Fermentation and aerobic stability of rehydrated corn grain silage treated with different doses of \textit{Lactobacillus buchneri} or a combination of \textit{Lactobacillus plantarum} and \textit{Pediococcus acidilactici}

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ABSTRACT

We investigated the effects of different types and doses of inoculants for ensiling rehydrated corn grain. Shelled corn was finely ground and rehydrated to 35% moisture. Treatments were as follows: (1) control (no additives); (2) \textit{Lactobacillus plantarum} and \textit{Pediococcus acidilactici} (LPPA) at a theoretical application rate of $1 \times 10^5$ cfu/g; (3) LPPA at $5 \times 10^5$ cfu/g; (4) LPPA at $1 \times 10^6$ cfu/g; (5) \textit{Lactobacillus buchneri} (LB) at $1 \times 10^5$ cfu/g; (6) LB at $5 \times 10^5$ cfu/g; and (7) LB at $1 \times 10^6$ cfu/g. We detected no effect of inoculant dose. Gas losses were greater in silages treated with LB compared with control and LPPA silages. Treating silages with LB reduced the concentrations of lactic acid and ethanol and increased silage pH and concentrations of acetic acid, propionic acid, and 1,2-propanediol. At silo opening, silages treated with LB had higher counts of lactic acid bacteria but lower yeast counts than the control silage. Aerobic stability was greater for silages treated with LB and lower for silages treated with LPPA compared with the control. The LB reduced dry matter (DM) losses during aerobic exposure, whereas LPPA increased them. Prolamin content was lower in silages treated with LB compared with the control, resulting in greater ruminal in situ DM degradability. Inoculating LB to a dose of $1 \times 10^5$ cfu/g increased aerobic stability and ruminal in situ DM degradability of rehydrated corn grain silage. The addition of LPPA did not alter the fermentation process and worsened the aerobic stability of rehydrated corn grain silage. Further studies are warranted to confirm these conclusions in other corn hybrids, inoculants, and their combinations.

Key words: fermentation end-product, ruminal in situ DM degradability, prolamin, corn grain

INTRODUCTION

Ensiling ground corn grain after rehydration is a promising technique to improve the nutritive value of corn kernels. The effect of this technique would be even greater in corn kernels with a high proportion of vitreous endosperm, such as flint hybrids, which are predominantly used in Brazil (Correa et al., 2002). This strategy may be affordable when production of high-moisture corn (HMC) is not possible due to logistic or land constraints (Ferraretto et al., 2015). However, mature grains contain variable concentrations of soluble carbohydrates and lower counts of lactic acid bacteria (LAB) compared with high-moisture corn (Carvalho et al., 2015), which would compromise the course of fermentation (McDonald et al., 1991).

Microbial additives containing homofermentative or facultative heterofermentative bacteria are able to rapidly reduce silage pH and inhibit the growth of undesirable microorganisms (Kung et al., 2003). However, inoculating HMC and corn silages with homolactic bacteria typically reduces aerobic stability (Kung and Ranjit, 2001; Kung et al., 2003; Kleinschmit et al., 2005). Conversely, obligatory heterofermentative LAB may prevent aerobic deterioration (Kung et al., 2003). \textit{Lactobacillus buchneri} is the most used heterofermentative LAB because of its capacity to convert lactic acid into acetic acid under anaerobic conditions (Oude Elferink et al., 2001) and improved aerobic stability at feed-out.

During silage fermentation, proteolysis of the hydrophobic protein matrix surrounding the starch granules can increase starch digestibility (Hoffman et al., 2011). Bacterial proteolysis is the main contributor to protein degradation (~60%) during fermentation of rehydrated corn grain silage (Junges et al., 2017). Therefore, the ef-
fect of inoculants on the microbial profile of rehydrated corn grain silage might influence starch availability. The effect of microbial inoculants on HMC is well documented (Taylor and Kung, 2002; Basso et al., 2012). However, the literature on rehydrated corn grain silage is scarce, and further investigation is needed, given the microbial and chemical dissimilarities between HMC and rehydrated corn grain silage. Based on previous literature, we hypothesized that homofermentative inoculants might improve the fermentation phase of rehydrated corn grain by a rapid decline in silage pH, whereas heterofermentative inoculants may enhance the aerobic stability of silages because of the increased production of acetic acid, an antifungal compound. The aim of this study was to determine the best type and dose of inoculant for ensiling rehydrated corn grain conserved in laboratory silos.

