniacal nitrogen analysis was performed
158 according to the methodology of Fenner (1965).

159

160 **Microbial population**

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

169

170 **Statistical Analysis**

171 Data regarding chemical composition, losses, ammoniacal nitrogen, pH, temperature,
172 microbial population, and cumulative gas production were analyzed in randomized blocks in a
173 3 × 2 factorial scheme with four replicates. The data were compared through the Tukey test
174 with a 0.05 significance level using the SAS MIXED package (SAS University Edition, SAS
175 Institute Inc., Cary, NC, USA).

176 We used the following statistical model:

$$177 \quad Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + b_k + e_{ijk}$$

178 Where: Y_{ijk} is the value observed for the variable under study referring to the k -th replicate of
179 the combination of the i -th level of factor α with the j -th level of factor β ; μ is the mean of all
180 experimental units for the variable under study; α_i is the use or not of additive in the silage
181 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

182 of additive and crops; b_k is the effect of the k -th block on the observation; and e_{ijk} is the error
183 associated with observation Y_{ijkl} .

184 The pH and temperature data (aerobic stability) were analyzed as repeated measures over
185 time, and we applied regression analysis with a significance level of 0.05 using the SAS
186 MIXED package (SAS University Edition, SAS Institute Inc., Cary, NC, USA).

187 We used the following statistical model:

$$188 \quad Y_{ijkl} = \mu + \alpha_i + \beta_j + \tau_k + \alpha\beta_{ij} + \alpha\tau_{ik} + \beta\tau_{jk} + \alpha\beta\tau_{ijk} + b_l + e_{ijkl}$$

189 Where: Y_{ijkl} is the value observed for the variable under study referring to the l -th replicate of
190 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
191 the mean of all experimental units for the variable under study; α_i is the use or not of additive
192 in the silage with $i = 1,2$; β_j is the crop effect, with $j = 1,2,3$; τ_k is the random effect of the
193 evaluation hours with $k = 0.24, \dots, 144$ for pH and $0.8, 16, \dots, 162$ for temperature; $\alpha\beta_{ij}$ is the
194 interaction between the use or not of additive and crops; $\alpha\tau_{ik}$ is the interaction between the
195 use of additive or not and the evaluation hours; $\beta\tau_{jk}$ is the interaction between crops and
196 evaluation hours; $\alpha\beta\tau_{ijk}$ is the interaction between the use of additive or not, crops, and
197 evaluation hours; b_k is the effect of the l -th block on the observation; and e_{ijkl} is the error
198 associated with observation Y_{ijkl} .

199

200 **Results**

201 **Chemical composition and gas production**

202 There was no interaction effect ($P > 0.05$) between additive and crops (Tables 1), except for
203 CP content ($P = 0.0357$). The use of additive increased the CP content ($P = 0.0062$) and
204 reduced the LIG content ($P = 0.0567$) in corn (19.65% [42.06/52.35]) and sorghum (14.43%
205 [50.15/58.61]) silages. The CF ($P = 0.7695$), NDF ($P = 0.0607$), and NCF ($P = 0.1429$)
206 contents did not show any statistical difference (Tables 1 and 2) between crops.

207 The gas production kinetics was not affected ($P > 0.05$) by the additive and neither between
208 crops (Tables 1 and 3).

209

210 **Aerobic stability test**

211 There was no interaction effect ($P > 0.05$) between additive and crops (Tables 1) and additive
212 and time for aerobic stability (Figure 2). Regarding the aerobic stability, we observed that the
213 additive did not affect the temperature of the corn ($P = 0.6419$) and pearl millet ($P = 0.8527$)
214 silages over the days. However, the additive affected ($P = 0.0123$) the temperature of the
215 sorghum silage (Figure 2C). The additive only affected the pH of the pearl millet silage ($P =$
216 0.0065) (Figure 2D).

217

218 **Fermentation parameters**

219 There was no interaction effect ($P > 0.05$) between additive and crops (Tables 1). The
220 temperature right after the opening of the silos and dry matter losses were not affected ($P >$
221 0.05) by the additive ($P > 0.05$) or between crops ($P > 0.05$) (Tables 1 and 4). The additive
222 decreased by 1.09% the pH of corn silage, 10.67% of sorghum silage, and 3.37% of pearl
223 millet silage. There was a statistical difference ($P < 0.0001$) between crops (Tables 1 and 4).

224 Ammoniacal nitrogen followed a similar behavior to pH. The additive increased 44.15% of
225 the ammonia nitrogen content in corn silage, 25.91% in sorghum silage, and decreased about
226 5% in pearl millet silage. The crops differed significantly ($P = 0.0095$) (Tables 1 and 4).

