

# Effect of rehydration with whey and inoculation with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* on the chemical composition, microbiological dynamics, and fermentative losses of corn grain silage

## Efeito da reidratação com soro de leite e inoculação com *Lactobacillus plantarum* e *Propionibacterium acidipropionici* sobre a composição química, dinâmica microbiológica e perdas fermentativas da silagem de grão de milho

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### Highlights:

Whey has the potential to be used in the rehydration of silage of grain corn.  
Rehydration with whey and inoculation improves microbiological dynamics.  
Corn grain silages rehydrated with whey and inoculant show less fermentative losses.  
Rehydration with whey is a strategy that avoids its disposal in the environment.

### Abstract

The objective of this study was to compare rehydrated corn grain silages using water or whey and inoculated (*Lactobacillus plantarum* and *Propionibacterium acidipropionici*) or not. We also verified whether rehydration with whey associated with the bacterial inoculant improves material conservation. The treatments were as follows: silages rehydrated with water without inoculant (SWa); silages rehydrated with water and inoculated (SWaI); silages rehydrated with whey without inoculant (SWe); silages rehydrated with whey and inoculated (SWeI). A completely randomized design was used, with three replications, treatments in a 2 × 2 factorial scheme (RE: rehydration with water or whey, and IN: inoculation or addition of water without chlorine), during the following storage periods (T): 0, 4, 8, 16, 32, and 64 days. There was an RE × IN × T interaction (P < 0.001) for dry matter (DM), with lower values over time for SWe and higher values in SWeI. An interaction between RE × IN (P < 0.001) and IN × T (P < 0.001) was observed for pH, with higher values for SWe at 64 days of storage and a reduction

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from the first days of ensiling for SWaI and SWeI. The microbiological variables showed an RE x IN x T interaction ( $P < 0.001$ ), with the highest counts of lactic acid bacteria for SWaI and SWeI up to 8 days of storage and subsequent higher counts in SWa and SWe. Higher counts were obtained in SWe. For the variables of fermentative losses, there was an RE x IN x T interaction ( $P < 0.001$ ), with SWeI showing lower losses. The inoculation associated with whey for rehydration of corn grain improved the fermentation profile of the silage, with lower pH values and reduced losses.

**Key words:** Lactic acid bacteria. Propionic acid bacteria. Fermentative profile. Grain silage.

## Resumo

O objetivo desse estudo foi comparar as silagens de grão de milho reidratado utilizando água ou soro de leite e inoculados (*Lactobacillus plantarum* e *Propionibacterium acidipropionici*) ou não, e verificar se a reidratação com soro associada ao inoculante bacteriano melhora a conservação do material. Os tratamentos foram: silagens reidratadas com água sem inoculante (SA); silagens reidratadas com água com inoculante (SAI); silagens reidratadas com soro de leite inoculante (SS); silagens reidratadas com soro de leite com inoculante (SSI). Foi utilizado um delineamento inteiramente casualizado, com três repetições, tratamentos em esquema fatorial  $2 \times 2$  (RE: reidratação com água ou soro de leite, e IN: inoculação ou adição de água sem cloro), durante os períodos de estocagem (T): 0, 4, 8, 16, 32 e 64 dias. Houve interação RE x IN x T ( $P < 0,001$ ) para variável matéria seca (MS) com menores valores ao longo do tempo para SS e valores mais elevados em SSI. Foi observada interação RE x IN ( $P < 0,001$ ) e IN x T ( $P < 0,001$ ) para pH, com maiores valores para SS aos 64 dias de estocagem e redução desde os primeiros dias de ensilagem para SAI e SSI. As variáveis microbiológicas apresentaram interação RE x IN x T ( $P < 0,001$ ), sendo a maior contagem de bactérias ácido láticas para SAI e SSI até os 8 dias de estocagem e após com maiores contagens em SA e SS; maiores contagens foram obtidas em SS. Para as variáveis de perdas fermentativas houve interação RE x IN x T ( $P < 0,001$ ), em geral, com SSI apresentando menores perdas. A inoculação associada ao uso de soro para reidratação de grão de milho melhorou o perfil fermentativo da silagem, com menores valores de pH e redução das perdas.

**Palavras-chave:** Bactérias ácido láticas. Bactérias ácido propiônicas, Perfil fermentativo. Silagem de grão.

## Introduction

Corn grain is one of the main ingredients used in diets for confined cattle (Oliveira & Millen, 2014), accounting for a considerable fraction of the food cost. Processing techniques to improve the efficiency in the use of the starch contained in the corn grain can decrease the production costs. This especially the case for hard corn, which is most commonly used in tropical regions, because of the greater vitreousness of the starch granule (Carareto et al., 2011; Arcari, Martins, Tomazi, & Santos, 2016). Ensilaging of the corn grain harvested at physiological maturity increases the breakdown of proteins (prolamines) that surround the starch granules, making them more accessible for fermentation by rumen bacteria (Carvalho et al., 2017; Silva et al., 2018). However,

in certain circumstances, there may be a delay in harvest, affecting the quality of the silage. Low moisture concentrations restrict fermentation, causing the deterioration of the ensiled material (Windle, Walker, Kung, 2014).

