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Lentilactobacillus buchneri LBu 01 reduces dry matter losses and increases the aerobic stability of whole-plant corn silage

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ABSTRACT - The objective of this study was to evaluate the LBu 01 - CGMCC 19250 strain of *Lentilactobacillus buchneri* (LB) in whole-plant corn silage (WPCS) during different storage periods. The experiment was conducted in a completely randomized design with a factorial arrangement of treatments (2 × 3), two inoculant levels (control – without inoculant, and experimental – inoculated with 9 log CFU LBu 01/kg of forage), and three storage periods (3, 15, and 62 days). Dry matter (DM) losses, aerobic stability, microorganism population, and nutritional composition were quantified. The pH was lower for the inoculated silage (3.68) than for the control silage (3.73) (P = 0.03). Dry matter loss was lower in LB silage (P<0.01) than in control silage after storage for 15 (6.3% control; 1.6% LB) and 62 (12.9% control; 4.8% LB) days. The digestibility of the NDF after 30 h of incubation was greater (P<0.01) in the inoculated silage (56.9% NDF) than in the control silage (54.9% NDF). At 62 days, the lactic acid concentration was greater in the control silage (9.64% DM) than in the inoculated silage (7.38% DM) (P = 0.03). At 62 days, the LAB population in the inoculated silage increased, and the aerobic stability was greater in the inoculated silage (42 h) than in the control silage (27 h). Silages inoculated with the *L. buchneri* LBu 01 strain showed promising results, mainly after 62 days of storage. This strain is recommended for use in whole-plant corn silage.

Keywords: aerobic stability, heterofermentative, lactic acid bacteria

1. Introduction

Whole-plant corn (*Zea mays* L.) silage (WPCS) is the world's most widely used forage source in dairy cow diets (USDA, 2014). Given its importance, strategies to enhance nutritive value and fermentation profile, reduce DM losses, and increase aerobic stability have been evaluated (Diepersloot et al., 2022). The most commonly adopted strategy to achieve these objectives is to use microbial inoculants, mainly containing lactic acid bacteria (LAB) (Ávila and Carvalho, 2020; Carvalho et al., 2020), which might improve fermentation by accelerating the drop in pH. These bacteria can produce acids, bacteriocins, and other compounds that inhibit undesirable microorganisms, increasing silage aerobic stability (Carvalho et al., 2020).

Obligate heterofermentative LAB can produce antifungal compounds, which can inhibit yeast and mold growth; thus, increasing aerobic stability (Arriola et al., 2021; Drouin et al., 2023). Among the obligatory heterofermentative LAB, the species *Lentilactobacillus buchneri* (LB) is the most studied silage inoculant (Kung et al., 2018). Arriola et al. (2021) revealed that LB inoculation increased aerobic stability, raised the concentration of 1,2-propanediol and acetic and propionic acids, reduced the lactic acid concentration, and lowered the populations of yeasts and filamentous fungi in several silages (corn, sorghum, temperate and tropical grass, sugarcane, alfalfa, grain, and high moisture corn). The meta-analysis also showed that the effects of LB inoculation were dependent on storage time, with a more pronounced increase in aerobic stability and reduction in yeast population observed after 90 days of storage (Arriola et al., 2021). However, da Silva et al. (2020) observed a reduction in the yeast population and an increase in aerobic stability with the inoculation of LB (40788) after 10 days of storage of high-moisture corn.

An important point to note is that the results depend on the strain used (Carvalho et al., 2020). To our knowledge, no studies have assessed the results of this strain inoculation in silages, warranting further investigation for specific strain intended for commercial use (Carvalho et al., 2020).

Another point for consideration is the need to evaluate the action of obligate heterofermentative strains for different storage periods, because some strains of LB have shown positive effects, even with short storage times (Arriola et al., 2021; Drouin et al., 2023).

The objective of the present study was to evaluate the effect of *Lentilactobacillus buchneri*, LBu 01 - CGMCC 19250 strain, on nutritive value and fermentation profile of WPCS under different storage periods. We hypothesized that microbial inoculation would be more pronounced acetic acid production and aerobic stability and that these effects would increase with storage length.

2. Material and methods

2.1. Silage preparation and treatments

Whole-plant corn forage (FS615 PWU; ForSeed®) was obtained from a commercial field in Lavras, Minas Gerais, Brazil (Latitude: 21°14'43" South, Longitude: 44°59'59" West). The forage was harvested in April, 2024, at 34.2% DM and chopped by a coupled forage harvester (JF machines - model JF 120C) set to a theoretical chop size of 3 mm. *L. buchneri* was evaluated after three storage periods (3, 15, and 62 days). The lyophilized LB strain (LBU01 - CGMCC 19250) was resuspended in water (1 g/1000 kg) according to the manufacturer's instructions (COANA Importação e exportação LTDA, Florianópolis, SC, Brazil), sprayed at an application rate of 2.3 L/ton, and homogenized manually into the forage. Sufficient forage for each experimental silo was weighed (3 kg) and the inoculant was applied individually. The control silage received the same treatment using water without inoculant.