**MATERIALS AND METHODS**

**Ensiling Process**

Shelled corn purchased in the local market (hybrid DKB 390 VT PRO 2; Dekalb, Uberlândia, Brazil) was finely ground through a 2-mm screen in a hammer mill and transferred to a mixer wagon (Silotomac, model S-2.3, São Carlos, SP, Brazil) and rehydrated with water from an artesian well to reach a moisture content of 35%. The material was constantly mixed during rehydration to ensure homogeneity. The rehydrated grain was split into 7 piles (150 kg per pile) and used for preparation of the following treatments: (1) control (no additives); (2) a combination of Lactobacillus plantarum (MA18/5U; 4 × 10^10 cfu/g) and Pediococcus acidilacticii (MA18/5M; 1 × 10^10 cfu/g) (LPPA; Bacto Silo Master Tropical, Lallemand, Aparecida de Goiânia, GO, Brazil) at a theoretical application rate of 1 × 10^6 cfu/g of rehydrated corn grain; (3) LPPA at 5 × 10^6 cfu/g; (4) LPPA at 1 × 10^6 cfu/g; (5) Lactobacillus buchneri (LB; NCIMB 40788, Lallemand) at 1 × 10^6 cfu/g; (6) LB at 5 × 10^5 cfu/g; and (7) LB at 1 × 10^6 cfu/g. Six replications per treatment were used. For all treatments, the inoculants were diluted in chlorine-free water and applied at 2 mL/kg using a manual sprayer (one sprayer per treatment). The control received the same amount of water. After rehydration, the DM content of the corn was 64.2%, and the concentrations of CP, ethanol-soluble carbohydrates, and prolamin were 8.26, 2.17, and 4.60% of DM, respectively. Buckets with a capacity of 15 L were used as experimental silos. Packing was performed manually, with a mean density of 1,085 ± 75 kg/m³. After packing, each silo was sealed with a plastic cap equipped with a lid to enable gas release.

**Fermentation Losses**

The laboratory silos were weighed immediately after closing and before opening to determine gas losses. After 124 d of storage, the content of each silo was homogenized and sampled for measuring chemical composition, fermentation profile, microbial counts, and aerobic stability.

**Aerobic Stability Test**

To monitor aerobic stability, approximately 3 kg of silage was placed in each polystyrene box (total of 42 boxes). A sheet of aluminum foil with small holes was placed on each box to decrease silage dehydration and dust contamination but allow air flow (Tabacco et al., 2009). The polystyrene boxes were kept in a closed room at ambient temperature (25.2 ± 2.9°C). The temperature of the silages and environment was recorded at intervals of 30 min with a data logger (Pro2.07.09; Escort Console, Buchanan, VA). Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 5°C above room temperature, a strategy adopted because the assay was conducted at uncontrolled room temperature. The peak of temperature was calculated. The buckets were weighed at 0 and 12 d of aerobic exposure and the silages were sampled for MS analysis to determine DM losses. An additional 3 kg of silage was placed in other polystyrene boxes (3 kg/box, 42 boxes) to determine the pH value (0, 2, 4, 6, 8, 10, and 12 d of aerobic exposure) and microbiological profile (0, 4, 8, and 12 d of aerobic exposure).

**Chemical and Microbiological Analyses**

The pre-ensiled material and silages, both in duplicate, were dehydrated in a forced ventilation oven at 55°C for 72 h and ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Sub-samples were analyzed for CP (method 2001.11; AOAC International, 2012), ethanol-soluble carbohydrates (Hall, 2000), and prolamin (Nellis et al., 2013). Silage pH and fermentation end-products were analyzed in an aqueous extract prepared with 30 g of sample material (fresh weight) and 270 g of distilled water, shaken for 4 min in Stomacher (400 Circulator, Seward Ltd., West Sussex, UK). The pH was measured with a pH meter (DM-22; Digimed, São Paulo, SP, Brazil), lactic acid was determined by colorimetry (Pryce, 1969), and ammoniacal nitrogen (N-NH₃) was measured according to Noel and Hambleton (1976). Alcohols, esters, volatile fatty acids, and acetone were measured by gas chromatography (GCMS QP2010 Plus; Shimadzu,
Kyoto, Japan) using a capillary column (Stabilwax; Restek, Bellefonte, PA; 60-m length, 0.25-mm outside diameter, 0.25-μm film thickness, crossbond carboxaw polyethylene glycol). Silage DM content was corrected for volatile compounds (Weissbach, 2009).