Correcting the humidity of the harvested and ground material to values close to 35% through the inclusion of water can improve fermentation (Rezende et al., 2014). To achieve this, whey can be used as it is rich in soluble proteins, lactose, minerals, and vitamins, contributing to the fermentation phase and improving the nutritional value of silage (Rezende et al., 2014; El-Shewy, 2016). In addition, serum could also be used for this purpose, as it is a potential cause of environmental damage and cannot be disposed of directly in ponds and rivers.

The use of inoculants with optional heterofermentative bacteria such as *Lactobacillus plantarum*, which are efficient in the formation of lactic acid and which produce acetic acid under specific conditions, can help reduce the loss of dry matter during ensiling by providing a rapid lowering of the pH (Amaral et al., 2020). *Propionibacterium acidipropionici* bacteria can convert lactic acid to acetic and propionic acid, both effective in controlling fungi and yeasts in ensiled material (Cardoso, Ribeiro, Marcondes, Pereira, & Weiß, 2019). Thus, the association of *Lactobacillus plantarum* and *Propionibacterium acidipropionici* can improve the fermentation profile and produce high-quality silage. However, there are few studies combining the use of microbial additives in ground grain silages with rehydration with whey, making further investigations necessary.

We hypothesized that silages rehydrated with whey and inoculated with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* will show a rapid decline in the pH of the medium due to the greater availability of substrates and the greater production of acids associated with the inoculated bacteria, resulting in less proliferation of undesirable microorganisms and, consequently, in lower fermentative losses. The aim of this study was to compare corn silages rehydrated with water or whey, inoculated or not with *Lactobacillus plantarum* and *Propionibacterium acidipropionici*, and to verify whether rehydration with serum associated with the bacterial inoculant improves the conservation of ensiled material.

## Material and Methods

### Silage process

The experiment was conducted at the Federal Institute of Education, Science and Technology of the Southeast of Minas Gerais, Campus Rio Pomba, and the analyses were performed at the Animal Nutrition Laboratory of the Zootechnics Department. Commercial corn DKB 390 Pro 2, Dekalb, Uberlândia, Brazil (Flint texture), was used, with a moisture content of 13% (considered dry), and subjected to an electric hammer disintegrator (MFW®, model T0 2) with a 4 mm sieve.

The silages were prepared manually in PVC silos with a diameter of 0.1 m, a height of 0.4 m, and a volume of  $3.14 \times 10^{-3} \text{ m}^3$ , equipped with “Bunsen” valves to allow the free escape of fermentation gases; compaction was carried out with a wooden stick and reached an average density of  $1,003 \pm 23 \text{ kg m}^3$  of natural matter. At the bottom of each silo, 800 g of dry sand were placed in bags made of non-woven fabric (TNT) to capture the effluent generated during the process; a nylon screen was used to separate the ensiled material from the effluent collection area.

The amount of water or whey necessary to achieve 35% moisture reconstitution was calculated according to Ferreira (1983). The whey used in the experiment is the result of enzymatic coagulation of milk (cheese making), obtained from the Department of Food Science and Technology of the Federal Institute of Education, Science and Technology of the Southeast of Minas Gerais, Campus Rio Pomba. The serum was homogenized and analyzed in the department’s Physical-Chemical Analysis Laboratory (Table 1).

**Table 1**  
**Characteristics of whey (natural matter)**

Item	Whole whey
Density, g/L at 15°C	1028.23
pH	5.37
Dry matter, %	8.89
Crude protein, %	0.94
Ether extract, %	0.21
Mineral matter, %	1.03

The ground corn was mixed manually and received water from an artesian well or whey at room temperature, reaching a moisture content of 35%; some samples received inoculant and were constantly mixed to ensure uniformity of the material. We investigated four different treatments: silages rehydrated with water without inoculant (SWa); silages rehydrated with water using inoculant (SWal); silages rehydrated with whey without inoculant (SWe); silages rehydrated with whey using an inoculant (SWeI). A completely randomized design was used with three replications and four treatments in a  $2 \times 2$  factorial scheme, using the following factors: RE: rehydration with water or whey (correcting for  $350 \text{ g kg}^{-1}$  of moisture) and IN: inoculation with *Lactobacillus plantarum* ( $3.0 \times 10^{10}$  CFU  $\text{g}^{-1}$ ) together with *Propionibacterium acidipropionici* ( $3.0 \times 10^{10}$  CFU  $\text{g}^{-1}$ ) or only adding chlorine-free water during the following storage periods (T): 0, 4, 8, 16, 32, and 64 days. The inoculant (LALSIL® Milho) was homogeneously solubilized in water without chlorine at the time of mixing, using the dosage recommended by the manufacturer for silage of rehydrated grains of  $5 \text{ g ton}^{-1}$ , with the following composition: *Lactobacillus plantarum* MA 18/5U ( $3.0 \times 10^{10}$  CFU  $\text{g}^{-1}$ ), *Propionibacterium acidipropionici* MA 26/4U ( $3.0 \times 10^{10}$  UFC  $\text{g}^{-1}$ ), and sucrose. The silos were kept in a covered area at room temperature.

