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Article

Effects of acid whey on the fermentative chemical quality and aerobic stability of rehydrated corn grain silage

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Abstract:

The objective was to evaluate the fermentative, chemical characteristics and aerobic stability of corn grain silages rehydrated with whey fluid (WF) or whey powder (WP) and water, with or without the addition of inoculant (I). The corn grain was ground and hydrated adding water without chlorine and/or whey to reach 35 % humidity and stored in silos of 4.36 kg. After 45 d of fermentation, samples of the silages were submitted to chemical-fermentative analyses in opening of silos and 240 h of exposure to air. The aerobic stability of the silages was evaluating during 240 h has considered to the loss when the temperature of the ensiled mass exceeded the ambient temperature by 2 °C. A reduction in the acid detergent fiber (ADF) and lignin content of the silages was observed with the use of WF and WP. The levels of ammoniacal nitrogen (NH3-N) were the lowest for WF and WP (0.7 and 0.9 g/kg TN) and pH was 4.31 for WF after 240 h of aerobic exposure. The use of inoculants provided higher levels of Ash, ether extract (EE), and low buffering capacity (BC), in addition to reductions in ADF levels. The inoculated silages showed higher levels of NH3-N and pH after 240 h. The silage of corn grains rehydrated with WF provided ideal pH values, low NH3-N content, reduced levels of ADF and lignin, and improved aerobic stability. In addition to being a sustainable alternative, the use of fluid whey to rehydrate corn grains adds nutritional value and improves silage fermentation.

Key words: Acid whey, Byproduct, Corn grain, Inoculant, Silage, Sustainability.

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Introduction

Corn grain is one of the most used energy ingredients in animal feed; furthermore, it can also be subjected to rehydration to be stored as silage. Rehydrated corn grain silage is a strategy used to guarantee the availability of feeds throughout the year(1) decrease logistical costs(2), and minimize the effects of fluctuations in the price of this commodity(3).

The process of milling the dry corn grain and its subsequent rehydration also aims to increase its digestibility, reflecting positively on animal performance(4). In particular, these resources are appropriate since the corn used in most countries is characterized as flint, which has lower digestibility.

Associated with rehydration, the fermentation of grain corn is an interesting process; dry grain is not suitable for ensiling due to its low moisture and sugar content, which results in limited production of total acids(5). Thus, rehydration, commonly conducted with water and aimed at reaching final levels between 35 to 37 % humidity(6,7), is a practical application.

The use of a liquid source with low added value or one that has polluting but non-toxic characteristics can also be used for the rehydration of dry grain corn. Byproducts such as acid whey fluid, which have considerable concentrations of lactic acid bacteria and lactose(8) as well as recognized nutritional value, constitute a suitable example for this purpose, with advantages given to the supply of more nutrients to the silage(9) and an appropriate final destination for this byproduct.

In order to improve fermentation, reduce nutrient losses, and inhibit the growth of undesirable microorganisms(5,9), microbial inoculants composed of homofermentative and heterofermentative bacteria are also incorporated into the ensiled mass, which can prolong the aerobic stability of moist and rehydrated corn grain silages(7,10). Given this context, our hypothesis was that the composition of whey fluid and its microbiological capacity promotes the improvement of silage quality, reduces the use of water for rehydration of grains, and contributes to an appropriate destination for this byproduct. For whey powder, in addition to considering the nutrient load of its composition as a liquid source for rehydration, it is available on a commercial scale for purchase. Finally, the addition of inoculants may help these two liquid sources to improve the fermentative quality and aerobic stability of the silage. Thus, the objective of the present study was to evaluate the effects of rehydration of corn grains with acid whey or whey powder and water, with or without the addition of an inoculant, on the chemical, fermentative characteristics and aerobic stability of the silages.

Material and methods

Corn grain and preparation of silage

The corn grains were obtained from the storage silos of Cooperativa Agropecuária Cocamar®, Londrina, Paraná, Brazil, and their genetic identity is not known. These grains were initially processed in a hammer mill to reach an average particle size of 1.5 mm, and submitted to moisture content evaluation according to the methodology described in AOAC[11], with an average value of 117 g/kg dry matter (DM). The acid whey was obtained from the dairy company Volpato®, in the city of Arapongas, Paraná, Brazil, during the processing of milk for the production of derivatives and was used shortly thereafter in natura to rehydrate the grains. The powdered whey used was purchased from Cooperativa Cativa®, in the city of Londrina, Paraná, Brazil.