227

228 **Microbial population**

229 There was no interaction effect ($P > 0.05$) between additive and crops (Tables 1). There was
230 no appearance of enterobacteria in the silages with or without the additive. The additive
231 increased ($P = 0.0441$) by 9.63%, 19.46%, and 31.27% the population of lactic acid bacteria

232 in the pearl millet, corn, and sorghum silages, respectively (Table 5). Corn silage presented a
233 more significant amount of fungi ($P < 0.0001$) than pearl millet and sorghum silages,
234 regardless of the use of additive (Table 5).

235

236 **Discussion**

237 The nutritional value of silage depends mainly on three factors: the genetic material (plant
238 biomass, grain production, drought tolerance, and disease resistance), the stage at which the
239 forage was harvested (it affects the composition and the final quality of the preserved forage,
240 and the stage indicated for ensiling is between the doughy and farinaceous stages) and the
241 microorganisms (epiphytic) present in the forage during the ensiling process (Oliveira et al.,
242 2011; Behling Neto et al., 2017). Thus, we observed the chemical composition of the silages
243 and noticed a difference between the crops, except for the CF, NDF, and NCF contents (Table
244 2). The lower DM content observed in pearl millet and sorghum silages can be explained by
245 the higher resistance to drought due to an adaptive process that prevents excessive
246 dehydration such as smaller stomata, early stomatal closure, low stomatal density, and
247 increased leaf serosity, retaining more water in the plant (Levitt, 1980). In addition to the
248 difference between crops, we also observed that the additive increased the CP contents (Table
249 2), but the additive could not reduce losses caused by proteolysis. Analyzing the ammoniacal
250 nitrogen (Table 4), we observed that the CP degradation was between 25 and 30%. For Kung
251 Jr et al. (2018), the rapid pH decline is crucial to reduce protein degradation during ensiling,
252 which probably happened in corn silage without additive, as it had a low degradation rate
253 (21.11% [8.88/42.06]). Another factor that may have influenced the protein degradation is the
254 material of the silo (polyethylene) in terms of oxygen permeability, i.e., gas exchanges
255 between the silage and the external environment, with oxygen entering even without the
256 plastic bags present any physical damage (Amaral et al., 2014) which may have allowed the

257 increase of undesirable microorganisms such as mold (Table 5). These microorganisms have
258 their activity intensified in the presence of soluble carbohydrates, acids, and proteins,
259 increasing the silage pH (Table 4). The hemicellulose degradation has been neglected for
260 many years (Ning et al., 2017), but some studies have shown this degradation occurs during
261 the ensiling (Muck, 1990; Chen et al., 2015). Melvin (1965) and Yahaya et al. (2001) reported
262 that the degradation products of hemicellulose (xylose) and starch (glucose) could be
263 substrates for microorganisms to produce acids during the ensiling; this fact was observed in
264 our study (Table 2). LIG and CEL contents were lower in corn silage (Table 2). These values
265 can be explained by the carbon translocation from the leaf to the formation and filling of
266 grains, causing an increase in starch contents (Di Fonzo et al., 1982). In our study, the NFC
267 contents were not different ($P = 0.1429$) between crops, but corn silage was 2.35% higher
268 than pearl millet silage and 11.36% higher than sorghum silage without additive (Table 2).
269 Corn silage presented the lowest ash content (Table 2). According to Ashbell (1995), the low
270 ash content indicates excellent forage conservation, as the occurrence of inadequate
271 fermentation results in losses of organic matter, increasing the share of ash in DM.

272 The *in vitro* gas production allows evaluating the kinetics of ruminal fermentation,
273 providing information on the rate and extent of feed degradation in the rumen (Theodorou et
274 al., 1994). In our study, the gas production was not affected ($P > 0.05$) by the additive and
275 neither between crops (Table 1 and Figure 1). For Bach et al. (2005), crude protein
276 concentrations below 70 g/kg may restrict microbial activity due to lack of nitrogen. In this
277 study (Table 2), these concentrations were from 40 to 60 g/kg. However, we observed that
278 although there was no difference ($P > 0.05$), the time taken for half of the asymptotic gas to
279 be produced (Parameter B) in the corn silage with or without additive was shorter than the
280 other silages (Table 3). Gas production rates peaked in the first hours of incubation, being

281 longer in the corn silage without additive (Figure 1C), but all silages had a final rate below
282 0.1 ml/h (Figure 1).

