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Abstract

The aim of this study was to assess the quality of ground maize grain silage rehydrated with varying proportions of whey, in comparison with silage rehydrated with water, to determine the potential of using whey during silage production as a strategy to enhance process sustainability. The treatments included four different rehydration methods for the ground maize, with the control treatment adding 30% water to the maize grain, and whey being used to rehydrate the maize at three different rehydration levels (20%, 30%, and 40% whey). The highest effluent losses were observed in the treatment with 40% whey rehydration (7.86 kg/t fresh matter), and the pH was highest in the control treatment (5.51). The silage rehydrated with water contained the fewest lactic acid bacteria (0.90 log colony-forming units/g) and the lowest lactic acid concentration (2.71 g/kg dry matter). Stability loss occurred fastest in the silage rehydrated with water (41.6 hours), followed by the silage rehydrated with 40% whey (46.4 hours). Rehydration with whey enhanced the quality of the ground maize silage, promoting greater sustainability in both the processing and production of grain silage.

Keywords: grain conservation, co-product, silage, *Zea mays* L.

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Introduction

Maize (*Zea mays* L.) is one of the most widely cultivated cereals globally and serves as a primary feed source for animals. It is estimated that around 70% of the total maize produced is used in animal diets, making it the main energy source responsible for maintaining animal production rates (Benini *et al.*, 2020). The maize hybrids grown in Brazil are predominantly flint or hard types, with a higher proportion of vitreous endosperm; the higher the vitreousness of the maize grain, the lower the rumen digestibility of the starch in the grain (Jacovaci *et al.*, 2021).

Grain processing provides an effective method for enhancing the availability of starch, and rehydrating maize grain silage can improve the digestibility of maize grain starch (Rezende *et al.*, 2014).

Maize grain silage rehydration is a process that involves rehydrating ground dry grain to approximately 35% moisture before storing it for preservation through fermentation. According to Arcari *et al.* (2016), the improvement in digestibility is attributed to the disruption of the protein matrix by microbial proteolytic activity, which enhances the exposure of starch granules to enzymatic degradation. Additionally, the ensiling of maize grain offers a suitable storage method that minimises damage from insects and rodents, which is commonly observed in dry grain storage (Daniel *et al.*, 2022). Moreover, ensiling maize grain reduces the reliance on substances that may pose potential health risks to both workers and animals.

The rehydration of maize for ensiling can be carried out using different hydrating additives, with water being the most common (da Cruz *et al.*, 2021). However, Carvalho *et al.* (2017) noted that maize grain silage rehydrated with water alone is difficult to ferment, as it usually has low counts of lactic acid bacteria (LAB) and low aerobic stability, which are strong indicators of inadequate fermentation. It is therefore important to find alternative additives for rehydration to improve these characteristics.

The use of hydrating additives in grains is widely employed to improve digestibility and silage quality (Saylor *et al.*, 2020). According to Faustino *et al.* (2018), whey is a co-product produced on a large scale by the dairy industry that is sometimes not used properly, and its improper disposal in the environment can cause serious environmental damage. In addition to grain rehydration, this co-product can contribute to improvements in the nutritional quality and fermentation process of maize silage, as it is rich in lactose, minerals, soluble proteins, and vitamins (Souza *et al.*, 2020). Whey is also rich in LAB, bacteria that stimulate lactic acid production and help reduce pH, thereby stabilising the silage. Therefore, the use of whey for the rehydration of maize grain is a viable option for the use and proper disposal of this by-product (Ávila *et al.*, 2019).

The purpose of this study was therefore to evaluate the quality of ground maize grain silage rehydrated with different levels of whey, compared to silage rehydrated with water.

Materials and methods

The study was conducted at the Department of Animal Science, within the Center for Agricultural Sciences at the Federal University of Piauí (UFPI), located in Teresina, Piauí, Brazil. The region's climate is classified as Aw (tropical savanna), with an average yearly precipitation of 1451 mm and an average annual temperature of 27.9 °C.

A completely randomised experimental design was implemented, consisting of four treatments and five replicates per treatment. The treatments were defined based on the method of rehydration of the ground maize grain, which included water addition (30% water, on an as-fed basis) or the incorporation of whey at different levels (20%, 30%, or 40% whey, on an as-fed basis).

The maize grains were sourced from a local supplier and processed using a forage chopper (TRF 700G Trapp®, Santa Catarina-SC, Brazil) fitted with a 3 mm sieve. Following grinding, the rehydration process was conducted using either water or whey, ensuring thorough homogenisation. The treated maize was then ensiled in experimental bucket silos with a storage capacity of 4 kg and a packing density of 800 kg/m³. To prevent effluent accumulation, a 1 kg layer of coarse sand was placed at the base of each silo, covered by a non-woven synthetic fibre sheet (TNT) to separate the silage from the absorbent material. After compaction, the silos were sealed with lids equipped with Bunsen-type valves to facilitate gas release during fermentation.

The water used for rehydration was hypothermic mineral water from the Indaiá brand (Paraíba, Brazil), with a pH of 4.64. The whey originated from the enzymatic coagulation of fresh bovine milk (sweet whey) during the production of traditional rennet cheese. The collected whey was stored in sealed containers from a single day's processing and subsequently analysed following the methodologies outlined by Zenebon *et al.* (2008). The chemical composition and characteristics of the ground maize grains and whey are presented in Table 1.

Silage quality was evaluated after 90 days of storage, with the following parameters being measured: fermentation losses through gaseous emissions and effluent production, and the resultant dry matter recovery (DMR) rate; ammonia nitrogen concentration; pH; organic acid profile; microbial population dynamics; aerobic stability; chemical composition; and degradability.