The corn grain was ground and subjected to hydration according to each treatment by adding water without chlorine and/or whey to reach 35 % humidity, with or without the addition of an inoculant defining five products, which were incorporated into the dry corn grain corresponding to the experimental treatments: Corn grain silage rehydrated with water (CON); Corn grain silage rehydrated with whey fluid (SWF); Corn grain silage rehydrated with fluid whey, plus inoculant (SWF + I); Corn grain silage rehydrated with water-reconstituted whey powder (SWP); and Corn grain silage rehydrated with powdered whey reconstituted with water, plus inoculant (SWP + I). The microbial inoculant added to the mass to be ensiled was previously diluted to 2.5 mL of the product to 7 L of water without chlorine and/or whey for each 20 kg of ground corn and manually homogenized. The inoculant used was Biotrato SLO® (SLO Biotecnologia & Agropecuária, Cambé, Paraná, Brazil) which consists of *Propionibacterium acidipropionici, Lactobacillus plantarum, Lactobacillus acidophilus, Pediococcus acidilactici, Enterococcus faecium, Lactobacillus buchneri,* and *Lactbacillus curvatus* at a concentration of 70×10^9 UFC/g and 8 % of cellulolytic enzymes.

Once hydrated, the mass of each of the five products was stored in six polyethylene silos with a capacity of 4 L each, determining units with an initial average weight of 4.36 ± 0.17 kg. Compaction was carried out manually, with an average specific density of 1,020 ± 0.04 kg natural matter (NM)/m^3. All silos were sealed with a lid and appropriate plastic tape and stored in a dry and ventilated place for 45 d until the opening date, when they reached a final weight of 4.28 ± 0.20 kg. The experimental design was completely randomized with five treatments and six replications corresponding to each silo.

Chemical analysis

Samples of corn grain prior to ensiling (883 g DM/kg in natural matter (NM), 92.7 g crude protein (CP)/kg DM, 11.5 g Ash/kg DM, 31.8 g ether extract (EE)/kg DM, 126.2 g NDF/kg DM, 25.8 g ADF/kg DM, and 11.3 g lignin/kg DM) and the ensiled mass after opening the silos (Table 1) were evaluated according methodologies to AOAC[(11)], and neutral detergent fiber (NDF) assayed with a heat stable alpha amylase and sodium sulphite (aNDF), acid detergent fiber (ADF) and lignin (lignin (sa)) using sulphuric acid and corrected for ash were evaluated according to the methodology described by Van Soest *et al*[(12)]. The values of total digestible nutrients (TDN) were calculated according to Sniffen *et al*[(13)], total carbohydrates (TCHO) according to the equation proposed by Chandler[(14)], and non-fibrous carbohydrates (NFC) according to Hall[(15)].

The composition of fluid whey before silage was: 60 g DM/kg NM, 865 g CP/kg DM, 3.40 g Ash/kg DM, 3.50 g EE/kg DM, pH 6.30, and acidity of 0.13 for lactic acid. The whey powder showed the following characteristics: 970 g DM/kg NM, 110 g CP/kg DM, 60 g Ash/kg DM, 15 g EE/kg DM, pH 6.30-6.80, and acidity of 0.13 for lactic acid. These analyses followed the procedures described by Zenebon *et al*[(16)]. Samples of the silages for each treatment were collected when the silos were opened to determine the chemical-fermentative composition using a near-infrared spectroscopy system (NIRS DS2500; Foss, Denmark) (Table 2) from the 3rlab® laboratory (Chapecó, Santa Catarina, Brazil). The analysis results shown in Table 2 are only exploratory and descriptive, since it is a characterization of silages without the application of statistical analysis.

Table 2: Chemical-fermentative composition of rehydrated corn grain silages determined by the NIRS system

	Treatment [1]				
Variables [2] (g/kg DM)	**CON**	**SWF**		**SWP**	
		Without	**With**	**Without**	**With**
DM, g/kg NM	652.9	609.4	655.0	641.5	621.7
Moisture	347.1	390.6	344.0	358.5	378.3
CP	97.2	98.5	100.1	100.7	95.1
Protein soluble g/kg CP	496.0	504.8	520.6	504.0	509.5
Protein available	96.2	97.9	99.2	100.1	94.4
ADIP	01.0	00.6	00.9	00.7	00.7
NDIP	02.8	02.2	02.9	02.8	02.3
ADIP g/kg CP	10.0	06.2	09.1	06.6	07.5
ADF	31.3	28.6	31.4	26.0	27.4
NDF	91.0	74.9	87.9	85.5	76.6
aFDNmo	85.4	69.3	82.1	78.7	69.4
Lignin	05.1	04.9	05.1	04.6	04.7
Lignin, g/kg NDF	56.3	64.9	58.5	53.7	61.8
Sugars (carbohydrates soluble in water)	44.5	50.5	49.0	50.4	48.3
Starch	698.8	703.9	688.9	704.7	699.9
Starch, g/kg NFC	919.7	905.1	905.0	916.4	904.4
Digestibility of rumen starch, g/kg starch 0h	466.4	394.1	417.6	354.0	458.1
Digestibility of rumen starch, g/kg starch 7h	701.9	709.2	701.6	686.4	740.7
Lipids	36.2	33.1	35.3	29.7	38.1
Ash	18.5	17.9	18.3	17.8	18.6
Calcium	00.5	00.5	00.5	00.5	00.5
Phosphorus	02.2	02.0	02.0	02.2	02.2
Potassium	03.9	03.7	03.9	04.0	03.8
Magnesium	00.9	00.8	00.9	00.9	00.9
Sulfur	00.9	00.9	00.9	00.9	00.9
Lactic acid	21.5	24.8	26.3	22.2	24.8
Acetic acid	03.2	05.0	03.6	03.0	04.2
Protein equivalent of NH3-N	03.3	03.6	03.1	02.9	03.8
NH3-N, g/kg of CP	33.6	37.0	31.3	28.8	40.1
pH	4.78	3.89	4.13	4.23	4.15
Kd of starch (using 3.7 h) %h	17.00	17.19	16.96	16.29	18.86