283 The aerobic stability of the silage is expressed as the resistance of forage mass to
284 deterioration after opening the silo, i.e., the speed at which the mass deteriorates after its
285 contact with the air (Jobim et al., 2007). Thus, we observed that the silages' temperature at the
286 opening was not affected by the additive ($P = 0.8911$) and neither between crops ($P =$
287 0.3196). However, pH was affected by the additive ($P = 0.0013$) and crops ($P < 0.0001$)
288 (Table 4). *Lactobacillus plantarum*, one of the inoculants in this study, aims to increase lactic
289 acid production, consequently reducing the pH of the ensiled mass and inhibiting the growth
290 of unwanted microorganisms (McDonald et al., 1991; Muck, 2010). Analyzing the
291 temperature over the days, we observed that in the first 36 hours, there was a peak
292 temperature in sorghum silage regardless of the additive. However, the silage with an additive
293 reduced its temperature more quickly (Figure 2C). For Woolford (1990), the initial increase in
294 temperature is caused by the growth of yeasts and filamentous fungi, but after some time,
295 according to Muck and Pitt (1992), the increase in pH (above 5.0) can favor the growth of
296 bacilli that can cause a second increase in the temperature, this fact was observed in our study
297 (Figures 2 B and C). The plastic bag (polyethylene) silos can present oxygen permeability at a
298 temperature of 25 °C. Gas exchange between the interior of the silo and the environment is
299 close to 1 liter/m², which is a value for an intact bag without any physical damage (Greenhill,
300 1964). This exchange can make the silage more prone to aerobic deterioration due to the
301 increase in the permeability of the bags, as the aeration of this mass allows the action of
302 yeasts that oxidize the silage's preservative organic acids triggering aerobic degradation and
303 increasing pH. In our study, the pearl millet, corn, and sorghum silages without additive
304 increased 2.0 points in pH in 24, 96, and 96 hours, respectively, whereas those with additive,

305 increased 2.0 points in pH in 48 hours (Figures 2D, E, and F). The aerobic stability loss of
306 silages is usually manifested by an increase in temperature and a change in pH.

307 Ammoniacal nitrogen (NH₃-N) indicates the amount of protein degraded during the
308 fermentation. It is an indicator of the extent of clostridial activity since it is produced in small
309 amounts by other microorganisms in the silage and plant enzymes (Borreani et al., 2018).
310 According to Blajman et al. (2020), the combination of hetero and homofermentative bacteria
311 favors reducing pH values, ammoniacal nitrogen, and fermentative losses in silages. The
312 NH₃-N levels were affected by the additive (0.0040) and between crops ($P = 0.0095$) (Table
313 4). According to Tomich et al. (2003), NH₃-N values below 10 g/kg CP indicate good
314 fermentation, and above 15 g/kg CP of NH₃-N in silage indicate a significant amount of
315 proteolysis. In the present study, only corn silage without additive (8.88 g/kg CP) presented
316 NH₃-N values below 10 g/kg CP. This fact may indicate a higher intensity of proteolysis,
317 especially by amino acid degradation by bacteria of the genus *Clostridium* (Diether and
318 Willing, 2019).

319 It is also essential to understand the microbial population, as the ensiling will preserve the
320 forage and inhibit undesired microorganisms (*Clostridium* sp, enterobacteria, yeasts, and
321 fungi), influencing the silage quality (Muck, 2010). In this study, we observed there were no
322 counts for enterobacteria in the silos. This fact is related to the active growth of lactic acid
323 bacteria (LAB) during the fermentation process, as the pH decrease to values between 3.35
324 and 3.75 (Table 4), it favors the population decline of enterobacteria rapidly, turning LAB the
325 main microorganisms in silage (McDonald et al., 1991). The additive increased the LAB
326 population and decreased the pH of the silos. *Lactobacillus plantarum* (one of the
327 microorganisms in the additive of this study) aims to increase the lactic acid production,
328 consequently reducing the pH of the ensiled mass and inhibiting the growth of unwanted
329 microorganisms (McDonald et al., 1991; Muck, 2010). Analyzing the crops, we observed that

330 corn silage presented the highest ($P < 0.001$) fungi population, about 37.75% more than pearl
331 millet silage and 29.27% more than sorghum silage (Table 5). This fact is probably because
332 the plastic bag (polyethylene) silos can present oxygen permeability, and most fungi are
333 strictly aerobic. They can use sugars (glucose, sucrose, and maltose) and more complex
334 compounds (starch, cellulose, and hemicellulose) as substrates for their growth (Wang et al.,
335 2020).

336

337 **Conclusion**

338 The bacterial additive did not improve the fermentation profile and nutritional value of corn,
339 pearl millet, and sorghum silages in plastic bag silos.