Table 1 Chemical composition and characteristics of ground maize and whey used for ensiling

Chemical composition and characteristics	Ingredients	
	Ground maize	Whey ¹
Dry matter (%)	86.91	
Crude protein (% dry matter)	7.54	
Neutral detergent fibre (% dry matter)	12.21	
Acid detergent fibre (% dry matter)	2.42	
Ash (% dry matter)	1.18	
Ether extract (% dry matter)	3.41	
Total soluble carbohydrates (% dry matter)	8.97	
Buffer capacity (e.mg NaOH/100 g dry matter) ²	5.68	
Dry matter (g/kg)		29
Crude protein (g/kg)		23.2
Ether extract (g/kg)		1.0
Ash (g/kg)		5.75
Acidity (°D)		13.9
Density (g/mL)		1.027
Cryoscopy (°H)		-0.535
pH		6.4

¹ Based on the methodologies established by Zenebon *et al.* (2008). ² mg equivalents of NaOH per 100 g of dry matter

The experimental silos were weighed at sealing and reopening to quantify the dry matter (DM) losses via gas release and effluent production, as well as to determine the DMR rate, using the equations established by Zanine *et al.* (2010). Gas losses were estimated based on the differences in dry forage mass before and after ensiling:

$$G = \frac{(FSW_c - FSW_o)}{(FMc \times DM_c)} \times 10000$$

where:

G: DM loss due to gas emissions (kg),

FSW_c: total weight of the silo at sealing (kg),

FSW_o: total weight of the silo at reopening (kg),

FM_c: forage mass at sealing (kg), and

DM_c: DM content of the forage at sealing (kg/kg).

Effluent losses were calculated using the following equation, based on the difference in sand weight and related to the mass of fresh forage at closure:

$$E = \frac{(ESW_o - St) - (ESW_c - St)}{FMc} \times 100$$

where:

E: effluent production (kg per tonne of ensiled material),

ESW_c: combined weight of the empty silo and sand at sealing (kg),

ESW_o: combined weight of the empty silo and sand at reopening (kg),

St: silo tare weight (kg), and

FM_c: forage mass at sealing (kg).

The following equation was used to estimate the DMR rate:

$$DMR = \frac{(FMO \times DMO)}{(FMC \times DMC)} \times 100$$

where:

DMR: DMR rate (/kg),

FMO: forage mass at opening (kg),

DMO: forage DM content at opening (/kg),

FMC: forage mass at closure (kg), and

DMC: forage DM content at closure (/kg).

The DM, ash, crude protein, and ether extract concentrations in the silage samples were determined following the methodologies described by the AOAC (1990), using method numbers 934.01, 930.05, 981.10, and 920.29, respectively. The concentrations of neutral detergent fibre and acid detergent fibre were determined using the procedure outlined by Van Soest *et al.* (1991), with the adaptations proposed by Senger *et al.* (2008), utilising an Ankom fibre analyser (Ankom Technology Corporation). All analyses were performed at the Animal Nutrition Research Laboratory and the Forage Science Laboratory at the Federal University of Piauí, Teresina, Brazil.

The buffer capacity of the silage was determined following the methodology established by Mizubuti *et al.* (2009). For this analysis, 10 to 20 g samples of macerated silage were homogenised in 250 mL of distilled water. The solution was titrated to pH 3.0 using 0.1 N HCl to release bicarbonates as carbon dioxide. Subsequently, it was titrated to pH 6.0 with 0.1 N NaOH, and the volume of NaOH required to shift the pH from 4.0 to 6.0 was recorded. The buffer capacity was then calculated using the appropriate equation:

$$BCAP = \frac{0.1 \times (Vs - Vb)}{SW} \times 100$$

where:

BCAP: buffer capacity in mg equivalents of NaOH per 100 g of DM (e.mg NaOH/100 g DM),

0.1: normality of NaOH,

Vs: volume of NaOH required to adjust the sample pH from 4.0 to 6.0,

Vb: volume of NaOH required to adjust the blank pH from 4.0 to 6.0, and

SW: dry sample weight, determined as: (sample weight × DM content) ÷ 100.

The concentration of total soluble carbohydrates was determined using the concentrated sulphuric acid method described by Dubois *et al.* (1956), with the modifications proposed by Corsato *et al.* (2008). Organic compounds were extracted using an ethanol solution, and the absorbance was measured at 490 nm with D-glucose as the standard. The total soluble carbohydrate content (in g/100 mL) was initially calculated based on the solution and subsequently adjusted according to the DM content of each sample.

The pH determination was performed in duplicate by adding 100 mL of distilled water to 25 g of maize grain silage sample from each treatment. After one hour of equilibration, the hydrogen potential was measured following the protocol established by Bolsen *et al.* (1992), using an Instruterm potentiometer (São Paulo, Brazil).

The quantification of the ammonia nitrogen concentration in the silage samples was carried out using the methodology described by Bolsen *et al.* (1992). For this analysis, 25 g of fresh silage was mixed with 200 mL of a 0.2 N H₂SO₄ solution and refrigerated for 48 hours. The sample was then filtered through filter paper to estimate the silage's DM content (Detmann *et al.* 2012).

Aerobic stability was evaluated by storing the silage samples in a temperature-controlled room maintained at 25 °C using an air conditioning system. Ambient temperature was monitored using a thermometer (Incoterm®, Rio Grande do Sul, Brazil) placed near the silage samples. After 90 days of storage, the silos were opened, and the silage was exposed to air for 96 hours. During this period, the surface and internal temperatures of the silage mass were recorded every four hours. The surface temperature of the silage was measured using a non-touch digital thermometer, while the temperature of the forage mass was measured using a digital immersion thermometer inserted 10 cm into the centre of the silage mass. The ambient temperature was controlled using a thermometer suspended in the air. Aerobic stability break occurred when the internal temperature of the silage increased to at least 2 °C above the ambient temperature (Taylor & Kung, 2002).

The quantification of the organic acid (lactic, acetic, propionic, and butyric acids) concentrations in the silage samples was performed using high-performance liquid chromatography (HPLC). For this analysis, 10 g of each silage sample was weighed in triplicate and combined with 90 mL of distilled water. The mixture was homogenised for one minute using a blender and then filtered through 0.22 µm polyvinylidene fluoride syringe filters. A 10 mL aliquot of the filtered solution was transferred to a centrifuge tube, followed by the addition of 1.0 mL of metaphosphoric acid and two drops of 50% sulphuric acid. The samples were centrifuged at $13\,000 \times g$ for approximately 15 minutes, and the resulting supernatant was collected in Eppendorf tubes and frozen for subsequent analysis.