[1] Treatment: CON= corn grain silage rehydrated with water; SWF= corn grain silage rehydrated with whey fluid; SWF + I= corn grain silage rehydrated with fluid whey, plus inoculant; SWP= corn grain silage rehydrated with water-reconstituted whey powder; SWP + I= corn grain silage rehydrated with powdered whey reconstituted with water, plus inoculant.

[2]Variables: DM= Dry matter (g/kg of natural matter); ADIP= acid detergent insoluble protein; NDIP= Neutral detergent insoluble protein; ADF= acid detergent fiber; NDF= neutral detergent fiber; aNDFmo= neutral detergent fiber with amylase and expressed excluding residual ash; NH3-N= ammoniacal nitrogen. Kd= fractional rate of degradation. Analyses determined by the laboratory 3rLab.

Fermentative analysis and aerobic stability

To evaluate the fermentation profile of the silages, buffering capacity (BC) and ammoniacal nitrogen (NH3-N) were determined according to the methodology of Playne and McDonald[17] at the opening of the silos and after 240 h of exposure to air (Table 3).

The aerobic stability of the silages was determined using 3 kg of the ensiled mass, homogenized and deposited in the silos according to each treatment, which remained in a closed room exposed on a bench at ambient temperature for 240 h. The ambient (25.43 ± 2.38 °C) and silage temperatures were obtained every 12 h using a digital thermometer (TP101 Xtrad 145 mm; Shenzhen Handsome Techn., Guangdong City, China). The thermometer rod was inserted 10 cm deep in the center of the mass for this measurement. The loss aerobic stability was considered when the temperature of the ensiled mass exceeded the ambient temperature by 2 °C[18].

The pH of the ensiled mass during aerobic exposure was measured using a potentiometer (AZ Temp Meter; AZ Instrument Corp., Taichung City, Taiwan) according to the methodology of Phillip and Fellner[19]. These analyses were delineated in subdivided plots, where the main portion was the treatment and the subplot was the time of aerobic exposure.

Statistical analysis

The data were analyzed according to a completely randomized design using the General Linear Model (PROC GLM) procedure of SAS (see 9.2; SAS Inst. Inc., Cary, NC, USA). Contrasts were used to verify scientific hypotheses using the CONTRAST command, making it possible to compare the impacts of using fluid whey and powdered whey on the investigated variables, as well as comparing the effects of using inoculants with these reconstituting agents. The proposed model was as follows:

$$\mathbf{Y_{ijkl}} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + \xi_{ijkl}$$

where:
$\mathbf{Y}_{ijkl}$ = observed value regarding level i of factor A, combined with level j of factor B and level k of factor C, in repetition l;
$\boldsymbol{\mu}$ = overall average;
$\boldsymbol{\alpha}_i$ = level effect i of factor A;
$\boldsymbol{\beta}_j$ = level effect j of factor B;
$\boldsymbol{\delta}_k$ = level effect k of factor C;
$(\boldsymbol{\alpha\beta})_{ij}$ = interaction effect of A with B;
$(\boldsymbol{\alpha\delta})_{ik}$ = interaction effect of A with C;
$(\boldsymbol{\beta\delta})_{jk}$ = interaction effect of B with C;
$\boldsymbol{\xi}_{\mathbf{ijkl}}$ = experimental error associated with Y_{ijkl} and considered independent and identically distributed, with distribution N(0, σ2).

The $(\alpha\beta\delta)_{ijk}$ interaction was initially tested, but due to its low magnitude, it was removed from the statistical model. The results are presented as means ± standard deviation, as well as the corresponding standard error. Significance was declared at P<0.01 and P <0.05, and trends were discussed when P<0.10. For the aerobic stability pH data, regression analysis (α= 0.05) was performed to split the time interaction per treatment in the RStudio statistical program (v. 3.6.0; 2019).

Results

Quality of chemical composition

The ADF contents of the corn grain silage rehydrated with SWP differed significantly (P<0.01) between treatments (Table 1), with a lower observed ADF value of 14.7 g/kg DM for the silage without inoculation, followed by SWP + I with 21.0 g/kg of DM. There was a significant interaction (P<0.01) between the liquid sources used, in which the SWP silages had the lowest ADF values.