Another silage sample was used for counting yeasts, molds, and LAB. Aqueous extracts were prepared with 30 g of sample material (fresh weight) and 270 g of sterile peptone water (0.1%) and homogenized for 4 min in the stomacher (400 Circulator, Seward Ltd.). Subsequent 10-fold dilutions were spread plated on yeast extract glucose chloramphenicol agar (Sigma-Aldrich, São Paulo, Brazil) to enumerate yeasts and molds (Tabacco et al., 2009), and the plates were incubated at 28°C for 3 d (yeasts) and 5 d (molds). To enumerate LAB, subsequent 10-fold dilutions were spread onto de Man, Rogosa, and Sharpe agar plates (HiMedia Laboratories, Mumbai, India), and the plates were incubated at 35°C for 3 d. During the aerobic stability test, samples were collected from the polystyrene boxes at 4, 8, and 12 d.

### Ruminal In Situ Degradability

For the ruminal in situ DM degradability assay, 5 g of ungrown dried sample (mean particle size of 1.68 mm) was placed in bags (model R1020; Ankom, Macedon, NY; pore size = 50 ± 15 μm), as described by Nocek (1988). Bags were incubated in the rumen ventral sac of 6 rumen-cannulated Nellore steers (635 ± 55 kg of BW) fed a TMR containing sugarcane bagasse (12.5% of DM), dried citrus pulp (17.0% of DM), dry ground corn (62.6% of DM), peanut meal (3.99% of DM), mineral premix (1.86% of DM), and urea (2.13% of DM). The use of the animals was approved by the Ethics Committee of FCAV/UNESP, Jaboticabal (Brazil; protocol no. 20858/13). The bags were incubated for 12 d of air exposure were analyzed as repeated measures using the following model: $Y_{ij} = μ + T_i + D_j + A_{ij} + T \times D_{ij} + e_{ij}$, where $μ =$ overall mean, $T_i =$ fixed effect of treatment ($i =$ control, LPPA $1 \times 10^5$ cfu/g, LPPA $5 \times 10^5$ cfu/g, LPPA $1 \times 10^6$ cfu/g, LB $1 \times 10^5$ cfu/g, LB $5 \times 10^5$ cfu/g, and LB $1 \times 10^6$ cfu/g); and $e_{ij} =$ error. Means were compared by orthogonal contrasts (C1: control vs. LPPA; C2: control vs. LB; C3: linear effect of LPPA dose; C4: quadratic effect of LPPA dose; C5: linear effect of LB dose; and C6: quadratic effect of LB dose).

The pH and counts of yeasts and molds over 12 d of air exposure were analyzed as repeated measures using the following model: $Y_{ijk} = μ + T_i + D_j + A_{ij} + T \times D_{ij} + e_{ijk}$, where $μ =$ overall mean, $T_i =$ fixed effect of treatment ($i =$ control, LPPA $1 \times 10^5$ cfu/g, LPPA $5 \times 10^5$ cfu/g, LPPA $1 \times 10^6$ cfu/g, LB $1 \times 10^5$ cfu/g, LB $5 \times 10^5$ cfu/g, and LB $1 \times 10^6$ cfu/g); $D_j =$ fixed effect of day of aerobic exposure (pH, $j = 0, 2, 4, 6, 8, 10, \text{ and 12}$; yeasts and molds, $j = 0, 4, 8, \text{ and 12}$), and $T \times D_{ij} =$ interaction between treatment and day of aerobic exposure, and the random effects of $A_{ij} =$ laboratory silo nested within treatment and $e_{ijk} =$ error. Covariance structures used for pH and yeast and mold count data were heterogeneous compound symmetry (CSH), banded [UN(1)], and UN(1), respectively. Matrices were chosen based on the lowest Bayesian information criterion value. All means and contrasts were compared using the $t$-test at 5% probability.

### Statistical Analysis

Outcomes with a single measurement for each experimental unit were analyzed using the Mixed procedure of SAS (v. 9.2; SAS Institute Inc., Cary, NC) as a completely randomized design with 6 replications, using the following model: $Y_i = μ + T_i + e_i$, where $Y_i =$ response variable; $μ =$ overall mean; $T_i =$ effect of treatment ($i =$ control, LPPA $1 \times 10^5$ cfu/g, LPPA $5 \times 10^5$ cfu/g, LPPA $1 \times 10^6$ cfu/g, LB $1 \times 10^5$ cfu/g, LB $5 \times 10^5$ cfu/g, and LB $1 \times 10^6$ cfu/g); and $e_i =$ error. Means were compared by orthogonal contrasts (C1: control vs. LPPA; C2: control vs. LB; C3: linear effect of LPPA dose; C4: quadratic effect of LPPA dose; C5: linear effect of LB dose; and C6: quadratic effect of LB dose).