The organic acid concentrations were determined using a SHIMADZU, SPD-10A VP HPLC system (Siegfried *et al.* 1984). The system was equipped with an ultraviolet detector and an Aminex HPX-87H column (BIO-RAD, CA, United States). A 0.005 M sulphuric acid solution was used as the mobile phase, with a flow rate of 0.6 mL/min. Detection was performed at 210 nm, and all analyses were conducted at the Soils and Animal Nutrition Laboratory of the Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas (CPCE), Brazil.

The microbiological evaluation was carried out following the protocol established by González & Rodrigues (2003) to determine the populations of LAB, enterobacteria, filamentous fungi, and yeasts. The microbial populations in the ground maize grain and whey samples are presented in Table 2.

Table 2 Microbiological populations of ground maize and whey samples prior to ensiling

Ingredients	Microorganisms (log CFU/g)			
	Lactic acid bacteria	Yeasts	Filamentous fungi	Enterobacteria
Whey	6.25	0.00	1.66	3.02
Maize	0.00	0.00	0.75	0.00

CFU: colony-forming units

For microbial analysis, 25 g of fresh silage was weighed, and 90 mL of distilled water was added. The sample was then homogenised using a blender for approximately 1–2 minutes. A 1 mL aliquot of the homogenate was obtained and subjected to serial dilutions from 10^{-1} to 10^{-9} . Each dilution was plated in duplicate onto selective culture media to quantify microbial populations under anaerobic conditions. Rogosa agar (Kasvi®, Pinhais, Paraná, Brazil) was used for LAB enumeration, after incubation at 37 °C for 48 hours; potato dextrose agar (Kasvi®, Pinhais, Paraná, Brazil) acidified with 1% tartaric acid was employed to quantify filamentous fungi and yeasts, after incubation at room temperature for 48 hours; and brilliant green bile agar (Kasvi®, Pinhais, Paraná, Brazil) was used for enterobacteria enumeration, after the samples were incubated for 24 hours at 35 °C. Plates with 30 to 300 colony-forming units (CFU) were considered countable, and the final microbial counts were determined based on the averages of the selected dilution plates. Yeasts and moulds were differentiated through visual assessment based on morphological characteristics, as yeasts are unicellular, whereas moulds exhibit a multicellular filamentous structure.

The Daisy II incubation system was used for *in vitro* digestibility assessments, following the manufacturer's guidelines and using the buffer solution described by McDougall (1948). Porous non-woven fabric bags (4 × 4.5 cm) were prepared according to the experimental treatments. Once the samples had been weighed, the bags were immediately sealed (Detmann *et al.*, 2012). Rumen fluid was collected from a recently slaughtered bovine at the municipal slaughterhouse in Patos, PB, along with approximately 300 g of rumen particulate material. The collected material was transported in a thermal container to the Animal Nutrition Laboratory at the Federal University of Campina Grande (UFCG), Patos Campus, Paraíba, Brazil. The rumen inoculum was prepared by blending the rumen fluid and particulate material in a Waring blender for two minutes under constant CO₂ infusion.

Prior to incubation, McDougall's buffer solution was prepared. Urea solution (5.5 g/100 mL) was added at a ratio of 5 mL per 300 mL of buffer and the pH of the solution was calibrated to 6.80 by bubbling CO₂ through it for 15 to 20 minutes. Fresh buffer solution was prepared immediately before each digestion assay by heating solutions A and B to 39 °C and mixing 20 mL of solution B per litre of

solution A. When necessary, small volumes (1–2 mL) of solution B were added to adjust the buffer pH to 6.8.

Replicates of each sample were placed in incubator jars, and for each battery, a 'blank' bag was placed in each jar. Next, 400 mL of rumen inoculum and 1600 mL of McDougall's solution (a 1:4 ratio of inoculum to buffer solution) were added to each jar. The free space in the jars was immediately saturated with CO₂, and the jars were then closed and placed inside their respective incubators, which had been previously heated to 39 °C. Degradability was analysed at 0, 3, 6, 12, 24, 48, 72, and 96 hours. At each specified time, the selected bags were immediately washed with hot distilled water, with light manual pressure exerted to remove the gases contained in them. After washing, all the bags were oven-dried at 105 °C for 24 hours and weighed to obtain the apparently undigested DM residue.

Silage quality was assessed using the scoring system proposed by Ribeiro *et al.* (2023). Scores ranged from one to four, with the best-performing silage for a specific quality characteristic receiving a score of one, and the second-best receiving a score of two, and so on. Thus, the silage with the lowest total score was regarded as the best treatment. Treatments that did not differ from each other received the same score. The parameters that showed significant differences between the treatments and had the greatest influence on silage quality were evaluated, namely: effluent production, DMR rate, hydrogen potential, DM content, total soluble carbohydrate content, LAB content, degradability, lactic acid content, and aerobic stability.

McDonald (1991) reported that a greater loss of effluents in silage production can indicate lower quality, so the treatment with the greatest loss of effluents received the highest score. According to Pacheco *et al.* (2014), higher DMR rates are associated with lower gas and effluent losses, so the treatment with the lowest score was the one with the highest DMR rate. According to McDonald (1981), silage of good conservation should have a pH of 3.8 to 4.2, and the treatments closest to this range received the lowest score. Rezende *et al.* (2014) noted that the moisture content of ground and rehydrated maize grain silage should be between 25% and 40% to provide suitable moisture conditions for good fermentation, so the treatments that obtained DM results within this range received the lowest scores.