The lignin content differed significantly (P<0.10) between the SWF silages and other treatments, with the highest lignin content observed for SWF + I at 7.8 g/kg DM, in addition to presenting a significant interaction (P<0.05) between SWF and SWP, with a lower observed lignin content for SWF silage (Table 1).

The EE value differed (P<0.05) for the silages where SWF was used as a liquid source to rehydrate corn kernels, with a higher content of this nutrient for SWF + I. There was a significant interaction (P<0.01) between the liquid sources of SWF and SWP used to rehydrate the grains (Table 1), in which the SWF source showed an increase in EE compared to that in the silages for the control and SWP treatments.

For levels of Ash a significance difference was observed for the sources of treatments (P<0.05), addition of inoculants (P<0.01) and interaction between the liquid sources (P<0.05). The corn grain silages that were rehydrated with SWP had the highest levels of Ash compared to those in the other sources of rehydration, and the addition of inoculant in the SWP and SWF treatments provided the highest levels of this component (Table 1). The DM, CP, NDF, TCHO, and NFC values of the silages were not influenced (P>0.05).by the liquid sources used or by the addition of inoculants.

Fermentation profile

The NH3-N of the silages after 240 h of exposure to air from the ensiled mass were significantly different between silages (Table 3), with and without inoculation (P<0.05), for treatments with the liquid sources SWP and SWF (P<0.01), and there was significant interaction between the sources (P<0.01). The silages with the addition of inoculant and the control treatment showed the highest levels of NH3-N at 240 h.

The pH values evaluated in the silages showed a significant difference both in the opening of the silos and after 240 h of exposure to air (Table 3). For the pH values during the opening of the silos, a significant difference was observed between the silages with the liquid sources of SWF (P<0.05) and SWP (P<0.01) without the addition of inoculant, with a lower pH value of 4.26 for SWF.

After 240 h of exposure, the pH values differed between the SWF and SWP treatments (P<0.01; 0.10, respectively), with a lower observed value of 4.31 for the WF and a tendency towards a lower pH value for the silage with SWP when compared to those in the control (Table 3). For the addition of inoculant, corn grain silages rehydrated with SWP showed a higher pH value compared to that in the control and SWF treatments (P<0.01). In addition, a significant interaction (P<0.01) was observed between the silages with SWP and SWF, in which the lowest pH values were observed for SWF, regardless of the addition of inoculant. The inoculated silages (P<0.05) that were independent of the liquid sources used showed the lowest BC values compared to those of the SWF and control sources.

Aerobic stability

The loss of aerobic stability differed significantly (P<0.05) between the corn grain silages rehydrated with water and SWF + I and those from the other silages, which broke the stability after 84 h of oxygen exposure, showing greater stability after opening the silos (Table 4). The time required for the ensiled mass of the treatments with water and SWF

+ I to increase the temperature of the ensiled mass by 2 °C above the ambient temperature was significantly shorter (76 and 75 h, respectively) than that in the other treatments (*P*<0.05), a behavior that characterizes the loss of aerobic stability and onset deterioration of silage.

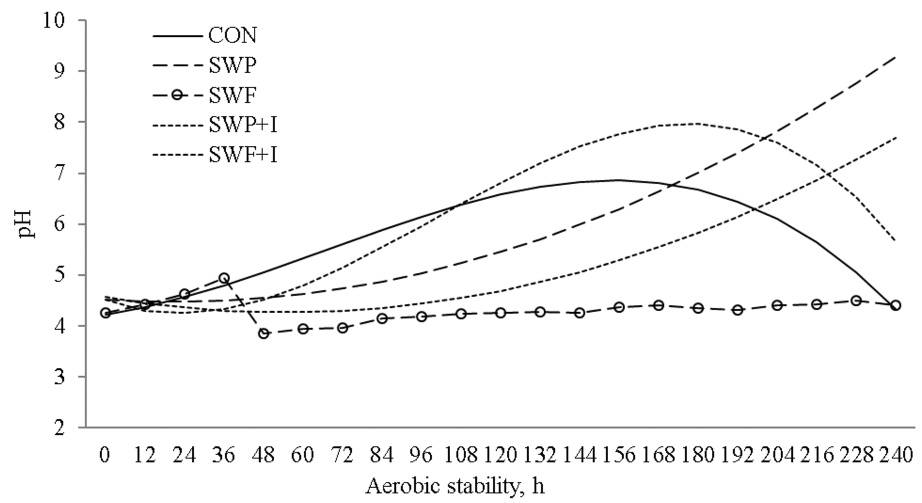
The time to reach the maximum temperature of the ensiled mass, except for the silage made with corn grain rehydrated with SWF (*P*<0.05), was longer than 200 h, with a significant difference between treatments in the final pH of the silages exposed to air for 240 h (Table 4, Figure 1).