#### *Microbiological dynamics*

To a sample of 25 g of silage, 225 mL of Ringer's solution were added, and the mixture was homogenized in an industrial blender for 1 minute, obtaining a  $10^{-1}$  dilution. Subsequently, successive dilutions were performed to obtain dilutions ranging from  $10^{-1}$  to  $10^{-7}$  for the cultivation in sterile Petri dishes. We used the pour-plating technique; plates with values between 30 and 300 colony-forming units (UFC) were considered countable. For the counting of lactic acid bacteria (BAL), plating was performed on MRS Agar (Fluka Analytical), and the plates were incubated at 35°C for 72 hours. The number of enterobacteria (ENT) was determined by plating on Violet Red Bile Glucose Agar (Fluka Analytical), incubated at 35°C for 48 hours. Fungi and yeasts were determined by plating on Potato Dextrose Agar (Acumedia), acidified with 10% (w / v) tartaric acid, after autoclaving and incubated at 25°C for a period of 5 days (Cherney & Cherney, 2003).

#### *Chemical composition and fermentative losses*

The pre-ensiled material and the silages (both in triplicate) were taken to a forced ventilation oven at 55° C for 72 hours, using 800 g per sample, to determine dry matter (DM). Subsequently, the samples were ground in a Willey mill with a 1-mm sieve for the determination of crude protein (CP),

ether extract (EE), and mineral matter (MM); the pH was analyzed using fresh samples without grinding, according to the Association of Official Analytical Chemists [AOAC] (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest, Robertson, & Lewis (1991), and hemicellulose (HEM) was calculated as the difference between NDF and ADF. Dry matter losses (DML), gaseous losses (GL), and effluent losses (EL), as well as dry matter recovery (DMR), were estimated according to the methodology described by Jobim, Nussio, Reis and Schmidt (2007).

### Statistical analysis

Data were analyzed as repeated measures over time, using PROC MIXED in SAS according to the following model:

$$Y_{ijk} = \mu + RE_i + IN_j + RE \times IN_{ij} + T_k + A_{j:i} + RE \times T_{ik} + IN \times T_{jk} + RE \times IN \times T_{ijk} + e_{ijk},$$

in which fixed effects are  $\mu$  = general average; RE = type of rehydration (water; whey);  $IN_j$  = inoculant (without; with *L. plantarum* ( $3.0 \times 10^{10}$  CFU  $g^{-1}$ ), and *P. acidipropionici* ( $3.0 \times 10^{10}$  CFU  $g^{-1}$ ); RE  $\times$   $IN_{ij}$  = interaction between type of rehydration and inoculants;  $T_k$  = storage times for silos (4, 8, 16, 32 and 64 days), time "0" was included in certain variables; RE  $\times$   $T_{ik}$  = interaction between type of rehydration and storage times;  $IN \times T_{jk}$  = interaction between inoculants and storage time; RE  $\times$   $IN \times T_{ijk}$  = interaction between type of rehydration, inoculants and storage time; and random effects of  $A_{j:i}$  (nested silos in treatment); and  $e_{ijk}$  = experimental error.

Storage time was included as the option of SAS REPEATED, with the silo was included as subject. Several covariance-to-error structures were investigated, and the structure that best fitted the data according to the lowest value of the Bayesian information criterion (BIC) was selected. The following covariance structures were tested for all

variables: UN, UN (1), CS, CSH, TOEP, AR, VC, TOEP (2), ARH (1), ARMA, FA (1), HF, ANTE (1), TOEPH and UNRA covariance structure VC was selected for pH, fungi and yeasts, EL, DM, CP, MM, NDF, and ADF. The HF was selected for LAB, enterobacteria, DML, GL, DMR, and EE. For hemicellulose, ARH (1) was selected. The results were considered significant at the level of  $P < 0.05$ . The data were analyzed in PROC MIXED of the SAS program (SAS Inst. Inc., Cary, NC), with previous proof of normal distribution (Shapiro-Wilk test) and homoscedasticity of variances (Bartlett test) with a significance level of  $P < 0.05$ .

### Results

The results obtained for chemical composition, microbiological dynamics, and fermentative losses are shown in Table 2. An interaction was observed between the studied factors (RE  $\times$  IN;  $P < 0.001$ ) for the pH of the silage, with higher values observed in silages rehydrated with milk without the use of inoculant (5.08) and lower values in silages rehydrated with water without inoculant (4.94), silages rehydrated with water using inoculant (4.84), and silages rehydrated with whey using inoculant (4.78). There also was an interaction between the inoculant (IN) and time (T) factors for the pH values ( $P < 0.001$ ), where inoculation promoted a more pronounced drop at the beginning and maintenance of the lowest values throughout the conservation, reaching values of 4.34 and 4.01 for treatments without and with *Lactobacillus plantarum* and *Propionibacterium acidipropionici*, respectively, at 64 days of storage (Figure 1).