The population of inoculated bacteria was verified by tenfold serial dilution of an aliquot of the suspension and enumeration after culture on De Man Rogosa Sharpe agar (MRS; HiMedia Laboratories, Mumbai, India) at 36 °C for 72 h. The colony-forming units (CFU) were counted and used to establish the inoculation rate of 9 log CFU/kg of forage. Plastic containers (5 L) (Injetsul, Lambari, MG, Brazil) were used as experimental silos. Approximately 3 kg of forage was compacted in each container to achieve a density of 590 (±5.6) kg FM/m^3. The silos were sealed, weighed, and stored protected from the sun and rain. Five replicates were prepared for each inoculant for each storage day (3, 15, and 62 days) (30 experimental units in total).

2.2. Sample preparation and analysis

After each storage period, the silos were reweighed, and the total DM loss was calculated by comparing the DM weights of fresh forage and silage. The silos were opened, their contents were homogenized, and divided into three subsamples. One subsample was immediately stored at −20 °C until further

analysis. Another subsample was immediately used to evaluate aerobic stability, and a third subsample was used to prepare an aqueous extract.

To obtain the aqueous extract, a 25-g sample of fresh forage or silage was blended in 225 mL of 0.1% sterile peptone water and homogenized in an orbital shaker for 20 min. Part of this extract was used for aseptic microbiological analyses; another part (2 mL) was acidified with 10 µL of 50% (vol/vol) H_2SO_4 and frozen for subsequent analysis of fermentation end products, including lactic, acetic, butyric, and propionic acids, ethanol, and 1,2-propanediol. The analyses were carried out on a Shimadzu high-efficiency liquid chromatography (HPLC) system equipped with a quaternary pump (model LC-20AT), diode array detector (DAD) (model SPDM-20A), degasser (model DGU-20A5), interface (model CBM-20A). Samples were automatically injected by an autosampler (model SIL-20A). The analytes were separated on a Supelcogel 8H (300 × 7.8 mm) column (cat. 59246-U) equipped with a Supelcogel 8H (10 × 7.8 mm) pre-column, with isocratic elution using 0.005 mol/L H_2SO_4 buffer solution as the mobile phase at a flow rate of 0.5 mL/min and a column temperature of 30 °C. The injection volume was 20 µL. Acids were analyzed at 210 nm, and sugars and alcohols with a refractive index detector. Compounds were identified by comparing retention times with known standards and quantified using external standardization. All the samples and standards were analyzed in triplicate.

After sampling for microbiological and chromatographic analysis, the remainder of the aqueous extract was used for pH determinations (DIGIMED®DM 20 Potentiometer, Digicrom Instrumentos, SP, Brazil). Chemical composition of samples (part 1) was determined by ESALQLab (Department of Animal Science, ESALQ/USP, São Paulo, Brazil). The samples were pre-dried in a forced-air circulation oven at 55 °C for 72 h, ground in a Willey mill (Solab, Piracicaba, SP, Brazil) with a 1-mm sieve, and then analyzed using the NIRS (Near-Infrared Reflectance Spectroscopy) method. The samples were placed in quartz cuvettes and scanned on a FOSS DS2500F spectrophotometer (Silver Springs, MD, EUA), using the NIR calibration (WinISI version 4.6.11, FOSS Analytical A/S, Denmark) developed by Dairy One Forage Lab (Ithaca, NY) and used in commercial routine of the ESALQLab (Piracicaba, SP) to determine the concentrations of dry matter (DM), crude protein (CP), soluble protein, ammonia-N (NH_3), neutral detergent fiber (NDF), starch, water-soluble carbohydrates (WSC), 7 h *in vitro* ruminal starch disappearance (StarchD), 30 h *in vitro* ruminal NDF digestibility (NDFD), 30 h *in vitro* ruminal undigested NDF (uNDF), and 24 h *in vitro* digestibility of DM.

2.3. Microbiological analysis

The other portion of aqueous extracts was sequentially diluted tenfold and used for microorganism counts. Yeasts and filamentous fungi were plated for counting on Dichloran Rose Bengal Chloramphenicol Medium (DRBC, Difco, Becton Dickinson, Sparks, MD, USA) and incubated at 28 °C for 72 h. Yeasts were distinguished from filamentous fungi by colony appearance and cell morphology. Lactic acid bacteria were counted by spreading on MRS agar (HiMedia Laboratories, Mumbai, India) plus nystatin (4 mL/L) and incubation at 36 °C for 72 h. Enterobacteria were counted on Eosin Methylene Blue agar (EMB - HiMedia Laboratories, Mumbai, India) plus nystatin (4 mL/L). Aerobic spore-forming aerobic bacteria (ASFB) were counted by maintaining a sample of aqueous extracts at 80 °C for 10 min, serial dilution, and plating on Nutrient Agar medium (NA; HiMedia Laboratories, Mumbai, India) plus nystatin (4 mL/L). The EMB and NA plates were incubated at 36 °C for 24 h. Colonies were counted on plates containing a minimum of 30 and a maximum of 300 CFU.