Regarding the carbohydrate levels, Gourley & Lusk (1978) reported that levels of 6% to 8% are necessary for the best preservation of silage, and the treatments with values closest to this range thus received the lowest scores. Pahlow (2003) recommended that well-fermented silages should have at least 5 log CFU LAB/g, and the treatments that contained this minimum amount therefore received the lowest scores. According to Roth & Undersander (1995), the lactic acid content for good-quality silage should be between 4% and 6% of the DM, and the treatments that were closest to this range received the lowest scores. Higher degradability may indicate better use of the feed by the animals, so the treatments with the highest degradability rates received the lowest scores. According to Coutinho *et al.* (2020), it is important for silages to exhibit high aerobic stability, which can be defined as the resistance of the ensiled material to deterioration after the onset of exposure to air. The treatments that showed greater stability received lower scores.

The data were analysed using analysis of variance, considering a significance threshold of $P < 0.05$, followed by linear regression. Mean comparisons were performed using Tukey's test at a 5% significance level ($P < 0.05$). Statistical analyses were conducted using SISVAR software, version 5.0 (Ferreira, 2011). The following statistical model was adopted:

$$Y_{ijk} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij} + \varepsilon_{ijk}$$

where:

Y_{ijk} : the observed parameter for rehydrated silage i at analysis time j ;

μ : general constant;

τ_i : effect of the type of rehydration i , where $i = 1, 2, 3$, and 4 (30% water, 20% whey, 30% whey, and 40% whey);

γ_j : effect of the time j , where $j = 1, 2, 3, 4, 5, 6, 7$, and 8 (0, 3, 6, 12, 24, 48, 72, and 96 hours);

$(\tau\gamma)_{ij}$: interaction between the different types of silage rehydration i and the times analysed j ; and

ε_{ijk} : random error associated with each type of silage rehydration and the time analysed.

Results and discussion

The silage rehydration method used significantly affected the effluent losses, DMR rates, ammonia nitrogen contents, and pH (Table 3). No effect ($P > 0.05$) was observed on gas losses (Table 3).

Table 3 Fermentation losses, pH, and ammonia nitrogen content of ground maize grain silage rehydrated with water or varying proportions of whey

Rehydration method	Effluents (kg/t fresh matter)	Gases (% DM)	DMR rate (% DM)	pH	N-NH ₃ (% total nitrogen)
30% water	2.77 ^b	1.49	97.76 ^a	5.51 ^a	0.02 ^c
20% whey	2.54 ^b	1.90	97.04 ^{ab}	4.79 ^b	0.02 ^c
30% whey	2.50 ^b	1.79	96.81 ^{ab}	4.38 ^c	0.03 ^b
40% whey	7.86 ^a	2.02	95.95 ^b	4.21 ^d	0.04 ^a
Mean	3.92	1.81	96.89	4.72	0.02
P-value	<0.01	0.18	<0.01	<0.01	<0.01
SEM	0.34	0.14	0.31	0.04	0.00

SEM: standard error of the mean, DM: dry matter, DMR: DM recovery, N-NH₃: ammonia nitrogen, ^{abc} Means in the same column with different lowercase superscript letters statistically differ from each other at $P < 0.05$, according to Tukey's test.

The highest effluent losses were observed in the silage samples rehydrated with 40% whey (7.86 kg/t fresh matter), whereas no significant differences were detected between the other treatments, which had effluent losses that ranged from 2.50 to 2.77 kg/t fresh matter. The increased effluent loss in the silage rehydrated with 40% whey can likely be attributed to its elevated moisture content, given that effluent production is directly correlated with silage moisture levels. Excessive effluent loss during ensiling can indicate a decline in silage quality, as effluents contain significant amounts of organic compounds, including proteins, organic acids, sugars, and other soluble components derived from the ensiled material (McDonald *et al.*, 1991). Mombach *et al.* (2019) reported similar findings, investigating the effects of varying water inclusion levels on the chemical composition and fermentation characteristics of rehydrated maize grain silage, with their results indicating that an increase in the water content (40%) led to higher effluent losses.

A significant effect was observed for the DMR rate, with the highest value observed in silage rehydrated with 30% water (97.76%), and the lowest value (95.95%) recorded for silage rehydrated with 40% whey. Overall, DMR values exceeded 95%, supporting the use of ground and rehydrated maize grain silage as an effective preservation method, because of its minimal DM losses during storage. According to Pinedo *et al.* (2022), the DMR rate is highly influenced by gaseous and effluent losses, meaning that silages with reduced fermentative losses exhibit higher DMR rates. The results observed in this study align with those reported by Diogénes *et al.* (2023), who determined the physicochemical composition and fatty acid profile of goat meat from animals fed ground maize silage rehydrated with different additives, and reported DMR values of 98.6% for silage rehydrated with water and 98.1% for silage rehydrated with whey.

The silage rehydrated with water exhibited the highest ($P < 0.01$) average pH (5.51), with the inclusion of whey as a rehydration medium resulting in lower silage pH values. This may be attributed to the enhanced proliferation of LAB, as shown in Table 2. This increased LAB activity likely improved the fermentation profile, leading to higher lactic acid production, which, in turn, lowered the pH and enhanced silage stability (França *et al.*, 2011). Furthermore, the acidity level observed in Table 1 (13.9 °D) suggests the presence of organic acids, which contribute to the acidification of the ensiled material. Similar findings were reported by Ávila *et al.* (2019), who investigated the chemical and microbiological attributes and fermentation losses of maize grain silage rehydrated with water or whey and supplemented with varying levels of tilapia by-products. They found a pH of 4.3 for silage rehydrated with 35% water and 4.2 for silage rehydrated with 35% whey, which aligns with the pH value recorded for the 40% whey treatment in this study.

The silage rehydrated with 40% whey exhibited twice the ammonia nitrogen concentration of the silages rehydrated with water and 20% whey. Increased whey inclusion in maize silage thus led to higher ammonia nitrogen levels; however, all the ammonia nitrogen levels found in this study were lower than those reported by Diog enes *et al.* (2023), who reported an ammonia nitrogen concentration of 0.61% total nitrogen (TN) in silage rehydrated with water and 0.70% TN in silage rehydrated with whey. The low ammonia nitrogen concentrations found across all the treatments suggest good silage quality, as lower ammonia nitrogen levels indicate reduced protein degradation by proteolytic enzymes, which are predominantly secreted by *Clostridium* spp. (Foskolos *et al.*, 2016). According to McDonald *et al.* (1991), high-quality silage should have less than 10% of its TN as ammonia nitrogen.