The DM concentration of the silage showed interaction between the factors evaluated (RE  $\times$  IN  $\times$  T) ( $P < 0.001$ ), in which silages rehydrated with whey without inoculant showing the lowest values over time (Figure 2, A). Less pronounced reductions were observed in silages rehydrated with whey using an inoculant.

There were no interactions between the factors (RE × IN) and time for the concentrations of crude protein (CP), ether extract (EE), mineral matter (MM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (HEM) ( $P \geq 0.054$ ). However, CP increased ( $P < 0.01$ ) due to rehydration with serum from 86.81 to 88.04 g kg<sup>-1</sup>. There was also a time effect on the concentrations of CP

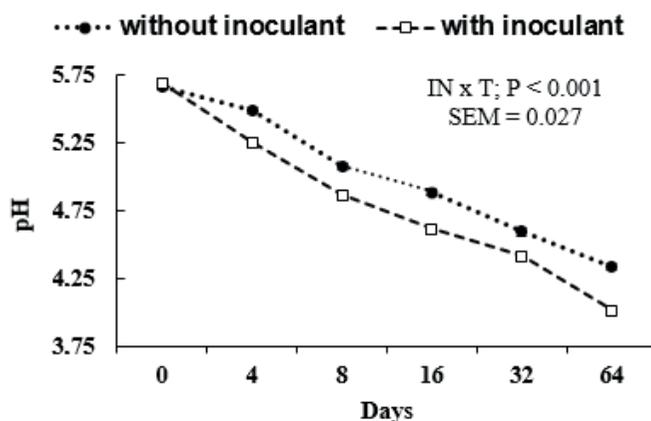
(Figure 2, B), NDF (Figure 2, C), and HEM (Figure 2, D) ( $P \leq 0.001$ ), where CP decreased over time from 90, 28 to 86.58 g kg<sup>-1</sup> in the storage periods from 0 to 64 days, respectively, while NDF and HEM had a pronounced reduction until day 4 from 136.15 to 133.05 g kg<sup>-1</sup> of the NDF and 100.39 to 97.20 g kg<sup>-1</sup> of HEM, with subsequent concentration stabilization.

**Table 2**

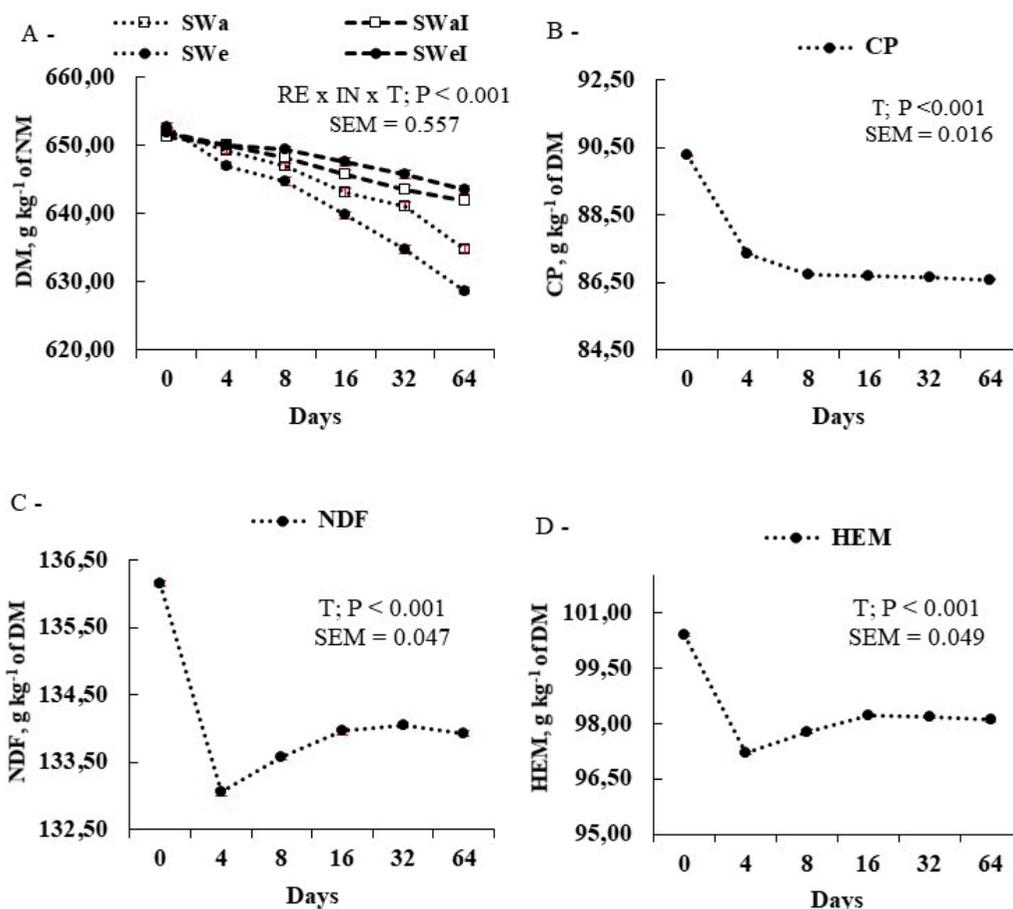
**Chemical composition, microbiological dynamics and fermentative losses of corn grain silages rehydrated with water or whey, using or not inoculating with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* and stored for 0, 4, 8, 16, 32, and 64 days**