340

341 **Declarations**

342 **Authors' contributions**

343 Conceptualization: A. M. Fernandes and T.S. Oliveira. Data curation: T.S. Oliveira. Formal
344 analysis: T.S. Oliveira. Investigation: E.F. Processi, and T.S. Oliveira. Methodology: S.E.E.
345 Bernardo, E.F. Processi, M.G. Camilo, D.F. Baffa, and P.H.B. Chrisostomo. Resources: T.S.
346 Oliveira. Supervision: T.S. Oliveira. Writing-original draft: T.S. Oliveira. Writing-review &
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355 **Code availability:** Not applicable.

356 **Research involving human participants and/or animals**

357 **Ethical Approval:** All experimental procedures were approved by the Ethics Committee on
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359 Ribeiro (UENF), registered under protocol 419-2017.

360

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Figures

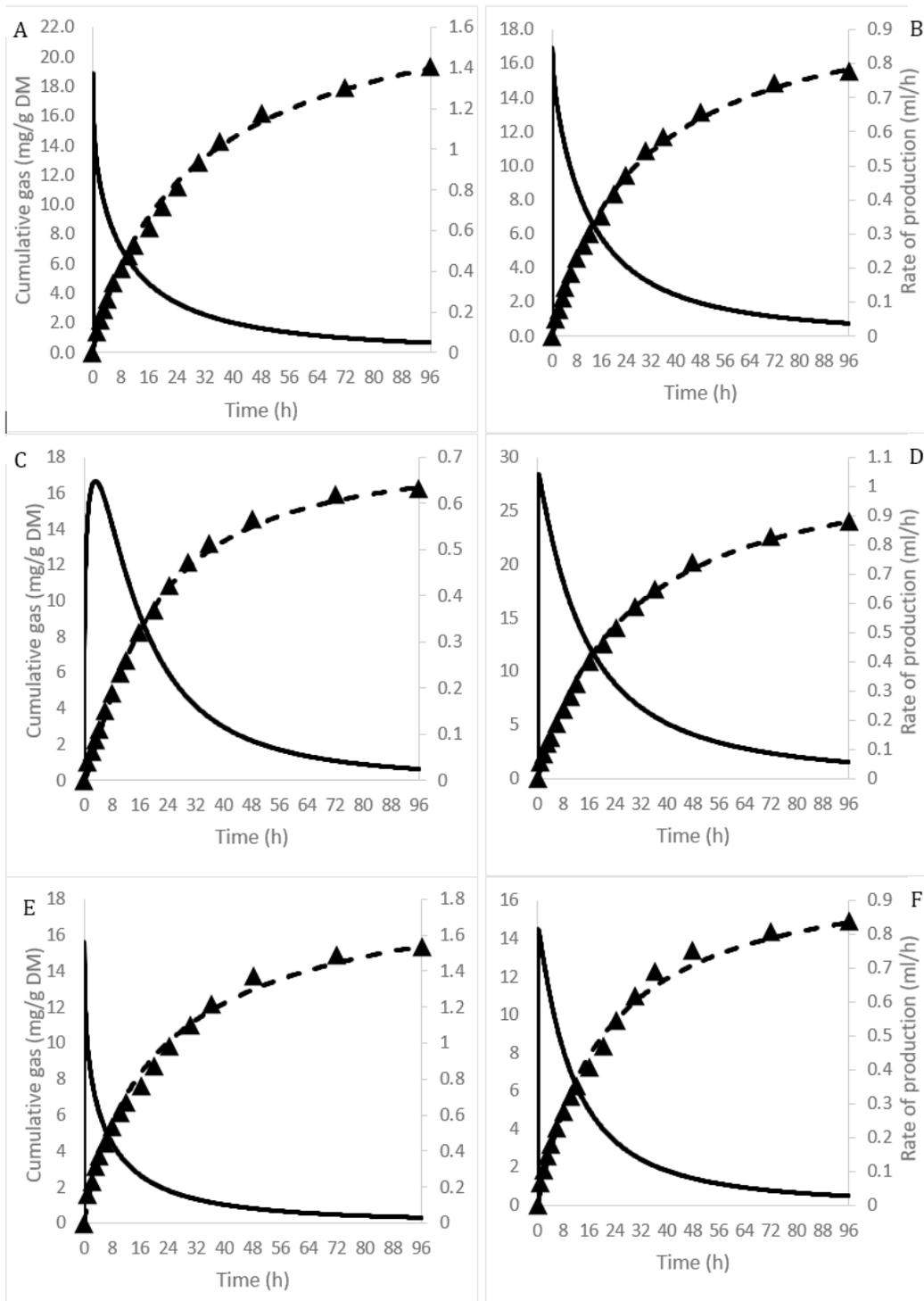


Figure 1

Cumulative gas production and rate of gas production profiles from pearl millet, corn, and sorghum silage with or not additives. On panel (1A) pearl millet without additive; (1B) pearl millet with additive; (1C) corn

without additive; (1D) corn with additive; (1E) sorghum without additive; and (1F) sorghum with additive [*Lactobacillus plantarum* (2.5×10^{10} cfu/g) and *Propionibacterium acidipropionici* (2.5×10^{10} cfu/g)].