The LPPA silages did not differ ($P = 0.08$) in DM content, CP, prolamin, or N-NH$_3$ levels from the control treatment (Table 1). Prolamin and N-NH$_3$ content linearly increased ($P < 0.01$) as LPPA dose increased (C3). Silages treated with LB had lower prolamin ($P = 0.02$) and higher N-NH$_3$ ($P < 0.01$) content compared with the control (C2). We detected no difference in DM ($P = 0.10$) or CP ($P = 0.99$) content of silages treated with LB compared with the control (C2). As LB doses increased, DM and CP contents linearly increased (C5, $P < 0.05$), N-NH$_3$ content linearly decreased (C5, $P < 0.01$), and prolamin content did not differ (C5 and C6, $P = 0.74$).

The LPPA silages did not differ ($P = 0.05$) in gas loss and had lower ($P = 0.01$) LAB counts than the control silage. Silages treated with LB had higher gas loss and higher LAB counts than those of the control treatment ($P < 0.01$). Increasing dose of LPPA and LB did not affect gas loss or LAB counts (C3, C4, C5, and C6; $P = 0.21$, Table 1).

Silages treated with LPPA (C1) had lower ($P < 0.01$) concentrations of acetic acid, ethyl lactate, and 2-butanol, and higher ($P < 0.05$) concentrations of butyric acid and valeric acid. There were no differences
among LPPA and control (C1, $P = 0.05$) for the other fermentation products (Table 2). As LPPA doses increased, 1,2-propanediol content linearly increased (C3, $P < 0.01$) and lactic acid and 2,3-butanediol linearly decreased (C3, $P < 0.05$). Silages treated with LB (C2) had lower ($P < 0.01$) concentrations of lactic acid, ethanol, ethyl lactate, and isovaleric acid, and higher ($P < 0.01$) concentrations of acetic acid, propionic acid, 1,2-propanediol, 2,3-butanediol, ethyl acetate, formic acid, isobutyric acid, 2-butanol, and propyl acetate. Increasing LB doses did not affect (C5 and C6, $P = 0.12$) the concentrations of major fermentation end-products (i.e., lactic acid, acetic acid, butyric acid, ethanol, and 1,2-propanediol), but propionic acid linearly decreased with higher doses of LB ($P < 0.01$).

**Aerobic Stability**

Rehydrated corn grain silage treated with LB increased (C2, $P < 0.01$) aerobic stability by 406% compared with the control treatment, whereas silages treated with LPPA reduced (C1, $P < 0.01$) aerobic stability by 26% compared with the control (Table 1). The peak temperature was lower ($P < 0.01$, Table 1) for silages treated with LB than that of the control. The peak temperature was higher ($P = 0.04$) for silages treated with LPPA (C1), and, as LPPA dose increased (C3, $P < 0.01$), peak temperature linearly increased. Compared with the control treatment, LB decreased DM losses during aerobic exposure (C2, $P < 0.01$); however, this was not affected by LPPA treatment (C1, $P = 0.51$). The greater aerobic stability of silages treated with LB reduced ($P < 0.01$) DM losses by 80.3% over the 12 d of aerobic exposure. In this way, we observed an interaction ($P < 0.01$) between treatment and day of aerobic exposure for silage pH (Figure 1). At silo opening (d 0) and after 2 d of exposure to air, silages treated with LB had higher pH than those of LPPA and control treatment. However, pH of LPPA and control silages increased steeply after 4 d and remained higher than that of LB silages. The pH of silages treated with LB remained stable during the 12 d of aerobic exposure.

We detected an interaction ($P < 0.01$) between treatment and day of aerobic exposure for the count of yeasts and molds (Figure 2). Applying LB resulted in a low count of yeasts during aerobic exposure compared with the control and LPPA treatments. Count of yeasts of silage treated with LB remained below the detection limit (<2.0 log cfu/g silage) during aerobic exposure. Count of molds were below the detection limit at silo opening (d 0) for all treatments and remained below this level in silages treated with LB during aerobic exposure.