RE	Water		Whey		SEM	P-value							
	IN	Without	With	Without		With	RE	IN	T	RE x IN	RE x T	IN x T	RE x IN x T
pH		4.94	4.84	5.08	4.78	0.015	0.008	<0.001	<0.001	<0.001	0.309	<0.001	0.115
Chemical composition, g kg <sup>-1</sup> of DM													
DM <sup>1</sup>		64.46	64.68	64.13	64.81	0.227	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	<0.001
CP		87.10	86.61	87.90	88.09	0.013	<0.001	0.106	<0.001	0.064	0.992	0.630	0.990
EE		35.11	35.62	35.46	35.24	0.028	0.650	0.523	0.622	0.150	0.770	0.688	0.560
MM		14.97	14.94	15.10	15.09	0.013	0.427	0.554	0.514	0.919	0.532	0.106	0.105
NDF		134.11	134.48	134.66	133.42	0.038	0.426	0.286	<0.001	0.054	0.284	0.707	0.311
ADF		35.81	35.88	35.90	35.84	0.009	0.756	0.516	0.964	0.843	0.994	0.977	0.988
HEM		98.30	98.60	98.76	97.58	0.039	0.400	0.385	<0.001	0.070	0.275	0.789	0.291
Microbiological dynamics, log CFU g <sup>-1</sup>													
LAB		6.10	6.02	6.28	5.97	0.020	0.002	<0.001	<0.001	<0.001	0.011	<0.001	<0.001
ENT		2.68	2.31	2.96	2.24	0.019	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MOFO		4.51	4.18	4.85	4.07	0.015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fermentative losses, g kg <sup>-1</sup> of DM													
DML		14.48	11.11	24.15	9.93	0.284	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	<0.001
GL		12.32	9.62	21.23	8.71	0.235	<0.001	<0.001	<0.001	<0.001	0.036	<0.001	<0.001
EL <sup>2</sup>		2.12	1.50	2.98	1.23	0.058	<0.001	<0.001	<0.001	<0.001	0.286	<0.001	0.098
DMR		985.52	988.89	975.85	990.07	0.284	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001

<sup>1</sup>Units in g / kg of natural material. <sup>2</sup>Units in kg / ton. of natural matter. RE: Rehydration; IN: Inoculant; DM: Dry matter; CP: Crude protein; EE: Ethereal extract; MM: mineral matter; NDF: neutral detergent fiber; FDA: Fiber in acid detergent; HEM: Hemicellulose; BAL: Lactic acid bacteria; ENT: Enterobacteria; MOFO: Fungi and yeasts; PMS: Loss of dry matter; GL: Gas loss; EL: Effluent loss; DMR: Dry matter recovery; SEM: Standard error of the mean. RE: effect of the rehydration source; IN: inoculant effect; T: effect of the storage period; RE × IN: interaction between source of rehydration and inoculant; RE × T: interaction between source of rehydration and storage period; IN × T: interaction between inoculant and storage period; RE × IN × T: interaction between rehydration source, inoculant and storage period.



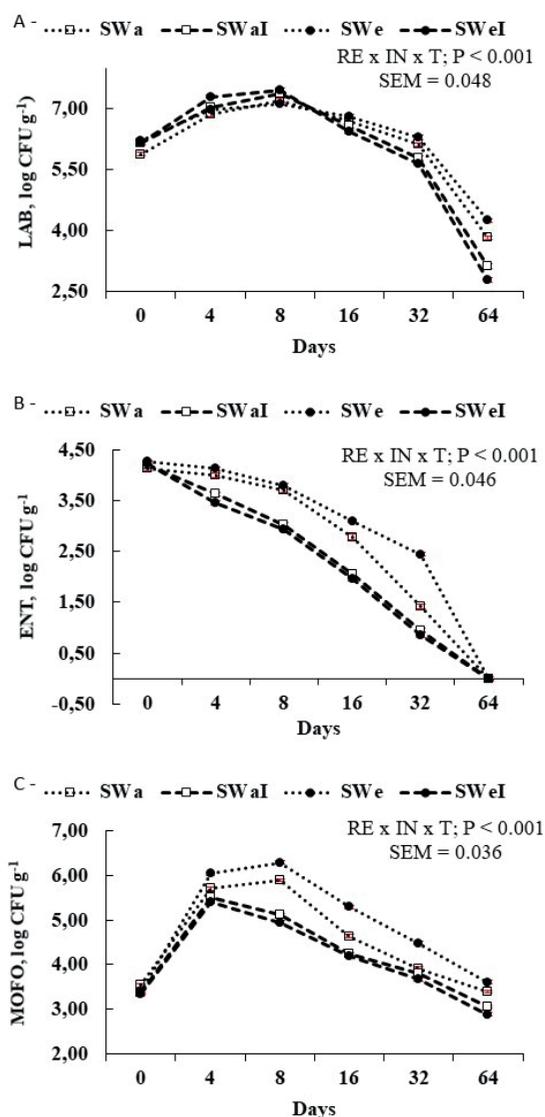
**Figure 1.** pH values of corn grain silages rehydrated with water or whey, with or without *Lactobacillus plantarum* and *Propionibacterium acidipropionici* and stored for 0, 4, 8, 16, 32, and 64 days.