Table 4: Aerobic stability parameters of corn grain silages submitted to rehydration

	Treatments					
Parameters	**CON**	**SWP**	**SWF**	**SWP+I**	**SWF+I**	***P*-value**
Aerobic stability, hour	76[b]	84[a]	84[a]	84[a]	75[b]	0.0007
Time to maximum temperature, hour	234[a]	234[a]	104[b]	204[a]	201[a]	0.0001
pH-240 h	5.50±1.2[ac]	7.35±1.2[b]	4.40±0.3[c]	6.71±1.6[ab]	6.88±1.4[ab]	0.0006

CON= corn grain silage rehydrated with water; SWF= corn grain silage rehydrated with whey fluid; SWF + I= corn grain silage rehydrated with fluid whey, plus inoculant; SWP= corn grain silage rehydrated with water-reconstituted whey powder; SWP + I= corn grain silage rehydrated with powdered whey reconstituted with water, plus inoculant.

Figure 1: pH values after aerobic exposure of rehydrated corn grain silages



CON: 6.06±1.18, $\hat{Y}=4.23+0.01x+0.0002x^2-0.000001x^3$, $R^2=0.72$, *P*=0.0011, CV=9.54; SWP: 5.38±1.18, $\hat{Y}=4.52-0.0042x+0.0001x^2$, $R^2=0.88$, *P*=0.028, CV=21.88; SWF: 4.31±0.27, *P*=0.123, CV=6.26; SWP+I: 6.37±1.60, $\hat{Y}=4.51-0.024x+0.0006x^2-0.000002x^3$, $R^2=0.92$, *P*=0.001, CV=25.17; SWP+I: 5.11±1.40, $\hat{Y}=4.57-0.011+0.0001x^2$, $R^2=0.89$, *P*=0.005, CV=27.35.

Discussion

Quality of chemical composition

It was observed that the inoculant contributed to ADF reduction when compared to that in the control treatment. These lower ADF values may be related to the dilution of the fiber content evaluated[20] and to the cellulolytic enzymes present in the inoculants, which degrade the fiber and alter the three-dimensional structure of the grain cell wall[9,21], determining positive results for the digestibility of this food.

The ADF and NDF results for the silages that used SWF were in agreement with Rezende *et al*[9], who also observed small reductions in the ADF levels in treatments with acid whey associated with inoculants, and in general observed lower NDF content for the treatments with rehydration of the corn grain with acid whey, without adding inoculant. For these variables and the liquid fluid whey source, it is still unclear how reductions in the levels of these nutrients occur. However, the whey acidity contributes to potentiating fermentation, which, together with the acidic hydrolysis of hemicellulose, can reduce the levels of these fibers.

The levels of lignin in all silages regardless of treatment could be considered low, since the content obtained in the corn kernels before rehydration was 11.3 g/kg DM. This reduction can occur after ensiling, due to the acid hydrolysis process of fibers and acidification that will weaken the complex lignin molecules[20] and thus obtain lower values of this component. However, the action of fluid and whey sources on reductions in lignin content in rehydrated corn grain silages requires more specific studies in terms of fiber structures, since this nutrient, as well as NDF and ADF, are directly related to feed digestibility.

The lignin levels determined in the present study for the control treatment differed from those obtained by Oliveira *et al*[1], who found values for lignin between 13.7 and 14 g/kg DM in corn grain silages rehydrated with water plus enzymatic additive. One factor that may explain this variation is that the levels of lignin present in rehydrated grain silages can vary widely due to the diversity of available corn cultivars, phase, and agronomic management.

The addition of the inoculant may have contributed to a higher value of content of EE for SWF + I, as this increase was also observed by Tres *et al*[22] and Arcari *et al*[3] in rehydrated corn grain silages inoculated with *L. buchneri*. The SWF source increased in EE compared to that in the silages for the control and SWP treatments. This increase can be explained by the microbiological potential and availability of nutrients that the SWF presents as a fresh product[8] at the time of rehydration, since there was an adjustment in the DM base of the tested liquid sources, for homogeneous distribution of the presented nutrient load. The EE levels determined in the present study for the control treatment were similar to the results obtained by Oliveira *et al*[1], Mombach *et al*[23], and Tres *et al*[22] who identified values of 53.1, 45.2, and 39.6 g EE/kg DM, respectively, in samples of grain silages rehydrated with water without adding inoculants.

The use of the SWP liquid source may have contributed to higher observed levels of Ash, due to its composition having 60 g Ash/kg DM, while SWF had 3.40 g Ash/kg DM. The DM, CP, NDF, TCHO, and NFC values of the silages were similar to the results observed by Rezende *et al*[9] in corn grain silages rehydrated with whey, and by Da Silva *et al*[10] and Oliveira *et al*[1], who evaluated corn grain silages rehydrated with water, plus inoculants, and their effects on nutrients.