### Table 1. Chemical composition, microbial counts, and aerobic stability of rehydrated corn grain silage after 124 d of fermentation

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>LPPA 1 × 10⁵</th>
<th>LPPA 5 × 10⁵</th>
<th>LPPA 1 × 10⁶</th>
<th>LB 1 × 10⁵</th>
<th>LB 5 × 10⁵</th>
<th>LB 1 × 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (% of DM)</td>
<td>62.8</td>
<td>63.2</td>
<td>63.0</td>
<td>63.8</td>
<td>62.9</td>
<td>63.3</td>
<td>63.9</td>
</tr>
<tr>
<td>CP (% of DM)</td>
<td>9.11</td>
<td>8.32</td>
<td>8.52</td>
<td>8.47</td>
<td>8.36</td>
<td>9.27</td>
<td>9.68</td>
</tr>
<tr>
<td>Prolamin (% of DM)</td>
<td>3.55</td>
<td>3.22</td>
<td>3.63</td>
<td>3.85</td>
<td>3.15</td>
<td>3.10</td>
<td>3.16</td>
</tr>
<tr>
<td>N-NH₃ (% of DM)</td>
<td>0.071</td>
<td>0.073</td>
<td>0.059</td>
<td>0.10</td>
<td>0.103</td>
<td>0.098</td>
<td>0.094</td>
</tr>
<tr>
<td>Lactic acid bacteria (log cfu/g)</td>
<td>3.36</td>
<td>2.41</td>
<td>2.84</td>
<td>2.53</td>
<td>6.46</td>
<td>6.35</td>
<td>6.31</td>
</tr>
<tr>
<td>DM losses during 12 d of air exposure (% of DM)</td>
<td>12.7</td>
<td>13.2</td>
<td>12.1</td>
<td>12.1</td>
<td>16.5</td>
<td>16.5</td>
<td>16.5</td>
</tr>
</tbody>
</table>

**Note:**
- Control = silage without inoculant; LPPA = combination of *Lactobacillus plantarum* (MA18/5U) and *Pediococcus acidilactici* (MA18/5M) at a dose of 1 × 10⁵, 5 × 10⁵, or 1 × 10⁶ cfu/g of rehydrated corn grain; LB = *Lactobacillus buchneri* (NCIMB 40788) at a dose of 1 × 10⁵, 5 × 10⁵, or 1 × 10⁶ cfu/g of rehydrated corn grain.
- C1 = control vs. LPPA; C2 = control vs. LB; C3 = linear effect of LPPA dose; C4 = quadratic effect of LPPA dose; C5 = linear effect of LB dose; C6 = quadratic effect of LB dose.
Table 2. Fermentation products (per kg of DM) of rehydrated corn grain silage after 124 d of fermentation