**Figure 2.** Dry matter content (A), crude protein (B), NDF level (C), and hemicellulose content (D) of corn grain silages rehydrated with water or whey, with or without *Lactobacillus plantarum* and *Propionibacterium acidipropionici* and stored for 0, 4, 8, 16, 32, and 64 days. Silages rehydrated with water without inoculant (SWa), silages rehydrated with water with inoculant (SWaI), silages rehydrated with whey without inoculant (SWe), silages rehydrated with whey with inoculant (SWeI).

In relation to microbiological dynamics, an interaction was observed between the factors studied (RE × IN × T) for lactic acid bacteria (LAB), enterobacteria (ENT), and fungi and yeasts (MOFO) ( $P \leq 0.001$ ). The LAB values were higher up to 8 days of storage in silages rehydrated with water using inoculants and in silages rehydrated with whey using inoculants. Subsequently, there was an inversion, with the highest LAB values for silages rehydrated with water without inoculants

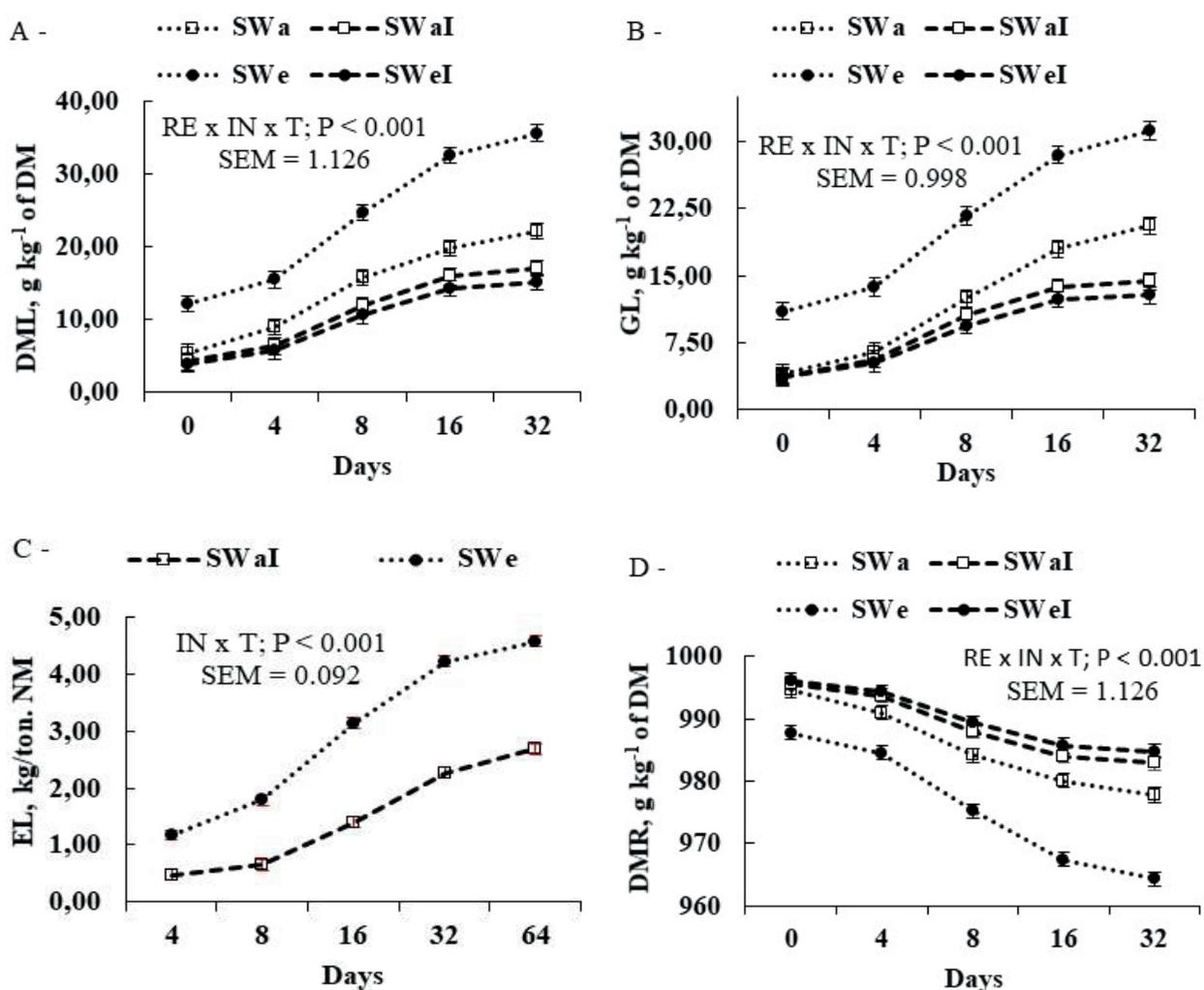
and silages rehydrated with whey without inoculants due to the decline in LAB (Figure 3, A). After the peak, with the use of the inoculant, the values of ENT (Figure 3, B) and MOFO (Figure 3, C) were lower over the storage time for silages rehydrated with water using inoculants and silages rehydrated with whey using inoculants; the highest ENT and MOFO counts were observed in silages rehydrated with whey without inoculant, followed by silages rehydrated with water without inoculant.



