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Effect of aerobic exposure time and use of inoculants on chemical, fermentative, and digestibility characteristics of relocated whole-plant corn silages

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ABSTRACT - The current study evaluated the impact of aerobic exposure time (ET) and microbial inoculants (MI) on the fermentative and microbiological characteristics and the nutritional value of relocated whole-plant corn silage (WPCS). Treatments were arranged in a 4 × 5 factorial design: microbial inoculants (without inoculant (WI); *L. plantarum* (DSM3676; DSM3677) + *L. buchneri* (DSM13573) (LPLB); *L. plantarum* (MA18/5U) + *P. acidipropionici* (MA26/4U) (LPPA); and *L. buchneri* (DSM13573) (LB)) and non-relocated (NR) or relocated after 12, 24, 48, and 60 h of ET. In the NR silage, the total storage time was 120 (not exposed-ET 0 h) or 210 (NR) days. Fresh forage that included an inoculant was inoculated with 1 × 10^5 CFU g^{-1} of forage. Samples were collected after 120 days of ensiling at various exposure times, and again after an additional 90 days of fermentation. For the samples collected at the initial opening (120 days), exposure time was not included as a factor in the statistical model. After 120 d of ensiling, inoculated silage exhibited higher lactic and propionic acid and lower ethanol concentrations, lower yeast counts, and reduced dry matter losses. During exposure, yeast counts increased (P<0.01) in WI silages after ET 0 (3.50 CFU g^{-1}) to 60 h (4.90 CFU g^{-1}). Post-relocation, the mold counts in WI silage were greater (P<0.01) when NR (4.02 CFU g^{-1}) and relocated at 12 h (4.1 CFU g^{-1}) and 24 h (4.2 CFU g^{-1}) compared with other silages. The *in vitro* dry matter digestibility of LB silage decreased (P<0.01) from NR (674.7 g kg^{-1} DM) to 60 h (592.6 g kg^{-1} DM). The LB silage exhibited reduced aerobic stability. *L. buchneri* inoculation enhances the digestibility of corn silage ensiled for 210 d, but the digestibility is reduced from 24 h of aerobic exposure.

Keywords: aerobic stability, heterofermentative bacteria, relocation, restriction

1. Introduction

The relocation of corn silage has become a common practice among ruminant animal producers worldwide. This practice enables the sale of preserved feeds and facilitates the supply of roughage to

farm animals. During relocation, the silo is emptied, and the silage is transported, packed, and sealed in a new silo (Chen and Weinberg, 2014; Cardoso et al., 2022). These operations must be conducted swiftly because corn silage is highly susceptible to aerobic deterioration, particularly in hot and humid climates (Kung et al., 2018).

When silages are exposed to oxygen, the yeasts within them become metabolically active, initiating a deterioration process that diminishes the nutritional value of the feed (Borreani et al., 2018). Losses during relocation hinge on exposure duration and microbial activity dynamics. Consequently, prolonged oxygen exposure can bolster the population of aerobic microorganisms, thereby undermining silage quality (Pahlow et al., 2003).

The literature indicates that relocation success hinges not only on air exposure duration but also on the initial properties of the silage, such as chemical composition, fermentative profile, and microorganism count (Mahana and Chase, 2003; Chen and Weinberg, 2014; Michel et al., 2017; Anjos et al., 2018; Queiroz et al., 2021; Santos et al., 2023). For instance, silages with elevated lactic acid levels may be less stable and incur greater losses during relocation. Consequently, using microbial inoculants (MI) with heterofermentative lactic acid bacteria (LAB) strains could enhance the stability of corn silages throughout the relocation process. This approach may mitigate aerobic deterioration issues, particularly when relocation involves extended air exposure (Muck et al., 2018).

The most commonly used inoculants in whole-plant corn silage (WPCS) contain heterofermentative bacteria, including strains of *L. buchneri* alone or in combination with *P. acidipropionici* strains. These may be combined with homofermentative bacterial strains (e.g., *L. plantarum*), which hasten lactic acid production and contribute to lowering the silage pH, thereby minimizing fermentative losses. Therefore, we hypothesized that WPCS with inoculants would present a better fermentation profile and lower yeast and mold counts, being safer for relocation process than silages without inoculant. Consequently, the relocated inoculated WPCS silages would present better characteristics than the relocated WPCS without inoculant (WI). The study aimed to evaluate the impact of aerobic exposure in silages with and without inoculants on the chemical, fermentation and microbiological characteristics, as well as on the digestibility, of relocated WPCS.

2. Material and methods

2.1. Ensiling process

The corn used was the Pioneer 4285 hybrid, which was planted in February 2017 at a density of 55,000 plants ha^{-1} in an area located in Paragominas, PA, Brazil (03°02'S, 47°20'W, 91 m a.s.l.). The climate of the growing region is classified as Aw, featuring two well-defined seasons: a rainy season from December to May and a dry season from June to November. The corn crop received two fertilization applications: the first at crop establishment with 400 kg/ha of 10-30−10 (N-P-K) and the second as a top dressing with 200 kg/ha of 20-00-20 (N-P-K), applied 28 days after sowing.