<table>
<thead>
<tr>
<th>Product</th>
<th>LPPA</th>
<th>LB</th>
<th>SEM</th>
<th>Contrast²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1 × 10⁵ 5 × 10⁵ 1 × 10⁶</td>
<td>1 × 10⁵ 5 × 10⁵ 1 × 10⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid (g)</td>
<td>27.6 28.1 24.3 23.4</td>
<td>12.0 11.4 15.1</td>
<td>1.48</td>
<td>0.18 &lt;0.01 0.03 0.35 0.12 0.27</td>
</tr>
<tr>
<td>Ethanol (g)</td>
<td>5.25 5.68 6.76 5.08</td>
<td>2.93 3.38 3.72</td>
<td>0.43</td>
<td>0.24 &lt;0.01 0.27 0.02 0.19 0.84</td>
</tr>
<tr>
<td>Acetic acid (g)</td>
<td>3.60 2.83 2.66 2.49</td>
<td>15.4 15.2 16.3</td>
<td>0.52</td>
<td>&lt;0.01 &lt;0.01 0.33 0.89 0.40 0.66</td>
</tr>
<tr>
<td>2,3-Butanediol (mg)</td>
<td>393 398 343 294</td>
<td>2,183 2,205 1,836</td>
<td>63.3</td>
<td>0.22 &lt;0.01 0.04 0.73 0.02 0.25</td>
</tr>
<tr>
<td>Ethyl lactate (mg)</td>
<td>172 146 173 153</td>
<td>39.4 45.1 64.1</td>
<td>3.88</td>
<td>&lt;0.01 &lt;0.01 0.37 &lt;0.01 &lt;0.01 0.24</td>
</tr>
<tr>
<td>1,2-Propanediol (mg)</td>
<td>175 4.53 18.4 14.5</td>
<td>11,305 11,216 12,216</td>
<td>287</td>
<td>0.09 &lt;0.01 &lt;0.01 &lt;0.01 0.25 0.58</td>
</tr>
<tr>
<td>Ethyl acetate (mg)</td>
<td>54.3 49.8 65.7 41.2</td>
<td>148 112 108</td>
<td>8.95</td>
<td>0.77 &lt;0.01 0.27 0.02 0.03 0.26</td>
</tr>
<tr>
<td>Methanol (mg)</td>
<td>31.4 37.1 36.1 25.9</td>
<td>32.2 30.0 33.2</td>
<td>3.36</td>
<td>0.67 0.91 0.02 0.35 0.80 0.52</td>
</tr>
<tr>
<td>Propionic acid (mg)</td>
<td>30.5 23.8 26.1 22.4</td>
<td>159.6 78.1 66.4</td>
<td>5.57</td>
<td>0.07 &lt;0.01 0.74 0.58 &lt;0.01 0.01</td>
</tr>
<tr>
<td>Acetone (mg)</td>
<td>7.75 5.57 6.23 6.30</td>
<td>7.64 7.91 7.15</td>
<td>0.74</td>
<td>0.05 0.83 0.51 0.72 0.62 0.59</td>
</tr>
<tr>
<td>2-Butanol (mg)</td>
<td>6.30 0.34 0.97 0.40</td>
<td>19.76 8.98 5.60</td>
<td>1.10</td>
<td>&lt;0.01 &lt;0.01 0.94 0.28 &lt;0.01 0.07</td>
</tr>
<tr>
<td>Isovaleric acid (mg)</td>
<td>5.26 5.00 4.85 4.98</td>
<td>2.66 2.40 1.78</td>
<td>0.29</td>
<td>0.35 &lt;0.01 0.98 0.70 0.03 0.72</td>
</tr>
<tr>
<td>Butyric acid (mg)</td>
<td>5.16 7.79 11.79 7.00</td>
<td>3.41 3.49 3.55</td>
<td>1.15</td>
<td>0.03 0.08 0.69 0.17 0.76 0.97</td>
</tr>
<tr>
<td>Isobutyric acid (mg)</td>
<td>1.87 3.12 2.22 3.10</td>
<td>20.3 20.3 20.4</td>
<td>0.77</td>
<td>0.07 &lt;0.01 0.93 0.08 0.93 0.98</td>
</tr>
<tr>
<td>Valeric acid (mg)</td>
<td>0.94 2.25 1.48 1.17</td>
<td>1.03 0.77 0.86</td>
<td>0.16</td>
<td>&lt;0.01 0.70 &lt;0.01 0.38 0.36 0.12</td>
</tr>
<tr>
<td>Formic acid (mg)</td>
<td>0.91 1.06 0.00 0.01</td>
<td>248 209 192</td>
<td>16.4</td>
<td>0.98 &lt;0.01 0.97 0.98 0.02 0.48</td>
</tr>
<tr>
<td>Isopropyl alcohol (mg)</td>
<td>0.71 0.41 0.61 0.59</td>
<td>0.91 0.79 0.80</td>
<td>0.12</td>
<td>0.21 0.39 0.32 0.42 0.52 0.64</td>
</tr>
<tr>
<td>Propyl acetate (mg)</td>
<td>0.40 0.47 0.59 0.31</td>
<td>12.09 8.43 7.41</td>
<td>0.49</td>
<td>0.75 &lt;0.01 0.40 0.47 &lt;0.01 0.16</td>
</tr>
<tr>
<td>1-Propanol (mg)</td>
<td>0.00 0.00 0.00 0.00</td>
<td>144 135 128</td>
<td>4.63</td>
<td>1.00 &lt;0.01 1.00 1.00 0.15 0.82</td>
</tr>
</tbody>
</table>

¹Control = silage without inoculant; LPPA = combination of Lactobacillus plantarum (MA18/5U) and Pediococcus acidilactici (MA18/5M) at a dose of 1 × 10⁵, 5 × 10⁵, or 1 × 10⁶ cfu/g of rehydrated corn grain; LB = Lactobacillus buchneri (NCIMB 40788) at a dose of 1 × 10⁵, 5 × 10⁵, or 1 × 10⁶ cfu/g of rehydrated corn grain.
²C1 = control vs. LPPA; C2 = control vs. LB; C3 = linear effect of LPPA dose; C4 = quadratic effect of LPPA dose; C5 = linear effect of LB dose; C6 = quadratic effect of LB dose.
Ruminal In Situ Degradability

The ruminal in situ DM degradability of DM after 12 and 24 h of incubation was higher (P < 0.05) for silages treated with LB compared with the control silage (Figure 3). The LPPA silages did not differ (P = 0.06) in ruminal in situ DM degradability of DM after 12 or 24 h of incubation from the control treatment. As LPPA and LB doses increased, ruminal in situ DM degradability of DM after 12 and 24 h of incubation did not change significantly (C3, C4, C5, and C6, P = 0.50).