2.4. Aerobic stability

Aerobic stability was examined by removing samples of approximately 1.8 kg after each storage period, placing them in buckets (5 L), and storing them in a room with ambient temperature monitoring. The buckets were covered with two layers of gauze to prevent insects from entering and excessive water loss. The temperatures were measured every 30 min using one data logger (model RC5+; Elitech, Rio Grande do Sul, Brazil) inserted into the center of the silage mass. The room and the silage temperatures

were measured for 192 h. Aerobic stability was defined as the time (hours) the silage remained stable before rising to more than 2 °C above the ambient temperature.

2.5. Statistical analysis

The experiments were carried out using a completely randomized design with a factorial arrangement (2 × 3), with two inoculant levels (uninoculated control and LB inoculated) and three storage periods (3, 15, and 62 days) with five replicates, for a total of 30 experimental units. The data were subjected to analysis of variance using the SISVAR® computer package (Variance Analysis System for Balanced Data) following the general mathematical statistical model:

$$Y_{ij} = \mu + I_i + S_j + (I \times S)_{ij} + \varepsilon_{ij},$$

in which μ = overall mean, I_i = inoculant effect (i = without inoculant or with *L. buchneri*), S_j = storage period effect (j = 3, 15, or 62 days), $(S \times T)_{ij}$ = effect of the interaction between inoculant and storage period, and ε_{ij} = error term.

The means were compared using Tukey's test at a probability level of $P<0.05$. The effect of the inoculant on aerobic stability was statistically analyzed in each storage period, given the different ambient temperatures between the periods used to evaluate the aerobic stability of the silages. Therefore, for this variable, the statistical model contained only the inoculant effect.

3. Results

Table 1 shows the chemical and microbiological compositions of the control and inoculated forage. Table 2 presents the probability values (P values) for the effects of inoculants, storage time, and their interactions.

The pH was lower for the inoculated silage (3.68) than for the control silage (3.73). At 15 days of storage, the pH reached its lowest value (3.50), whereas it increased after 60 days (4.00) (Table 3). An interaction between inoculation and storage period was evident for silage DM concentration and DM losses (Table 2). After three days of storage, no difference was detected between the DM concentration of the control silage and the inoculated silage; however, after 15 and 62 days, the DM concentration was greater for inoculated than for control silage (Figure 1A). The DM concentration of the inoculated silage

Table 1 - Chemical and microbiological characteristics of fresh, uninoculated whole-plant corn

Item	Control	LB[1]
pH	5.68	5.61
Dry matter$_{corr}$ (DM; %)	34.1	34.3
Crude protein (CP; % DM)	6.90	7.40
Soluble protein (% CP)	45.0	45.0
Ammonia (% DM)	0.29	0.31
Starch (% DM)	29.2	28.4
Water-soluble carbohydrates (% DM)	9.8	8.7
Starch digestibility 7 h (% starch)	49.0	50.0
Neutral detergent fiber (NDF) (% DM)	47.9	50.1
In vitro NDF digestibility 30 h (% NDF)	55.0	55.0
Non-digestible NDF 30 h (% DM)	17.9	18.6
In vitro DM digestibility 24 h (% DM)	77.0	76.0
Bacteria in MRS (log CFU/g)	7.06	7.76
Yeasts (log CFU/g)	6.24	5.94
Filamentous fungi (log CFU/g)	5.47	5.51
Bacteria in EMB (log CFU/g)	6.88	6.94
Aerobic spore-forming bacteria (log CFU/g)	5.99	5.71

[1] Strain: LBu01.

did not vary from three (33.4%) to 15 days of storage (33.9%); however, the DM of the inoculated silage was lower (32.9%) at 62 days than after 15 days of storage. The DM of the control silage decreased consistently from three (33.9%) to 62 (30.1%) days of storage. The percentage of DM losses showed an inverse pattern to the DM concentration (Figure 1B). Dry matter losses were lower in LB silage than in control silage after storage for 15 (6.3% control; 1.6% LB) and 62 (12.9% control; 4.8% LB) days. The DM loss in the inoculated silage increased from 15 to 62 days of storage, whereas the loss in the control silage consistently increased at each period (Figure 1B).

The silage CP concentration (Table 2) was not affected by either inoculation or the storage period and showed a mean of 7.32% DM. The concentrations of soluble protein and ammonia-N increased from three to 62 days of storage, with values of 51.20 and 60.10 (% CP) and 0.42 and 0.63 (% DM), respectively (Table 3).