Fermentation profile

The corn grain silage rehydrated with SWF showed superior fermentation quality compared to that of the silage with SWP and water, with the lowest value of NH3-N at 0.7 g/kg TN after exposure to air for 240 h. According to McDonald *et al*[20], as the pH rapidly decreases in the silage, the protein fraction is preserved and the concentrations of NH3-N will be lower, thus characterizing an adequate fermentation. An increase in NH3-N concentrations in silages may be related to the proteolytic activity of microorganisms from the epiphyte and/or inoculated population during silage, which will affect the decomposition of the prolamine of the cereals, as well as the digestibility of starch, and as a consequence there will be higher levels of protein available for NH3-N production[21].

In the present study, the use of fluid whey may have contributed to a rapid reduction in pH and preservation of the protein fraction during storage and exposure to air due to the low proteolytic activity of lactic acid bacteria[9,24] present in the microbiological profile of whey[8]. Conversely, the inclusion of the inoculant may have altered the profile of the bacterial population of the silo[10,24] and consequently reduced the prolamine content of the inoculated silages during exposure to air, as higher concentrations of soluble protein and NH3-N were observed at the opening of the silos (Table 2) and after 240 h of exposure to air (Table 3), respectively, in the silages in which the inoculants were incorporated, which may indicate greater proteolysis[5,21]. This hypothesis was supported by the results obtained by others[10], who, when evaluating the addition of *L. buchneri* in moist and reconstituted corn grain silages, found that the bacterial profile of the silage was modified, increasing the concentration of lactic acid bacteria as the silage storage period increased, with a higher concentration of NH3-N after 90 d of storage in both types of silages.

The lower pH value of 4.26 for SWF in opening silos reinforced the hypothesis that the microbial profile of fluid whey preserved the protein in corn kernels due to its low proteolytic activity of lactic acid bacteria, in addition to contributing to a rapid drop in pH after ensiling. It is known that the starch-protein matrix of corn grains presents greater degradation due to microbial activity than from simple solubilization of final fermentation products, such as acids[21].

The values of pH observed after 240 of exposure to air showed the action of source WF in silages and inoculants. The modification of the bacterial profile in silos can be due to the addition of inoculants with L. *buchneri*, that can creating ecological niches and to benefit proteolytic bacteria in convert lactic acid into acetic acid, consequently increasing the pH during the fermentation process in this type of environmental[21,24]. This fact observed in the inoculated silages, which presented higher pH values after 240 h of exposure to air (Table 3).

The pH of an ensiled sample is a measure of its acidity, which corresponds to the sum of the concentrations of acids present in the ensiled mass, whose main acids were acetic, propionic, and lactic acid. Lactic acid is produced by lactic acid bacteria and has a higher concentration, contributing more to the decline in pH during fermentation[25]. Some authors[12,20] consider pH values from 3.8 to 4.2 as ideal; however, the pH itself is not able to inhibit the action of undesirable microorganisms, which are also dependent on the speed of pH reduction, observed through the BC of the silages. The concentration of lactic acid in SWF and SWF + I were more expressive in samples obtained for the silages at the opening of the silos (Table 2), which presented the lowest pH values after 240 h of exposure to air.

The corn grain silage rehydrated with SWF maintained the pH at the opening and after the exposure to air, which was close to the values considered ideal for fermentative quality of the silage. This fact may also be due to the composition of the serum, which included sugars that were used as substrates by the lactic acid bacteria in the fermentation process, contributing to a rapid drop in pH. Considerable values were also observed for SWP, unlike water, which presented higher pH values at the opening of the silos. The results obtained for the control treatment were in accordance with those from Oliveira *et al*[1], who found pH values of 4.25 at the opening of the silo and 6.50 on the fifth day of exposure to air in corn silage rehydrated with water.

The inoculated silages that were independent of the liquid sources used showed the lowest BC values compared to those of the SWF and control sources (Table 3), which could be characterized as an important action of the inoculant in terms of fermentation right after ensiling, with a direct effect of inoculation on the speed of pH reduction and consequent improvement in silage BC. According to Jobim *et al*[26], this measurement depends on the composition of the plant regarding the contents of CP, inorganic ions, and the combination of organic acids and their salts, in addition to providing information on the speed of pH reduction, which must be low to facilitate this acidification during fermentation, culminating in improved conservation and silage quality. In the present study, these characteristics (Table 2) could be considered for a positive BC of rehydrated corn grain silages.

Aerobic stability

The loss of aerobic stability in corn grain silages rehydrated with water and whey occurred after 55 h[9]; however, in this work it was obtained greater aerobic stability with values of 75 to 84 h of exposure without losing stability, which characterizes a positive effect of rehydration. One of the factors that can influence the deterioration of the silages is the humidity of the ensiled mass, as there is a favoring of the medium so that undesirable microorganisms develop when the moisture content and acetic acid concentration are high[9]. In the present work, the moisture content was between the recommended intervals of 35 to 37 for a high quality of rehydrated grain silage[6,7], and the concentrations of acetic acid were similar between silages. In addition, the use of mandatory heterofermentative bacteria in inoculants, such as *L. buchneri*, increases the aerobic stability of moist and rehydrated corn grain silages[5,7,27], which justifies the results achieved for the loss of stability of the silages inoculated at 84 h of exposure to air.