**Figure 3.** Counts of lactic acid bacteria (A), enterobacteria (B), and fungi and yeasts (C) of corn grain silages rehydrated with water or whey, with or without *Lactobacillus plantarum* and *Propionibacterium acidipropionici* and stored for 0, 4, 8, 16, 32, and 64 days. Silages rehydrated with water without inoculant (SWa), silages rehydrated with water with inoculant (SWaI), silages rehydrated with whey without inoculant (SWe), silages rehydrated with whey with inoculant (SWeI).

For fermentative losses, interactions were verified between the factors (RE × IN × T) ( $P \leq 0.001$ ) for the variables dry matter losses (DML), gas losses (GL), and dry matter recovery (DMR). Throughout the experimental period, DML (Figure 4, A) and GL (Figure 4, B) were higher for silages rehydrated with whey without inoculants, with lower RMS values. In contrast, silages rehydrated with whey and inoculants showed lower DML and GL values, with higher DMR values (Figure 4, D). There was an interaction between the factors (RE and IN;  $P \leq$

0.001) for effluent losses (EL) (Figure 4, C), and the lowest losses were verified in inoculated silages, with a lower value for silages rehydrated with whey and inoculant. The highest EL values were obtained in silages rehydrated with whey without inoculant. There was also an interaction between the IN factor and T for EL ( $P \leq 0.001$ ), where the inoculated treatments had a lower PE until 8 days of storage; subsequently, the value remained below those of the other treatments until the end of the experiment.



**Figure 4.** Dry matter losses (A), gas losses (B), effluent losses (C), dry matter recovery (D) of corn grain silages rehydrated with water or whey, with or without *Lactobacillus plantarum* and *Propionibacterium acidipropionici* and stored for 0, 4, 8, 16, 32, and 64 days. Silages rehydrated with water without inoculant (SWa), silages rehydrated with water with inoculant (SWaI), silages rehydrated with whey without inoculant (Sw), silages rehydrated with whey with inoculant (SWeI).

## Discussion

The increase in the concentration of acids, mainly acetic and propionic acids, favors the beginning of the pH reduction inside the silo and directly influences the population dynamics of bacteria. Given this, homofermentative bacteria can emerge, which are more efficient in the production of lactic acid, promoting a faster drop in pH and ensuring favorable conditions for the conservation of the ensiled mass (Mombach, Pereira, Santos Pina, Pereira, & Bolson, 2018). In this way, *Lactobacillus plantarum* is characterized as an optional heterofermentative bacterial species with efficient lactic acid production, providing a reduction in pH. *Propionibacterium acidipropionici* is a heterofermentative bacterium that efficiently produces propionic acid, acting in the control of fungi and yeasts and maintaining low pH values (Yitbarek & Tamir, 2014; Michel et al., 2017). For better nutrient conservation, the pH should reach values between 3.8 and 4.2 (McDonald, 1981). In the present study, at 64 days of storage, only the silage rehydrated with whey without inoculant did not present values within this ideal range, indicating a higher number of undesirable microorganisms in storage, consequently, greater losses.

Whey contains high levels of soluble carbohydrates (lactose) (Nishanthi, Vasiljevic, & Chandrapala, 2017). It is possible to consider that lactic acid bacteria use the soluble carbohydrates of whey for their growth, resulting in a greater production of lactic acid and in a lower pH value.

Lactic acid bacteria are classified into three groups: homofermentative, mandatory heterofermentative, and facultative heterofermentative. Homofermentative bacteria use the Embden-Meyerhof-Parnas pathway, with the final fermentation product being only lactic acid and metabolizing hexoses. Mandatory heterofermentatives use the 6-phosphogluconate/phosphoquetolase pathway, resulting in 1 mol of lactic acid, 1 mol of CO<sub>2</sub>, and 1 methanol or acetic

acid. Furthermore, pentoses can only be metabolized by heterofermentative pathways, while optional heterofermentatives can use homofermentative and heterofermentative pathways, use hexoses and, under specific conditions, can ferment pentoses (Vinderola, Ouwehand, Salminen, & Von Wright, 2019).