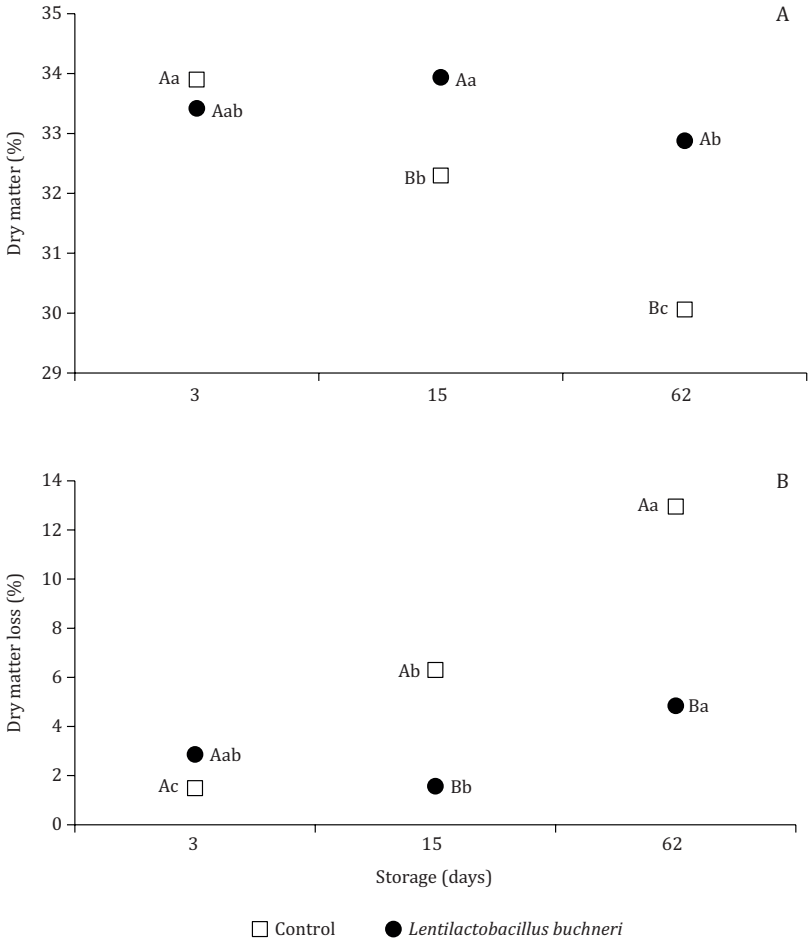
Table 2 - Probability values of the effects contained in the statistical model

Item	Inoculant (I)	Storage (S)	I×S
pH	0.03	<0.01	0.87
Dry matter (DM)	<0.01	<0.01	<0.01
DM loss	<0.01	<0.01	<0.01
Crude protein	0.79	0.21	0.90
Soluble protein	0.96	<0.01	0.68
Ammonia	0.63	<0.01	0.13
Starch	0.03	0.55	0.05
Water-soluble carbohydrates	0.62	<0.01	0.26
Starch digestibility 7 h	0.75	0.41	0.81
Neutral detergent fiber (NDF)	0.25	0.14	0.43
In vitro NDF digestibility 30 h	<0.01	<0.01	0.67
Non-digestible NDF 30 h	<0.01	<0.01	0.02
In vitro DM digestibility 24 h	<0.01	<0.01	0.16
Lactic acid	0.11	<0.01	0.03
Acetic acid	0.33	<0.01	0.31
Ethanol	0.55	0.91	0.20
Bacteria in MRS	<0.01	0.04	<0.01
Yeasts	0.15	0.33	0.23
Aerobic spore-forming bacteria	0.23	0.12	0.34

Table 3 - Effects of inoculation with *Lentilactobacillus buchneri* (LB) and storage period on whole-plant corn silage

Variable	Inoculant		SEM	Storage (days)			SEM
	Control	LB		3	15	62	
pH	3.73B	3.68A	0.012	3.61b	3.50c	4.00a	0.014
Crude protein (% DM)	7.33	7.30	0.087	7.20	7.47	7.28	0.107
Soluble protein (% CP)	55.47	55.53	1.000	51.20b	55.20b	60.10a	1.225
Ammonia (% DM)	0.53	0.54	0.012	0.42c	0.55b	0.63a	0.015
Water-soluble carbohydrates (% DM)	4.69	4.45	0.346	6.40a	3.71b	3.60b	0.423
Neutral detergent fiber (NDF) (% DM)	49.23	48.35	0.531	49.29	49.39	47.7	0.650
Starch digestibility 7 h (% starch)	59.8	60.3	1.189	61.5	60.0	58.7	1.457
In vitro NDF digestibility 30 h (% NDF)	54.9B	56.9A	0.463	54.2b	57.3a	56.3a	0.567
In vitro DM digestibility 24 h (% DM)	75.9B	77.2A	0.186	75.5b	76.8a	77.4a	0.227
Acetic acid (% DM)	0.96	1.06	0.063	0.77b	0.85b	1.41a	0.077
Ethanol (% DM)	0.66	0.61	0.052	0.65	0.64	0.62	0.063
Yeasts (log CFU/g)	5.93	5.60	0.152	5.82	5.54	5.94	0.186
Aerobic spore-forming bacteria (log CFU/g)	5.14	5.36	0.126	4.99	5.49	5.27	0.155