Regarding the variable time to reach the maximum temperature of the ensiled mass, as the heating rate was obtained through the maximum temperature records divided by the time to reach the maximum temperature, it was observed that the maximum temperature was reached from the eighth to the ninth day of exposure to air, except for the SWF, which reached the maximum temperature on the fourth day, although the maximum temperature values reached for this silage were low. In general, the rehydrated corn grain silages, regardless of the liquid source used for rehydrating the grains, were efficient in the time to reach the maximum temperature in this process. This justification may be related to the fermentative profile of these silages and the increased effect of the bacterial inoculant used, which improves the aerobic stability of the silages. Despite SWF reaching the maximum temperature before the other silages, with a final pH value close to the ideal (4.40) in terms of quality, after 240 h of exposure to air, the silage was still superior in qualitative terms and fermentations to those in the other silages. These results were similar to those obtained by Rezende *et al*[9], who observed that after 40 h of aerobic exposure, rehydrated grain silages increased the temperature, regardless of the liquid used for rehydration.

Although the loss of aerobic stability for the corn grain silage rehydrated with SWF occurred at 84 h of exposure, and it was observed that these silages, when challenged with the time of exposure to air, also presented average pH values lower than those of the silages made with the other sources of rehydration (Figure1). This characteristic of maintaining stability after opening the silos can be explained by the microbial profile of the liquid source used for rehydration, which has low proteolytic activity of lactic acid bacteria[24] and acceleration of grain fermentation by a rapid fall in pH after ensiling due to the presence of lactic acid (Table 2).

The treatments that received an increase in microbial inoculant also showed a final pH value, in aerobic exposure, lower than that of the SWP, but not lower than that of the SWF without the inoculant and the control silage (Table 4). This shows that although the inoculation was efficient at prolonging the time to reach the heating rate of the silages, the pH values could still be considered above the ideal values, such as the value obtained for SWF. A significant increase in pH was also observed for the treatments with water, SWP, and SWP + I after 60 h of exposure (Figure 1). The pH values of the silages increased with exposure to air, due to the action of yeasts that can use lactate as carbon and energy sources, favoring an environment for the growth of molds and aerobic bacteria, which are responsible for the deterioration of silage[(20)].

The pH values for the corn grain silage rehydrated with SWF remained more stable during the 240 h of exposure and were close to the values at the opening of the silo, when compared with those of the other treatments. This behavior can explain the significant difference in the final pH value in the aerobic exposure of this treatment. However, corn grain silage rehydrated with water, which also had a lower pH value at the end of the exposure, did not show stable behavior during the exposure. Conversely, the corn grain silage rehydrated with SWP showed a constant pH increase during the exposure and reached a higher final pH value (7.35). These results are similar to those observed by Oliveira *et al*[(1)], who found that the pH behavior of corn grain silages rehydrated with water showed a constant increase from 48 h to 120 h of exposure to air. The final pH of the silages is influenced by several factors, but according to Kung Junior *et al*[(25)], is more related to the concentration of lactic acid and TC in ensiled food, as shown in Table 2, in which the highest concentrations of lactic acid at the opening of the silos were for SWF, followed by the inoculated silages. A previous study[(10)] showed that increased aerobic stability with increased storage period for rehydrated and moist corn grain silages inoculated with *L. buchneri* are due to the accumulation of fermentation products such as acetic and propionic acid, which have antifungal properties.

The corn grain silages rehydrated with SWF were superior according to the quality parameters evaluated in the present study. In addition, the use of fluid whey in the rehydration of the grain can be considered as an alternative liquid source to water that provides conservation of ensiled food and an appropriate destination for this byproduct for preservation of the environment. Although whey powder showed improvements in the chemical and fermentative quality compared to those of the commonly used liquid source, it may not be an appropriate alternative option for rehydrating grains, as it requires water to dilute the powder before rehydrating the grain and distances itself from the production of food in a sustainable way. Another fact that may disadvantage the use of this source is the process becoming more expensive, since the product is acquired through purchase and the production of whey powder involves several processes that add value to the product to be sold.

Conclusions and implications

The use of fluid whey presents itself as a suitable alternative to the use of water for rehydration of corn grains for silage, because in addition to being a sustainable alternative and that to preserve the environment, it also justifies its use by adding chemical, fermentative improvements and aerobic stability to the silage. In addition, these data suggest that more specific research is needed regarding the action of whey on the reduction of rehydrated corn grain silage fibers and the microbiological potential of the product to be used as a possible biological additive in the conservation of these silages.

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Conflicts of interest

The authors declare no conflict of interest.