The change in the profile of lactic acid bacteria in which the mandatory heterofermentatives start fermentation is the replacement by homofermentatives (Vinderola et al., 2019). In the first 60 days after ensiling, homofermentatives dominate; afterwards, facultative heterofermentatives start to gain importance due to the greater resistance to low pH levels and the reduction of the substrate for homofermentatives (Zhou, Drouin, & Lafrenière, 2016).

The ideal pH for the growth of enterobacteria is between 6.0 and 7.0; they do not develop at pH levels below 5.0 (Muck, 2010; Gharechahi et al., 2017) and are inhibited by stable temperatures and low humidity (Yitbarek & Tamir, 2014). Still, lactic acid bacteria compete for substrate with undesirable microorganisms such as fungi, bacteria of the genus *Clostridium*, and enterobacteria at the beginning of the fermentation process (Yitbarek & Tamir, 2014).

The stable phase of the silage occurs at the moment when the ensiled mass reaches the pH necessary to reduce and control microbial activities (Anjos et al., 2018); otherwise, there will be a possibility of breaking stability (Pires et al., 2013). There is evidence of changes in the nutritional quality of the ensiled material during the conservation period due to the presence of microorganisms resistant to low pH values, such as yeasts (Leão et al., 2017). The bacterium *Propionibacterium acidipropionici* can ferment sugars and lactate to acetate and propionate; these short-chain aliphatic acids inhibit yeast growth (Cardoso et al., 2019).

Working with wet corn gluten bran, França et al. (2015) observed that a longer ensiling period reduced the yeast and fungus counts, decreasing

linearly from 7.2 to 5.5 log UFC g<sup>-1</sup> of silage between 1 and 42 days of ensiling, due to the reduction of the concentration of oxygen in the silo. This is because the counts of fungi and yeasts during the first 8 days of silage are related to the epiphytic population in the material to be ensiled (Ávila, Bravo Martins, & Schwan, 2010). Borreani and Tabacco (2010) observed higher counts of fungi and yeasts in the peripheral regions of silos, areas with higher concentrations of oxygen.

One of the reasons for greater dry matter losses is the loss of gas and effluent, resulting in nutrient losses (Ferraretto, Crump, & Shaver, 2013). The lower the dry matter losses, the greater the dry matter recovery of the ensiled material. When comparing the values of gas losses, Rabelo et al. (2012), working with corn silage at different stages of maturity, observed a small variation in losses by effluents (12.2 to 13.5 kg t<sup>-1</sup> of natural matter) due to bacterial inoculation; effluent losses were influenced by the humidity of the ensiled material. Therefore, in our study, the recommended humidity was 35%, preventing high effluent losses.

The formation of gas in the silage is the result of secondary fermentation promoted by enterobacteria, *Clostridium* species, and aerobic microorganisms, which normally grow in environments with a higher pH (Muck, 2010). The reduction of gas losses is probably due to the reduction of the action of these gas-producing microorganisms that develop in the silage under unfavorable conditions, gases from the conversion of proteins and carbohydrates by microorganisms that produce several components from them, such as organic acids, ethanol, water, ATP, and CO<sub>2</sub> (Edvan et al., 2013; Rigueira, Pereira, Ribeiro, Mantovani, & Agarussi, 2013). According to Muck (2010), several species of enterobacteria use nitrate as an electron acceptor instead of oxygen, reducing nitrate to nitrite or even nitrogen oxide, which has a low volatilization point, increasing gas losses during the fermentation process.

Bacterial inoculants containing homofermentative bacteria increase the recovery of dry matter during

fermentation due to the use of more efficient metabolic pathways in the production of lactic acid, rapidly reducing silage pH (Muck, 2010). The dry matter contents were higher for silages rehydrated with whey with the use of inoculants due to lower dry matter losses, the greater supply of solid compounds contained in whey in relation to rehydration with water, and mainly because of the improvement with the use of inoculants. The reduction in crude protein can be explained by the increased loss of dry matter through microorganisms, causing the dilution of the protein in dry matter (Campos, Lana, Galvão, Souza, & Tavares, 2017). The contents of NDF and hemicellulose decreased over time, which may be related to the solubilization of the cellulose and hemicellulose fractions. Rezende et al. (2014) observed that the NDF contents were reduced during the fermentation process due to the reduction of hemicellulose, which was consumed during fermentation. Therefore, these differences can be attributed to the biochemical variations that occur during the ensiling process. The reduction in the hemicellulose content can be associated with its acid hydrolysis, which results from the activity of the enzyme hemicellulase, produced by bacteria present in the ensiled material; hydrolysis occurs through the presence of acids produced during fermentation (Araújo et al., 2020).

## Conclusion

The rehydration of corn grains with whey, associated with the use of inoculant (*Lactobacillus plantarum* and *Propionibacterium acidipropionici*), improves the fermentation profile of the silage, with lower pH values and fermentation losses.

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