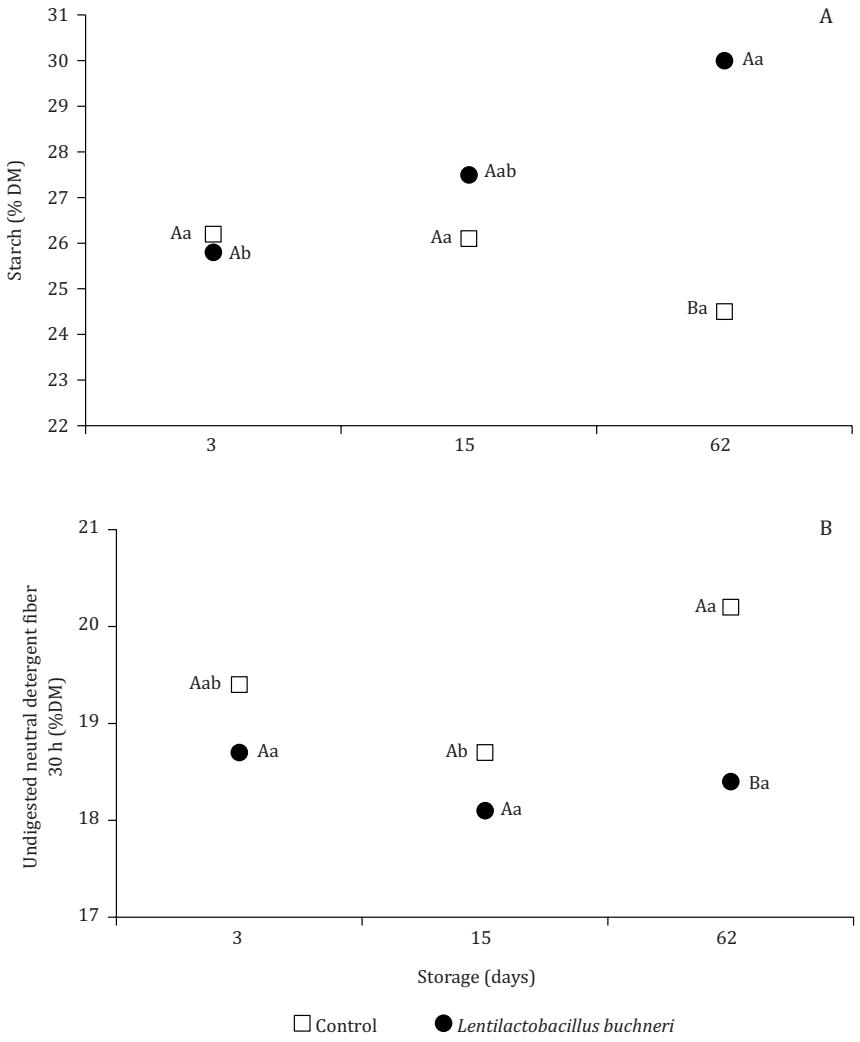
The inoculant mean values with different capital letters are significant at P<0.05 according to Tukey's test. The inoculant mean values with different lowercase letters are significant at P<0.05 according to Tukey's test.



SEM - DM = 0.295; SEM - DM loss = 0.850.
SEM - standard error of the means.
Capital letters compare the inoculant within storage period; lowercase letters compare the storage periods.

Figure 1 - Interaction between the use of *Lentilactobacillus buchneri* LBu01 and storage period on dry matter (DM) concentration (A) and percentage of DM losses (B) in whole-plant corn silage.

The WSC concentration decreased from 6.40 to 3.71 (% DM) from three to 15 days of storage and remained stable for up to 62 days (Table 3). The evaluated factors did not affect starch digestibility (Tables 2 and 3). However, an interaction was noted between inoculation and storage period (P = 0.05) regarding starch concentration (Figure 2A). The starch concentration was similar between the inoculated and control silages after three (26.0% DM, on average) and 15 (26.8% DM, on average) days of storage. However, after 62 days, the starch concentration was higher in the LB silage (30.0%) compared with the control silage (24.5%). The NDF concentration was not modified by inoculation; however, the NDF digestibility after incubation for 30 h was higher in the inoculated silage (56.9% NDF) than in the control silage (54.9% NDF). This variable increased after 15 days of storage and then remained stable until the final evaluation at 62 days (mean 56.8% NDF). An interaction was detected between the inoculant and storage time on the percentage of undigested NDF (30 h) (*in vitro*) (Table 2). The percentage of undigested NDF was lower in the inoculated silage than in the control silage after storage for 62 days (Figure 2B). During the evaluation period, this variable remained constant in the LB silage but decreased in the control silage. Dry matter digestibility (*in vitro*) was higher in inoculated silage (77.2% DM) than in control silage (75.9% DM) (Table 3). An increase in DM digestibility (*in vitro*) was observed after 15 days of storage, and the value was then maintained for the remainder of the experiment.