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Table 1: Chemical quality (g/kg DM) of grain corn silages rehydrated with water, powdered whey and fluid whey

Variables [2]	Treatment [1]					SEM [3]	SWF	SWP	I	SWF × SWP [4]
	CON	SWF		SWP						
		Without	With	Without	With					
DM	633.4 ± 0.38	628.3 ± 0.53	637.0 ± 0.34	629.8 ± 0.68	625.7 ± 1.84	0.97	ns	ns	ns	ns
Ash	11.3 ± 0.25	11.1 ± 0.06	11.8 ± 0.05	12.0 ± 0.07	12.7 ± 0.06	0.06	ns	**	*	**
CP	103.0 ± 0.19	104.7 ± 0.42	103.2 ± 0.27	101.3 ± 0.17	104.7 ± 1.74	0.31	ns	ns	ns	ns
EE	37.0 ± 0.55	37.3 ± 0.35	41.8 ± 0.47	35.4 ± 0.30	43.6 ± 0.30	0.40	**	ns	ns	*
NDF	122.1 ± 2.07	112.8 ± 0.77	128.6 ± 1.42	137.4 ± 2.21	101.4 ± 0.96	1.44	ns	ns	ns	ns
ADF	23.0 ± 0.46	20.2 ± 0.23	22.1 ± 1.00	14.7 ± 0.75	21.0 ± 0.21	0.27	ns	*	**	*
Lignin	3.4 ± 0.57	3.3 ± 0.27	7.8 ± 0.14	3.4 ± 0.23	4.2 ± 0.31	0.24	***	ns	ns	**
TCHO	847.7 ± 0.77	847.0 ± 0.46	843.2 ± 0.22	851.3 ± 0.36	845.9 ± 2.01	1.04	ns	ns	ns	ns
TDN	812.9 ± 0.02	813.1 ± 0.01	812.8 ± 0.05	813.3 ± 0.04	813.0 ± 0.01	0.02	ns	***	***	**
NFC	718.8 ± 2.64	734.2 ± 1.01	714.6 ± 1.29	714.0 ± 2.39	744.5 ± 2.49	2.12	ns	ns	ns	ns

[1] Treatment: CON= corn grain silage rehydrated with water; SWF= corn grain silage rehydrated with whey fluid; SWF + I= corn grain silage rehydrated with fluid whey, plus inoculant; SWP= corn grain silage rehydrated with water-reconstituted whey powder; SWP + I= corn grain silage rehydrated with powdered whey reconstituted with water, plus inoculant.

[2] DM= Dry matter (g/kg of natural matter); Ash= mineral matter; CP= crude protein; EE= ether extract; NDF= neutral detergent fiber; ADF= acid detergent fiber; TCHO= carbohydrates total; TDN= Total digestible nutrients; NFC= non-fibrous carbohydrates. [3] SEM= standard error of the mean. [4] Interaction "SWF x SWP".

*P<0.01; ** P<0.05; *** P<0.10; ns= no significant.

Table 3: Fermentative quality (g/kg DM) of grain corn silages rehydrated with water, powdered whey and fluid whey

Variables [2]	Treatment [1]					SEM [3]	SWF	SWP	I	SWF × SWP [4]
	CON	SWF		SWP						
		Without	With	Without	With					
NH3-N-0 h	0.6 ± 0.04	0.5 ± 0.02	0.4 ± 0.01	0.5 ± 0.009	0.4 ± 0.03	0.02	ns	ns	ns	ns
NH3-N-240 h	1.9 ± 0.08	0.7 ± 0.01	1.3 ± 0.04	0.9 ± 0.03	2,20 ± 0.08	0.05	*	*	**	*
pH-0 h	4.53 ± 0.07	4.26 ± 0.09	4.43 ± 0.03	4.38 ± 0.14	4.35 ± 0.12	0.10	**	*	ns	ns
pH-240 h	6.13 ± 0.08	4.31 ± 0.11	5.14 ± 0.70	5.44 ± 0.41	6.47 ± 0.18	0.33	*	***	*	*
BC	259.9 ± 1.78	276.8 ± 5.27	266.3 ± 1.73	231.6 ± 3.03	243.4 ± 1.67	3.11	ns	ns	**	ns

[1] Treatment: CON= corn grain silage rehydrated with water); SWF= corn grain silage rehydrated with whey fluid; SWF + I= corn grain silage rehydrated with fluid whey, plus inoculant; SWP= corn grain silage rehydrated with water-reconstituted whey powder; SWP + I= corn grain silage rehydrated with powdered whey reconstituted with water, plus inoculant.

[2] BC= buffering capacity (e.mg/100g DM); NH3-N= ammoniacal nitrogen g/kg of total nitrogen) and pH in opening (0h) and exposure to air (240 h);

[3] SEM= standard error of the mean;

[4] Interaction "SWF x SWP".

* P<0.01; ** P<0.05; *** P<0.10; ns= no significant.