The rehydration method used had a significant influence on the DM, ether extract, neutral detergent fibre, and total soluble carbohydrates concentrations, as well as on the buffer capacity of the silage. No significant differences between the treatments were observed for the crude protein, ash, or acid detergent fibre contents (Table 4).

Table 4 Chemical composition of ground maize grain silage rehydrated with water or varying proportions of whey

Rehydration method	DM (g/kg)	Ash (g/kg DM)	EE (g/kg DM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	TSC (g/kg DM)	BCAP (e.mg NaOH/100 g/DM)
30% water	727.9 ^a	17.7	34.8 ^b	79.9	109.9 ^a	17.7	80.1 ^b	3.59 ^d
20% whey	727.2 ^a	16.9	47.1 ^a	80.9	66.8 ^b	17.8	110.2 ^a	12.54 ^c
30% whey	687.9 ^b	16.5	47.8 ^a	80.6	69.6 ^b	16.6	126.0 ^a	15.20 ^b
40% whey	636.3 ^c	16.1	48.7 ^a	82.8	66.8 ^b	16.0	131.6 ^a	19.73 ^a
Mean	694.8	16.8	44.6	81.0	78.3	17.0	112.0	12.77
P-value	<0.01	0.73	<0.01	0.40	<0.01	0.47	<0.01	<0.01
SEM	0.33	0.10	0.12	0.12	0.36	0.09	0.67	0.54

SEM: standard error of the mean, DM: dry matter, EE: ether extract, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, TSC: total soluble carbohydrates, BCAP: buffer capacity. ^{abc} Means in the same column with different lowercase superscript letters statistically differ from each other at *P* < 0.05, according to Tukey's test.

In terms of the DM content, the silages rehydrated with 30% water and 20% whey had the highest levels among the treatments (727.9 and 727.2 g/kg, respectively). Those rehydrated with 30% and 40% whey had average values of 687.9 and 636.3 g/kg, respectively. All DM contents were within the appropriate range, as the moisture content in ground and rehydrated maize silage should be between 25% and 40%, according to Rezende *et al.* (2014), to provide adequate moisture conditions for good fermentation.

The silage rehydrated with water had the lowest ether extract content (34.8 g/kg DM), while the silages rehydrated with 20%, 30%, and 40% whey had average values of 47.1, 47.8, and 48.7 g/kg DM, respectively. Rehydration with whey increased the ether extract content of the silages, which may be attributed to the presence of fat in the composition of whey (Carter & Drake, 2018). This can be seen in the chemical composition of the whey before ensiling (Table 1), with this co-product containing 1.0 g/kg of ether extract. Water, on the other hand, has no fat in its composition, which explains the higher ether extract levels in the treatments rehydrated using whey. The higher concentration of ether extract in the treatments using whey than in the silage rehydrated with water corroborates the results of  vila *et al.* (2019), who evaluated maize grain silages rehydrated with 35% water and found an ether extract concentration of 3.3%, while the silage rehydrated with 35% whey contained 5.6% ether extract.

The neutral detergent fibre concentration was higher in the silage rehydrated with water, averaging 109.9 g/kg DM. This data corroborates the results of Rezende *et al.* (2014), who found greater reductions in the neutral detergent fibre contents of silages when whey was used to rehydrate the ground maize grains than when they were rehydrated with water.

Higher total soluble carbohydrate concentrations were observed in the silages rehydrated with 20% (110.2 g/kg DM), 30% (126.0 g/kg DM), and 40% (131.6 g/kg DM) whey, while rehydration with

30% water produced the lowest total soluble carbohydrate content (80.1 g/kg DM). The increase in total soluble carbohydrate values in the silages rehydrated with whey is explained by the presence of carbohydrates such as lactose in this co-product, with lactose being the main sugar present in whey (Ávila *et al.*, 2019).

Rehydration with water resulted in a significantly lower buffer capacity in the silage, averaging 3.59 e.mg NaOH/100 g DM. The silage rehydrated with 20% whey had the lowest buffer capacity of the silages rehydrated with this co-product (12.54 e.mg NaOH/100 g DM), while the silages containing 30% and 40% whey had buffer capacity values of 15.20 and 19.73 e.mg NaOH/100 g DM, respectively. Higher whey inclusion rates were thus associated with higher buffer capacity values, which may be attributed to the fact that whey is rich in various minerals, including calcium, potassium, phosphorus, and magnesium (González-Weller *et al.*, 2023). The chemical composition of whey before ensiling (Table 1) confirms these assertions, as 5.75 g/kg of ash was found in this co-product. In addition, the organic acids present in whey, such as citric acid, have a buffering effect, preventing the pH of the ensiled mass from decreasing and explaining the higher buffer capacity found in the silages rehydrated with whey.

Regarding the microbial population of the silages (Table 5), the populations of LAB, yeasts, and enterobacteria in the ground maize silages differed significantly between those rehydrated with water and those rehydrated with varying proportions of whey. In contrast, no significant effect was observed on the populations of filamentous fungi.

Table 5 Populations of microorganisms in ground maize grain silage rehydrated with water or varying proportions of whey

Rehydration method	Lactic acid bacteria (log CFU/g)	Yeasts (log CFU/g)	Filamentous fungi (log CFU/g)	Enterobacteria (log CFU/g)
30% water	0.90 ^c	3.74 ^a	5.72	0.86 ^a
20% whey	5.81 ^a	2.52 ^c	5.62	0.50 ^b
30% whey	3.08 ^b	2.28 ^c	5.73	0.60 ^b
40% whey	2.73 ^b	2.94 ^b	5.37	0.54 ^b
Mean	3.13	2.87	5.61	0.63
P-value	<0.01	<0.01	0.37	<0.01
SEM	0.24	0.16	0.16	0.03

SEM: standard error of the mean, CFU: colony-forming units. ^{abc} Means in the same column with different lowercase superscript letters statistically differ from each other at $P < 0.05$, according to Tukey's test.