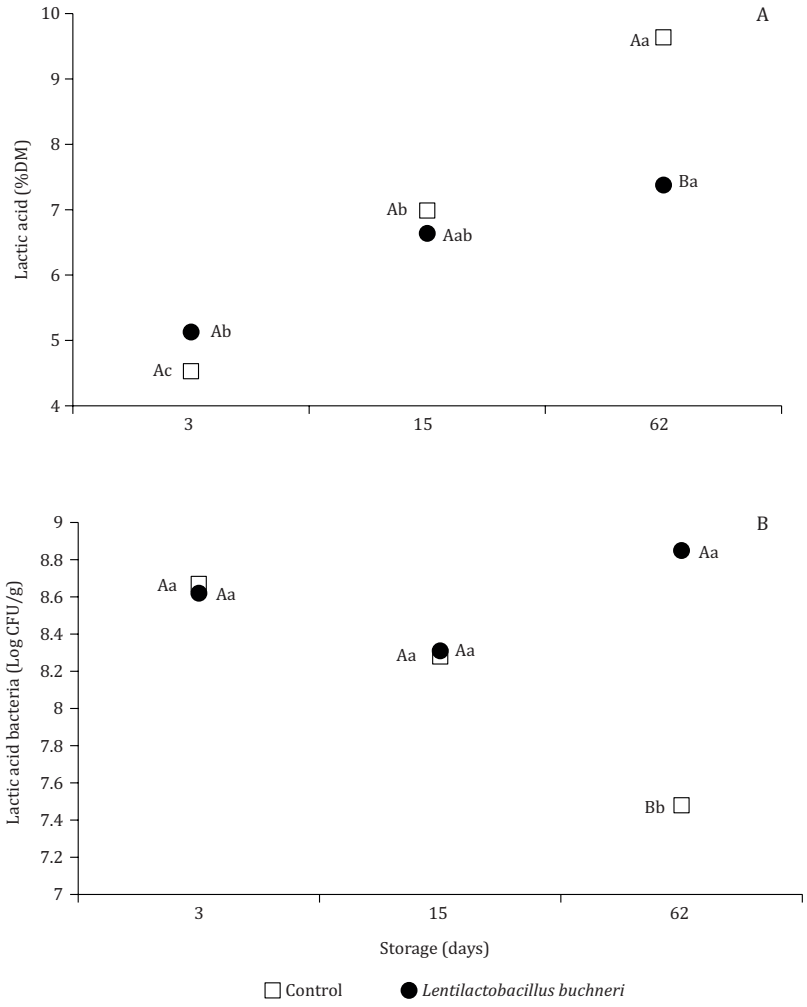
SEM - starch concentration = 1.162; SEM - undigested NDF = 0.850.
SEM - standard error of the means.
Capital letters compare the inoculant within storage period; lowercase letters compare the storage periods.

Figure 2 - Interaction between the use of *Lentilactobacillus buchneri* LBu01 and storage period on starch concentration (A) and percentage of undigested neutral detergent fiber (B) at 30 h in whole-plant corn silage.

The butyric acid, propionic acid, and 1,2-propanediol concentrations were below the detection level (0.006 mg/mL). An interaction between the inoculant and storage period was evident for lactic acid (P = 0.03; Figure 3A), as the lactic acid concentration was similar between the inoculated and control silages at three and 15 days of storage. However, at 62 days, the concentration of lactic acid was higher in the control silage (9.64% DM) than in the inoculated silage (7.38% DM). The lactic acid concentration increased in the control silage at each storage period, but the increase in the lactic acid concentration in the inoculated silage only showed a statistical difference after storage for three (5.13% DM) and 62 (7.38% DM) days (Figure 3A). Inoculation had no observable effect on the acetic acid concentration (Tables 2 and 3), although the acetic acid concentration increased (1.14% DM) after 62 days of storage in both silage treatments. Neither inoculation nor storage period had any effect on ethanol concentrations or yeast and ASFB populations (Tables 2 and 3).

The populations of filamentous fungi and enterobacteria were below the minimum detectable levels (2 log CFU/g). The LAB population was affected by the interaction between inoculation and storage period (Table 2). Evaluations made after storage for three and 15 days did not reveal any differences

in the LAB population between the inoculated and control silages. However, after storage for 62 days, the LAB population showed a small increase in the inoculated silage (8.85 Log CFU/g versus 7.48 Log CFU/g for the control silage) (Figure 3B).

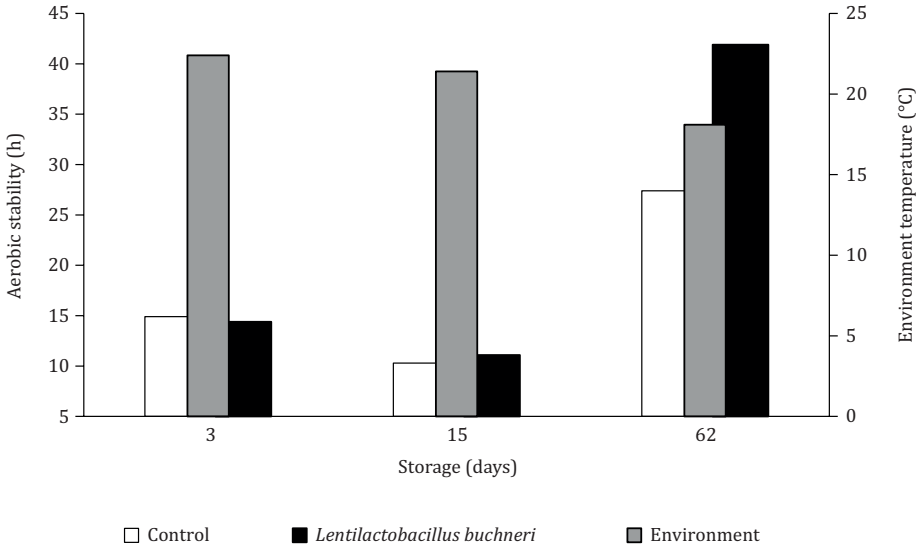


SEM - LA concentration = 0.477; SEM - LAB = 0.173.
SEM - standard error of the means.
Capital letters compare the inoculant within storage period; lowercase letters compare storage periods.