DISCUSSION

An issue of rehydrated corn grain is the potential for silage fermentation. During corn crop development, soluble carbohydrates are polymerized as starch in kernel endosperm, leaving only small amounts of water-soluble carbohydrates at dry grain harvesting, which are the main substrates for the growth of LAB in silage (McDonald et al., 1991). In our study, we did not observe any fermentation problems with the rehydrated corn grain silage. The concentrations of the main fermentation products and silage pH were similar to those reported in the literature for HMC (Sebastian et al., 1996; Taylor and Kung, 2002; Kung et al., 2014). In contrast to the findings of Carvalho et al. (2015), the production of butyric acid was low in the present study, indicating low growth of undesirable microorganisms (Pahlow et al., 2003). The low concentration of acetone was another indication of the low growth of undesirable microorganisms, given that acetone seems to be a metabolite of clostridia pathways (Rooke and Hatfield, 2003). However, the control silage exhibited high temperature peaks, increases in pH, and low aerobic stability during aerobic exposure, suggesting that rehydrated corn grain silage is prone to spoiling after feedout, which is similar to what is observed for HMC (Taylor and Kung, 2002; Kung et al., 2007).

As expected, LB changed the fermentation profile of silages. A reduction in lactic acid concentration associated with an increase in acetic acid and 1,2-propanediol concentrations are typical responses to the activity of L. buchneri (Oude Elferink et al., 2001; Nishino et al., 2003; Silva et al., 2014). The degradation of lactic acid by L. buchneri serves as a self-protection mechanism against the decline in pH mediated by lactic acid, permitting the survival of these bacteria for long periods (Oude Elferink et al., 2001). This fact explains the increase of 89.6% in LAB counts in silages inoculated with LB compared with the control at silo opening. The silages inoculated with LB contained higher levels of propionic acid and 1-propanol compared with the control, probably because of the fermentation of 1,2-propanediol to propionic acid and 1-propanol by Lactobacillus diolivorans (Krooneman et al., 2002).

Silages with LB have a high concentration of 2,3-butanediol and low concentrations of ethanol and lactic acid.
The concentration of 2,3-butanediol has been found to be negatively correlated with ethanol and lactic acid (Daniel et al., 2013). It is possible that the 2,3-butanediol pathway becomes significant for electron acceptance when ethanol and lactic acid production is suppressed. The high concentration of 2,3-butanediol may be associated with the high LAB count in the silages inoculated with LB, because 2,3-butanediol is produced by bacteria (enterobacteria, LAB, and bacilli; McDonald et al., 1991). Although clostridia also produce 2,3-butanediol (Siemerink et al., 2011), it is unlikely that clostridia thrived in these silages, as suggested by the low content of butyric acid. Esters with low molecular weight are formed in silage by esterification of an alcohol with a carboxylic acid. Thus, the content of alcohols (e.g., ethanol) and carboxylic acids (e.g., lactic and acetic acids) has been found to be correlated with esters in silage (Weiss et al., 2016). Similarly, in our study, the concentration of ethyl lactate was lower in silages inoculated with LB as a result of the reduced concentration of ethanol. However, ethyl acetate showed the opposite trend, likely because the higher content of acetic acid in silages inoculated with LB.

Due to the predominance of heterolactic fermentation with concomitant formation of CO₂ (Driehuis et al., 1999, 2001), LB increased DM losses during fermentation. The highest pH values for silages treated with LB.
can be explained by the conversion of lactic acid (acid dissociation constant $pK_a = 3.86$) to acetic acid ($pK_a = 4.76$), which has a low acidification power (Pahlow et al., 2003). Acetic acid and propionic acid possess high antifungal activity (Kleinschmit et al., 2005; Tabacco et al., 2011). A decrease in the number of yeasts leads to a reduction in ethanol concentration in silages inoculated with LB, because yeast ferment sugars to ethanol in anaerobic environments (Rooke and Hatfield, 2003). The presence of large amounts of acetic acid in the LB silages reduced the yeast count, which persisted throughout the period of aerobic exposure, resulting in greater aerobic stability compared with the control silage. We observed other parameters that indirectly demonstrated the highest aerobic stability of silages treated with LB such as low counts of molds, stable pH, and low peak of temperature during aerobic exposure, as previously described by Kung et al. (2007), Reis et al. (2008), and Basso et al. (2012) for HMC.

In general, the fermentation process of rehydrated corn grain silage remained unchanged by inoculation with homofermentative bacteria, but acetic acid concentration was significantly decreased by LPPA. Therefore, silages treated with LPPA showed the lowest aerobic stability and highest temperature peaks. Kleinschmit et al. (2005) also showed a reduction in aerobic stability of silage inoculated with homofermentative bacteria. The lower concentration of antifungal compounds capable of preventing the growth of spoilage microorganisms in silages treated with LPPA may have led to the increase in yeasts and molds during the first days of aerobic exposure.