Lactic acid bacteria were most prevalent in the silage rehydrated with 20% whey, with an average of 5.81 log CFU/g, while rehydration with water resulted in the lowest average LAB population (0.90 log CFU/g). These results suggest that whey supplied LAB to the silage, as shown in Table 2. The LAB counts observed may explain the better pH values found in the silages rehydrated with whey, proving the effectiveness of LAB in promoting a decline in silage pH (Muck *et al.*, 2018). In this study, only the silage rehydrated with 20% whey had an LAB count exceeding the minimum limit of 5 log CFU/g, which was recommended by Pahlow (2003) for good silage fermentation.

The highest average yeast population was recorded in the silage rehydrated with water (3.74 log CFU/g), while the lowest counts were observed in the maize grain silages rehydrated with 20% and 30% whey, with values of 2.52 and 2.28 log CFU/g, respectively. Yeasts are considered undesirable in silage because of their inability to contribute to pH reduction and their role in increasing ethanol and CO₂ production, which leads to increased gas losses. (Behling Neto *et al.*, 2017). In fact, yeast populations in silage with adequate fermentation should not exceed 5 log CFU/g, according to Woolford (1990), and neither the silage rehydrated with water nor the silages rehydrated with whey exceeded the range recommended by these authors.

The four silage types had similar populations of filamentous fungi, ranging from 5.37 to 5.73 log CFU/g. According to Macêdo *et al.* (2017), filamentous fungi can cause damage to silage because they consume soluble sugars and lactic acid, metabolise cellulose and other cell wall

components, and produce mycotoxins that can be harmful to animals. Therefore, high populations of filamentous fungi are not desirable in good-quality silage.

The highest average enterobacteria count was observed in the silage rehydrated with 30% water (0.86 log CFU/g). According to Maia *et al.* (2021) enterobacteria find it difficult to proliferate at a low pH, and this aligns with the findings of this study, as the highest average population of enterobacteria was found in the silage rehydrated with water, which had the highest average pH (5.51). This reflects the positive effects of using whey to rehydrate the ground grain, as this co-product acidified the medium, thereby controlling the proliferation of enterobacteria, which are harmful to the ensiled material. The population of enterobacteria normally decreases as the population of LAB increases (Macêdo *et al.*, 2017). This is also supported by our findings, as the silages rehydrated with whey showed higher populations of LAB and lower populations of enterobacteria than the silage rehydrated with water.

The type of silage rehydration used significantly affected the concentrations of lactic acid, acetic acid, and propionic acid, but had no effect ($P > 0.05$) on the concentration of butyric acid (Table 6).

Table 6 Organic acid concentrations in ground maize grain silage rehydrated with water or varying proportions of whey

Rehydration method	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	Propionic acid (g/kg DM)	Butyric acid (g/kg DM)
30% water	2.71 ^c	1.89 ^c	0.21 ^d	1.00
20% whey	4.20 ^b	6.22 ^b	2.93 ^a	1.07
30% whey	7.87 ^a	8.02 ^a	1.12 ^c	1.10
40% whey	8.09 ^a	6.12 ^b	2.44 ^b	1.17
Mean	5.72	5.56	1.67	1.08
<i>P</i> -value	<0.01	<0.01	<0.01	0.93
SEM	0.14	0.18	0.08	0.18

SEM: standard error of the mean, DM: dry matter. ^{abc} Means in the same column with different lowercase superscript letters statistically differ from each other at $P < 0.05$, according to Tukey's test.

The highest lactic acid concentrations were observed in the silages rehydrated using whey at 30% (7.87 g/kg DM) and 40% (8.09 g/kg DM). The lowest concentration of lactic acid, which was 2.71 g/kg DM, was found in the silage rehydrated with water. The higher lactic acid concentrations in the ground maize silages rehydrated with higher proportions of whey can be attributed to the acidifying power of whey, as shown in Table 1, and because this co-product serves as a substrate for LAB, which are the microorganisms responsible for producing lactic acid (McDonald *et al.*, 1991). These results are favourable, given that lactic acid contributes significantly to the preservation of silage by promoting a decrease in pH, thereby enhancing its stability and creating unfavourable conditions for the growth of spoilage microorganisms (Santos *et al.*, 2018).

Acetic acid was found at lower concentrations in the silage rehydrated with water (1.89 g/kg DM), while the highest concentration of this acid was observed in the treatment including 30% whey (8.02 g/kg DM). The presence of acetic acid in silage is important because this acid inhibits the growth of filamentous fungi and yeasts in the ensiled material, thus giving the silage greater aerobic stability after exposure to air (Muck, 2010). It was observed that the silage containing the highest concentration of acetic acid (30% whey) also had the lowest average yeast population (2.28 log CFU/g), which corroborates this statement. According to Kung *et al.* (2018), silages containing very low concentrations of acetic acid may have lower aerobic stability when exposed to air.

The highest propionic acid concentrations were found in the silages rehydrated with 20% (2.93 g/kg DM) and 40% (2.44 g/kg DM) whey, while the silage rehydrated with water contained the lowest concentration of this acid (0.21 g/kg DM). Good-quality silages should contain low concentrations of propionic acid (Kung *et al.*, 2018), and the treatments that used different levels of whey for rehydration showed concentrations within the acceptable range for this acid (1–10 g/kg DM), as proposed by Freitas *et al.* (2006).

The concentration of butyric acid did not vary significantly between the different rehydration treatments, and ranged from 1.00 to 1.17 g/kg DM. The presence of butyric acid in silage is highly undesirable, as it is an indication of metabolic activity by microorganisms of the *Clostridium* genus, which leads to large losses of energy and DM (Pahlow *et al.*, 2003). Butyric acid concentrations are considered adequate when they are lower than 1.0 g/kg DM, and the findings of this study are thus close to the acceptable values for silage with adequate fermentation (Vieira *et al.* 2004).