Figure 3 - Interaction between the use of *Lentilactobacillus buchneri* LBu01 and storage period on lactic acid concentration (A) and lactic acid bacteria population (B) in whole-plant corn silage.

3.1. Aerobic stability

The mean ambient temperature during the aerobic stability assessment periods was 22.4 °C (±0.5) at three days, 21.4 °C (±0.7) at 15 days, and 18.1 °C (±0.4) at 62 days. After storage for three or 15 days, none of the treatments led to statistical differences in silage aerobic stability (Figure 4), maximum silage temperature (mean 35.1 and 33.0 °C, in three or 15 days, respectively), or time to reach maximum temperature (mean 35 h in three and 15 days). After storage for 62 days, the aerobic stability was greater in the inoculated silage (42 h) than in the control silage (27 h) (Figure 4). However, the maximum temperature of the silage (mean 26.4 °C) and the time it took for the silage to reach the maximum temperature (61 h) did not differ between the inoculated and control silages.



Gray bars indicate the average room temperature within assessment period.

Figure 4 - Effect of *Lentilactobacillus buchneri* LBu01 on the aerobic stability of whole-plant corn silage after three (P inoculant = 0.40), 15 (P inoculant = 0.55), and 62 (P inoculant <0.01) days of storage.

4. Discussion

The chemical and microbiological characteristics of the forage examined in the present study are similar to those commonly reported in the literature for corn hybrids used in Brazil (Costa et al., 2021; Rossi et al., 2023). The pH observed in the silage indicate desirable fermentation. The pH of the inoculated silage was slightly lower than that of the control silage highlighting a favorable point for the strain under study. Generally, inoculation of silage with heterofermentative LAB results in an increase in the silage pH (Agarussi et al., 2022; Rossi et al., 2023). However, as observed in this study, depending on the strain used, silages inoculated with *Lentilactobacillus buchneri* may have a pH equal to or lower than that of uninoculated control silages (Agarussi et al., 2022; Rossi et al., 2023). This result may be related to the metabolism of LBu01 or a change in the diversity of other bacteria naturally present in the silage due to inoculation (Bai et al., 2022; Guo et al., 2023). More detailed studies of the metabolism and effect of LBu01 on microbial diversity should be carried out in the future.

Contrary to the findings of this study, inoculation with heterofermentative strains is associated with the highest percentage of fermentative losses. However, several studies have shown that losses are lower following inoculation with some of these strains than in uninoculated silages or silages inoculated with some homofermentative strains (Arriola et al., 2021; Costa et al., 2021; Agarussi et al., 2022). According to the literature, high pH and DM loss in silages inoculated with heterofermentative LAB strains are explained by a metabolism that produces more acetic acid because this acid has a lower capacity to reduce pH and its production pathway generates CO_2 (Ávila and Carvalho, 2020). Inoculation with strain LBu01 resulted in silages with lower pH and lower DM loss without changing the acetic acid concentration. Arriola et al. (2021) and Agarussi et al. (2022) reported that the losses related to inoculation with LB depend on the dose applied, the type of forage, the combination with other LAB, and the strain of LB used. These observations reinforce the need for the systematic isolation and selection of strains intended for inoculation in silage (Carvalho et al., 2020). An increase in fermentative losses is common throughout the storage period; however, the LBu 01 strain evaluated in this study was able to minimize the losses that occurred in the control silage. This loss reduction is generally associated with a reduced presence of undesirable microorganisms, such as yeast. However, the populations of

the undesirable microorganisms studied (yeasts, filamentous fungi, enterobacteria, and ASFB) did not change significantly, but the LAB population increased after 62 days of storage. Some inoculants (*Lacticaseibacillus rhamnosus* IMI 507023; *Lactiplantibacillus plantarum* IMI 507026; *Pediococcus pentosaceus* IMI 507025) can reduce N-NH_3 and soluble protein levels, but this is mainly observed in silages that are predisposed to have high values of these variables (e.g., silages with low DM) (Kung et al., 2018; Gonda et al., 2023). In the present study, the soluble protein concentration increased from 15 to 62 days, and the NH_3 concentration increased at all periods evaluated. For silages, slight increases in ammonia concentration (<15% total N) and soluble protein (<60% total N) during the storage period are acceptable and may reflect the action of plant enzymes and microorganisms. In corn grain silage, an increased soluble protein concentration is associated with prolamin degradation and increased starch digestibility. Although an increase in the soluble protein concentration was observed in the present study, starch digestibility did not increase as the storage period progressed, in agreement with previous findings by Rossi et al. (2023).

The increase in starch concentration observed with the interaction between LB inoculation and longer storage time was unexpected. Drouin et al. (2023) observed an increase in starch concentration after 180 days of storage of high-moisture corn silage, but did not observe an effect of inoculation with *L. buchneri* NCIMB 40788 on this nutrient. Kok et al. (2024) reported that inoculating *Lactococcus lactis* and *Lentilactobacillus buchneri* (SiloSolve FC®) showed a tendency to increase starch concentration (38.4% DM) compared with silage without inoculation (36.9% DM). However, these previous studies did not discuss the possible causes of their results. Tavares et al. (2024) found that inoculation with a microbial inoculant (*Lentilactobacillus buchneri* and *Lactiplantibacillus plantarum*) increased the starch concentration of WPCS (+2.9% DM), and they attributed this increase to the efficiency of the inoculant in preserving nutrients through fermentation. In the present study, we associated the increased in starch content (2.5%) with the lower loss of DM in the inoculated silage. In other studies, in which LB was inoculated alone, the increase in starch concentration was not observed. This may be because no reduction in DM losses was observed with inoculation (Drouin et al., 2023; Rossi et al., 2023).