The increase in N-NH$_3$ concentration in silages treated with LB was associated with a decrease in prolamin concentration and consequent improvement of ruminal in situ DM degradability at 12 and 24 h of incubation. As starch is the main component of corn kernel, and starch digestibility is strongly correlated with corn digestibility (Zinn et al., 2002), the greater ruminal in situ DM degradability was likely triggered by higher starch availability, which in turn was induced by proteolysis during silage fermentation. The protein matrix surrounding the starch granules consists of a hydrophobic layer, which is responsible for the reduced digestibility of starch granules, especially in vitreous portions of corn endosperm (Owens et al., 1986; Correa et al., 2002). The prolamins can be broken down during ensiling by enzymes of the plant and silage microorganisms through proteolysis and solubilization by acids produced during the fermentation process (Hoffman et al., 2011). The main factor responsible for proteolysis in rehydrated corn grain silage is the proteolytic activity of bacteria; kernel enzymes, fungi, and fermentation products contribute less (Junges et al., 2017).

![Figure 3](image-url)

**Figure 3.** Ruminal in situ DM degradability (%) after 12 and 24 h of incubation of rehydrated corn grain silage without inoculant (control, C) and inoculated with *Lactobacillus buchneri* (LB; NCIMB 40788) at a dose of $1 \times 10^5$ cfu/g of rehydrated corn grain (D1), $5 \times 10^5$ cfu/g (D2), or $1 \times 10^6$ cfu/g (D3), or with a combination of *Lactobacillus plantarum* (MA18/5U) and *Pediococcus acidilactici* (MA18/5M) (LPPA) at a dose of $1 \times 10^6$ cfu/g of rehydrated corn grain (D1), $5 \times 10^5$ cfu/g (D2), or $1 \times 10^6$ cfu/g (D3) after 124 d of fermentation. SEM = 1.13 at 12 h; SEM = 1.32 at 24 h. Effect of contrasts for 12 h of incubation: Control vs. LPPA, $P = 0.06$; Control vs. LB, $P < 0.01$; linear at the LPPA doses, $P = 0.79$; quadratic at the LPPA doses, $P = 0.70$; linear at the LB doses, $P = 0.07$; quadratic at the LB doses, $P = 0.71$. Effect of contrasts for 24 h of incubation: Control vs. LPPA, $P = 0.16$; Control vs. LB, $P = 0.02$; linear at the LPPA doses, $P = 0.50$; quadratic at the LPPA doses, $P = 0.63$; linear at the LB doses, $P = 0.89$; quadratic at the LB doses, $P = 0.57$. Error bars indicate SEM for 12 and 24 h.
Therefore, it is possible that the type of microorganism added in the silage modifies the intensity of proteolysis. Ferraretto et al. (2015) suggested that solubilization may not be the primary mechanism for reducing zein degradation during ensiling, and Hoffman et al. (2011) indicated proteolysis to be the main mechanism responsible for prolamin degradation. However, Hoffman et al. (2011) found no differences in zein degradation in HMC treated with different inoculants. We did not observe any differences in prolamin concentration, N-NH$_3$, or ruminal in situ DM degradability after 12 and 24 h of incubation in silages inoculated with LPPA. On the other hand, LB induced prolamin degradation and, in turn, increased ruminal in situ DM degradability. Although the mechanism is unknown, LB may have benefited other proteolytic microorganisms, because LAB have low proteolytic activity (McDonald et al., 1991). Other authors reported an increase in N-NH$_3$ in silages inoculated with LPPA. On the other hand, LB induced prolamin degradation and, in turn, increased ruminal in situ DM degradability. Hence, it is possible that the type of microorganism added in the silage modifies the intensity of proteolysis.

Inoculation with *Lactobacillus buchneri* at a dose of 1 × 10$^5$ cfu/g is a feasible strategy to increase the aerobic stability of rehydrated corn grain silage. Treating rehydrated corn grain with homolactic bacteria did not alter the fermentation process and decreased the aerobic stability of silage. The results of increased ruminal in situ DM degradability in silages treated with LB are promising. It would be interesting to evaluate the effects of inoculating rehydrated corn grain silage with *L. buchneri* in long- and short-term storage and its effects on animal performance. Likewise, further studies are warranted to confirm these conclusions in other corn hybrids, silage inoculants, and their combinations.

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**REFERENCES**