In the degradability analysis, no interaction effect ($P > 0.05$) between the different rehydration methods of ground maize grain silage and the incubation times evaluated was observed. However, there were isolated effects ($P < 0.01$) of both the type of rehydration and the hours of incubation analysed. A linear increase in degradability was observed across the analysed incubation periods of 0, 3, 6, 12, 24, 48, 72, and 96 hours (Figure 1), with degradability increasing as the incubation period increased. Average degradability values of 6.52% (0 hours), 35.43% (3 hours), 44.65% (6 hours), 55.74% (12 hours), 76.87 (24 hours), 83.04% (48 hours), 84.00% (72 hours), and 87.72% (96 hours) were found. Regarding the total degradability observed at the end of 96 hours of incubation, the silages rehydrated with 20% (91.76%) and 30% (90.75%) whey had the highest degradability, while the silages rehydrated with water or 40% whey had lower degradability values, of 84.24% and 84.12%, respectively (Table 7).

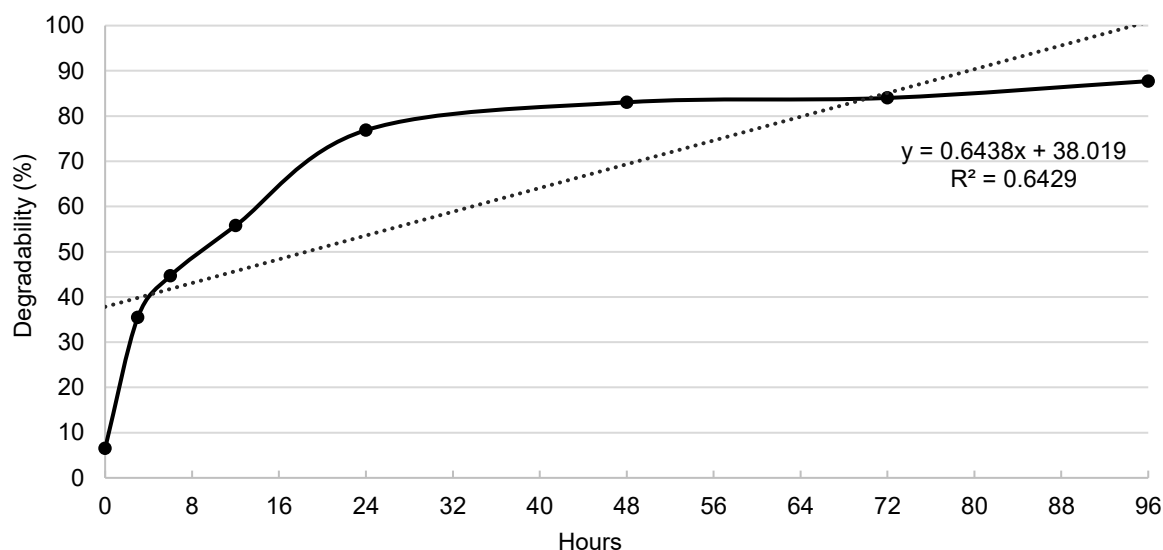


Figure 1 Degradability of ground maize grain silage rehydrated with water.

Table 7 The degradability of ground maize grain silage rehydrated with water or varying proportions of whey after 96 hours of incubation

Rehydration method	96-hour degradability (%)
30% water	84.24 ^b
20% whey	91.76 ^a
30% whey	90.75 ^a
40% whey	84.12 ^b
SEM	0.97
<i>P</i> -value (rehydration)	<0.01
<i>P</i> -value (hours)	<0.01
<i>P</i> -value (rehydration x hours)	>0.05

SEM: standard error of the mean. ^{abc} Means in the same column with different lowercase superscript letters statistically differ from each other at $P < 0.05$, according to Tukey's test.

The evaluation of silage degradability is important as this parameter serves as an indicator of silage quality, determining the amount of nutrients that animals will be able to absorb in the gastrointestinal tract (Pires *et al.* 2010). The results suggest that rehydration with whey at 20% to 30% improves the degradability of ground maize silage, in comparison to rehydration with water or higher levels of whey. This is promising, as greater degradability may indicate better feed utilisation by the animals, thereby improving their production rates.

Lucci *et al.* (2008) evaluated the effective *in situ* degradability of maize processed using different methods: broken maize, coarse ground maize, and fine ground maize. The authors found lower rates of DM degradability when maize was fed broken (35.43%) than when it was coarse ground (63.14%) or fine ground (71.68%). Cação *et al.* (2012), evaluated the effective rumen DM degradability of maize grain in the form of silage, extruded, and dried maize, and concluded that ensiling produced the best results, reporting average degradability values of 90.2% for silage, 66.56% for extruded maize, and 53.43% for dried maize, for the passage rates evaluated.

Ensiling is thus an effective process for improving the digestibility and degradability of maize grains (Daniel *et al.*, 2022). According to Arcari *et al.* (2016), the increase in the degradability of rehydrated and ensiled maize grain is due to the breakdown of the protein matrix, mediated by the proteolytic activity of microorganisms, which exposes the starch granules to enzymatic degradation. The results obtained in this study support the claims made by those authors, as the degradability obtained with the silage technique was higher than that obtained using the other methods of processing maize grain analysed by the aforementioned authors. In addition, using 20% to 30% whey to rehydrate the maize grain proved to be more effective in improving degradability than using water, highlighting the importance of using this co-product for rehydration.

Regarding the aerobic stability of the silages, the rehydration medium used significantly affected the stability break, internal temperature, and pH at break time (Table 8).