The *in vitro* NDF digestibility at 30 h and DM at 24 h were highest in the inoculated silage. Other studies have reported increases in NDF digestibility when *L. buchneri* was applied alone or combined with whole-plant corn silage (Weinberg et al., 2007; Rossi et al., 2023; Kok et al., 2024). This result can be associated with the production of the ferulic acid esterase (FAE) enzyme. Some strains of *L. buchneri* are known to produce this enzyme, which hydrolyzes the ferulic acid ester groups present in the bond between hemicellulose and lignin (Muck et al., 2018). The inoculated silages in the present study showed an increase of 3.1 (% NDF) and 1.3 (% DM) in the digestibility of NDF and DM from three to 15 days of storage. Considering the role of LAB in increasing the digestibility of the fibrous fraction, we speculate that FAE requires some time for production and activation. However, the ability of the LBu01 strain used in this study to produce FAE was not evaluated; therefore, future studies should be conducted to confirm this possibility. The increase in DM digestibility observed following LB inoculation may be associated with increased NDF digestibility.

The lactic acid concentrations (means of 7.05 and 6.38% in control and LB silage, respectively) and LAB population (means of 8.14 and 8.59 Log CFU/g in control and LB silage, respectively) found in the present study can be considered high compared with the variations described in the literature for corn silage (3–6% DM) (Kung et al., 2018; Arriola et al., 2021; Agarussi et al., 2022). The high lactic acid concentration could be associated with the high LAB population. At 62 days, fermentation is expected to be stable and the LAB population lower, and these features were observed in the control silage. However, the LAB viability remained stable in the inoculated silage. Heterofermentative LAB, such as *L. buchneri*, have been reported to survive longer than other LAB in silage, which would explain the larger LAB population in the inoculated silage after storage for 62 days (Carvalho et al., 2017).

The acetic acid concentrations in the present study were within the limits previously reported for corn silage (1–3% DM) (Kung et al., 2018). Inoculation with LB (LBu01) did not increase the acetic acid concentrations over those seen in the uninoculated control silage. A study evaluating eleven strains of *L. buchneri* showed that some strains produced silages with a lower acetic acid concentration (0.66%

DM) than was observed in the control treatment (1.24 DM) (Agarussi et al., 2022). Agarussi et al. (2022) also showed that inoculation with the LB-56.25 strain resulted in silage with a lower acetic acid concentration but greater aerobic stability than in uninoculated control silage. In the present study, the acetic acid concentration and yeast populations did not differ between the inoculated and control silages, but the inoculated silage showed greater aerobic stability. Acetic acid in silage reduces the populations of yeasts and filamentous fungi, which are the main microorganisms associated with aerobic deterioration (Ávila and Carvalho, 2020). Yeasts that can metabolize lactic acid are known to have negative effects on the aerobic stability of silage (Ávila and Carvalho, 2020); therefore, the present results suggest that the yeasts in the inoculated silage had a low capacity to metabolize lactic acid. Thus, the aerobic stability of this silage was lower.

5. Conclusions

Whole-plant corn silage demonstrated improved aerobic stability after storage for 62 days when inoculated with the *L. buchneri* LBu01 strain than without inoculation. Although *L. buchneri* LBu01 did not increase acetic acid concentration, the inoculated silages had lower pH, reduced dry matter loss, higher *in vitro* digestibility of neutral detergent fiber and dry matter, and improved aerobic stability. These findings indicate that *L. buchneri* LBu01 can be used in whole-plant corn silage.

Data availability

The data generated or analyzed during this study are included in this published article.

Author contributions

Conceptualization: Carvalho, B. F. and Ávila, C. L. S. **Data curation:** Ávila, C. L. S. **Formal analysis:** Carvalho, B. F. and Ávila, C. L. S. **Funding acquisition:** Ávila, C. L. S. **Investigation:** Carvalho, B. F.; Souza, V. C.; Schwan, R. F. and Ávila, C. L. S. **Methodology:** Carvalho, B. F.; Souza, V. C.; Sousa, L. H. L.; Schwan, R. F. and Ávila, C. L. S. **Project administration:** Ávila, C. L. S. **Resources:** Ávila, C. L. S. **Supervision:** Carvalho, B. F.; Schwan, R. F. and Ávila, C. L. S. **Validation:** Carvalho, B. F. and Ávila, C. L. S. **Visualization:** Ávila, C. L. S. **Writing – original draft:** Carvalho, B. F.; Souza, V. C.; Sousa, L. H. L.; Schwan, R. F. and Ávila, C. L. S. **Writing – review & editing:** Carvalho, B. F.; Schwan, R. F. and Ávila, C. L. S.

Conflict of interest

The authors declare no conflict of interest.

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