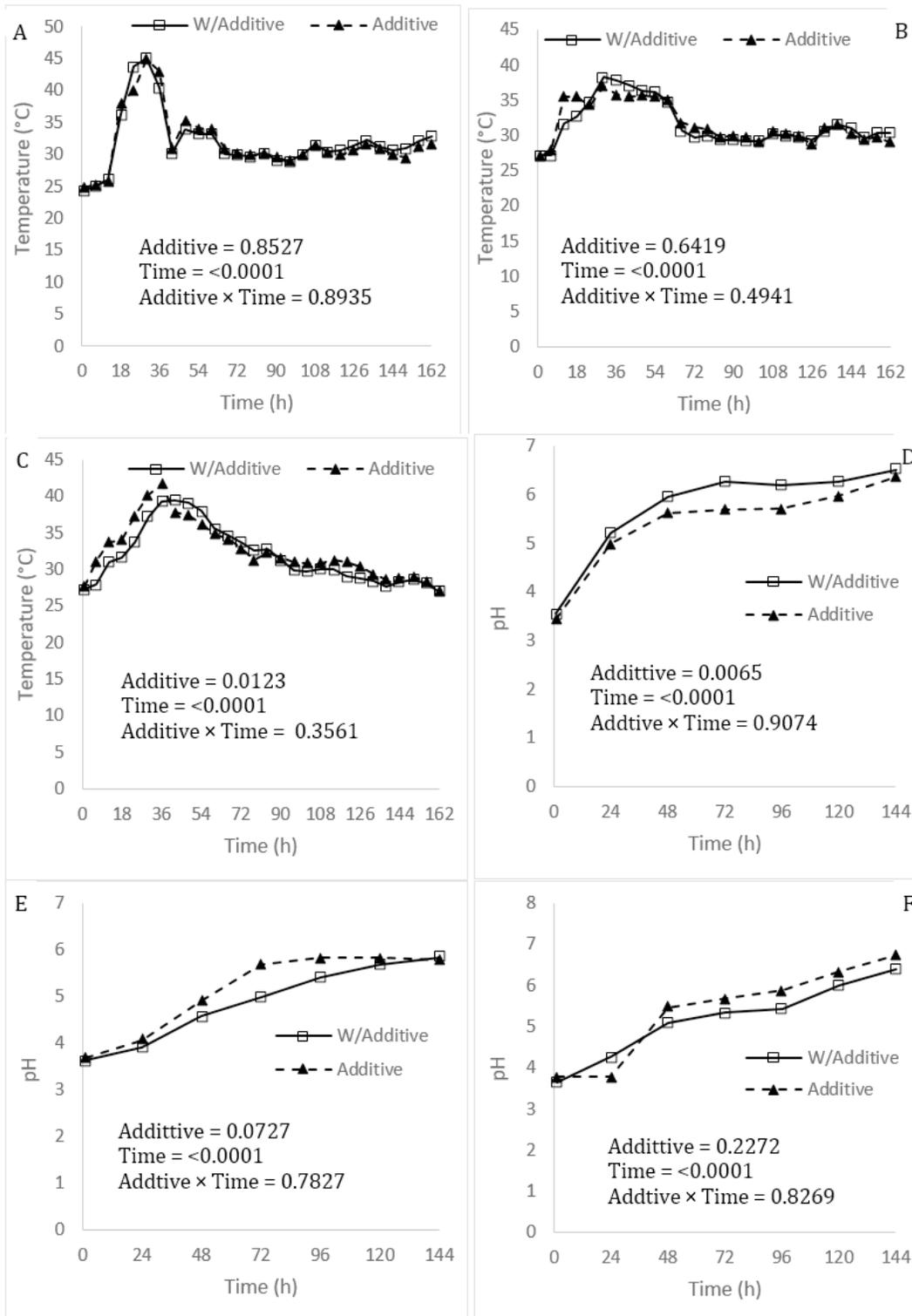


Figure 2

Aerobic stability of pearl millet, corn, and sorghum silage with or not additive. 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.