Table 8 Aerobic stability of ground maize grain silage rehydrated with water or varying proportions of whey

Rehydration method	Break (hours)	Internal temperature (°C)	pH (at break)
30% water	41.60 ^c	27.20 ^a	5.84 ^a
20% whey	96.00 ^{a*}	25.00 ^b	4.30 ^b
30% whey	75.20 ^b	27.20 ^a	3.67 ^c
40% whey	46.40 ^c	27.20 ^a	3.60 ^c
Mean	64.80	26.65	4.35
P-value	<0.01	<0.01	<0.01
SEM	1.63	0.16	0.02

SEM: standard error of the mean. * no break in stability. ^{abc} Means in the same column with different lowercase superscript letters statistically differ from each other at $P < 0.05$, according to Tukey's test.

The stability break occurred more quickly in the silage rehydrated with water and with 40% whey, occurring at 41.60 and 46.40 hours after the silages were exposed to air, respectively. No break in aerobic stability was observed after 96 hours for the silage rehydrated with 20% whey. A break in stability is caused by the action of aerobic microorganisms that become metabolically active on the exposure of the silage to air. This microbial activity produces heat by consuming the residual soluble carbohydrates and organic acids in the silage, resulting in a reduction in the quality of the material (Coutinho *et al.*, 2020). Kung *et al.* (2018) stated that silages containing low concentrations of acetic acid may have lower aerobic stability when exposed to air, and this was confirmed in the present study. The ground maize grain silage rehydrated with water contained the lowest concentration of this acid (1.89 g/kg DM) and broke stability more quickly, whereas the rapid break in aerobic stability of the treatment rehydrated with 40% whey may have been due to the high concentration of total soluble carbohydrates observed in this treatment (Table 4).

The internal temperature values were higher in the silages rehydrated with 30% water, 30% whey, and 40% whey, with these three treatments having the same average temperature at break

(27.20 °C). In contrast, the silage rehydrated with 20% whey had an average temperature of 25.00 °C. Taylor & Kung (2002) defined the break of aerobic stability as the time at which the internal temperature of the silage exceeds the ambient temperature by at least 2 °C. Thus, it can be seen that aerobic stability was broken in the 30% water, 30% whey, and 40% whey treatments when their internal temperatures exceeded the ambient temperature by more than 2 °C. The temperature of the silage rehydrated with 20% whey did not increase by 2 °C compared to the ambient temperature (25 °C), and there was thus no break in aerobic stability in this treatment.

The pH at the time of the break of aerobic stability was highest in the silage rehydrated with water, with a value of 5.84, while the lowest values were observed in the silages rehydrated with 30% (3.76) and 40% (3.60) whey. During the aerobic deterioration process, the metabolisation of residual carbohydrates and lactic acid results in an increase in silage pH (Woolford *et al.*, 1982). In this study, this was only observed for the silage rehydrated with water, which had a higher pH at the moment of aerobic stability break than at the time of opening the silo. In contrast, the silages rehydrated with different levels of whey had lower pH values at the point of aerobic stability breakdown than they had when their silos were initially opened.

The silages rehydrated with 20% and 30% whey obtained the lowest total scores for the silage quality assessment (Table 9), with 14 points each, while the silages rehydrated with water and 40% whey had the highest scores, with 19 and 17 points, respectively.

Table 9 Parameters for the quality evaluation of ground maize grain silages rehydrated with water or varying proportions of whey

Rehydration method	Effl.	DMR rate	pH	DM score (1 to 4)	TSC	LAB	LA	Degrad.	Stab.	Total
30% water	1	1	4	1	1	3	3	2	3	19
20% whey	1	2	3	1	2	1	2	1	1	14
30% whey	1	2	2	1	2	2	1	1	2	14
40% whey	2	3	1	1	2	2	1	2	3	17

Effl: effluents, DMR: dry matter recovery, DM: dry matter, TSC: total soluble carbohydrates, LAB: lactic acid bacteria, LA: lactic acid, Degrad: degradability, Stab: stability.

The results obtained corroborate those of Rezende *et al.* (2014), who stated that whey has great potential for improving the fermentation of rehydrated maize grains, as it contains several nutrients that are beneficial to the ensiling process. Thus, the results of this study justify the use of whey for the rehydration of maize as an alternative for the proper disposal of this residue. This is because the use of this co-product for the rehydration of ground maize grain resulted in improvements in silage quality compared to rehydration with water.

The score obtained for the silage rehydrated with 40% whey corroborates the results of McDonald (1991), who reported that higher effluent losses in silage production can indicate lower silage quality. This was observed in the present study, as this treatment had the highest effluent loss and, consequently, a higher score in the silage quality assessment, indicating that the silage produced was of lower quality than those produced using lower levels of whey rehydration. This is a satisfactory result, as it is thus not necessary to use a large amount of whey to rehydrate the ground maize grain. The need for smaller quantities of this co-product will reduce producers' costs for purchasing whey and for labour.

Conclusions

Rehydration with whey improved the quality of ground maize grain silage, when compared to rehydration with water. The use of 20% whey as-fed for rehydrating ground maize grain is recommended, because it reduces costs for the producers.

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Authors' contributions

L.F.R.C.: Formal analysis, investigation, methodology, visualisation, writing (original draft, review, and editing). R.L.E.: Conceptualisation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, visualisation, writing (review and editing). R.R. do N.: Formal analysis, investigation, methodology, visualisation, writing (original draft, review and editing). S.F.S.: Investigation, methodology, visualisation. J.P.M.P.: Investigation, methodology, visualisation. L.S.B.: Investigation, methodology, visualisation. C.B.M.S.X.: Investigation, visualisation, methodology. F.N.P.F.: Investigation, visualisation, methodology. A.F.P.: Investigation, project administration, methodology, resources, visualisation, supervision, writing (review and editing). M.M.R.: Investigation, methodology, visualisation, resources, supervision, writing (review and editing). D.B.: Investigation, methodology, visualisation, resources, supervision, writing (review and editing). L.R.B.: Investigation, methodology, visualisation, resources, supervision, writing (review and editing). E.M.S.: Investigation, methodology, project administration, resources, supervision, visualisation, writing (review and editing).

Conflict of interest declaration

The authors declare no conflicts of interest.

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