The crop was harvested when the corn plants reached an average dry matter (DM) content of 344.5 ± 2.0 g kg^{-1}, 95 days after planting, at a height of 20 cm from the ground, using a self-propelled harvester (FX40, New Holland Agriculture, Italy). In the field, samples of fresh corn plants were collected to determine the chemical composition of the material prior to ensiling. Fresh forage that included an inoculant was inoculated with 1×10^5 CFU g^{-1} of forage. The inoculant, diluted in distilled water at room temperature, was sprayed onto the forage at a dosage of 1 mL kg^{-1} of fresh forage and then manually mixed in, except for treatment without inoculant. The forage for each silo was inoculated individually. Experimental silos were created using plastic buckets (20 L). Three kilograms of white sand (washed and dried in a forced-circulation oven at 55 °C) were placed at the bottom of each experimental silo, and a nonwoven fabric sheet was placed above the sand to avoid the contact between the sand and the forage. Approximately 9.00 ± 0.20 kg of fresh forage was compacted into each silo, achieving an average density of 536.00 ± 30.43 kg m^{-3}. The silos were weighed, sealed, and stored for 120 days.

The sand was kept into the silos and used to calculate the effluent losses and not used in the aerobic exposure time (ET).

The trial was conducted using a completely randomized experimental design in a 4 × 5 factorial arrangement (MI × ET), with four replications. Treatments included microbial inoculants (WI; *L. plantarum* (DSM3676; DSM3677) + *L. buchneri* (DSM13573) (LPLB); *L. plantarum* (MA18/5U) + *P. acidipropionici* (MA26/4U) (LPPA); and *L. buchneri* (DSM13573) (LB)) and non-relocated (NR) or relocated after 12, 24, 48, and 60 h of ET before repackaging. Note that in NR silage, the total storage time was 120 (not exposed-ET 0 h) or 210 (NR) days.

After 120 days of ensiling, eighty silos were opened, the silage was removed, and individual piles were formed. Only sixteen experimental silos corresponding to ET 0 were opened, and the silages were sampled before aerobic exposure. After sampling, these silages were discarded. The other experimental silos (sixteen) corresponding to ET 0 remained closed for 210 days and represented the NR silages. The remaining silages were then exposed to air for 12, 24, 48, and 60 h. Ninety-six experimental silos were used. During the exposure period, the silages remained in a barn with a ceramic tile roof and open sides. They stayed on a tarp, and no measures were taken to prevent drying. During aerobic exposure, silage and environmental temperatures were measured punctually before repackaging. At the time of opening, at 07:00 h, the ambient temperature was 26.5 °C; after 12, 24, 48, and 60 h of exposure, the ambient temperature was 26.9, 27.8, 27.8, and 26.9 °C, respectively.

After the exposure period, the silages were individually homogenized and sampled before starting the repackaging process in the original silo, where they were stored for another 90 days, totaling 210 days of storage (ensiling plus relocation). After the total storage period, all silos were opened, and the NR and relocated silages were sampled for evaluation.

2.2. Chemical, microbiological composition, and *in vitro* dry matter digestibility (IVDMD)

The silage samples were pre-dried in a forced-air oven at 55 °C for 48 h and then ground using a Willey knife mill (Thomas Wiley Mill Model 4; Thomas Scientific, Swedesboro, NJ, USA) fitted with a 1-mm sieve. Only the DM content was determined for samples collected before (0 h) and after exposure to air (12, 24, 48, and 60 h).

To determine the DM content, the samples were dried in an oven at 105 °C for 16 h (AOAC Official Method 967.03; AOAC, 1990). The samples were analyzed for ash content (AOAC Official Method 942.05), ether extract (EE) (AOAC Official Method 920.39), and total nitrogen (AOAC Official Method 984.13; AOAC, 1990). The crude protein (CP) was calculated using the following formula: CP = total nitrogen × 6.25. The neutral detergent fiber (NDF) analysis was carried out according to Mertens (2002), using thermostable alpha-amylase without sodium sulphite. The non-fibrous carbohydrate (NFC) content was determined with the following equation:

$$NFC = 100 - (CP + EE + ash + NDF) \quad (1)$$

Yeasts, molds, and LAB counts were conducted using an aqueous extract (225 mL of 0.1% sterile peptone water + 25 g of silage) and homogenized for 4 min at 200 rpm in a Stomacher. Decimal dilutions were prepared to quantify microbial groups using the spread-plate technique (Tabacco et al., 2009). Yeasts and molds were enumerated on YGC Agar medium (Fluka, Sigma Aldrich Química Brasil LTDA), with colonies identified based on macromorphological characteristics. Plates were incubated at 28 °C for three and five days for yeast and mold counting, respectively. The LAB counts were performed on MRS Agar medium (MAN, ROGOSA, and SHARPE, Merck KGaA) with the addition of nystatin (4 mL L^{-1}). Plates were incubated at 35 °C for 72 h.

The IVDMD was determined according to Holden (1999) using DAISY II equipment (ANKOM Technology Corp, Fairport, NY, USA). Samples were dried at 55 °C for 48 h and ground to 1 mm. Rumen fluid was collected 2 h after the first feeding from two non-lactating Nellore cows (450 ± 29 kg) that were cannulated and fed WPCS, and energy protein concentrate twice daily. The diet consisted of

80% WPCS and 20% concentrate. Filter bags (5 × 5 cm) were prepared from non-woven fabric with a porosity of 100 µm. These bags were washed twice with neutral detergent for 15 min, immersed in acetone for 5 min, and dried in a forced-air oven at 55 °C for 48 h. An average of 0.80 ± 0.003 g of the sample was placed in each bag and incubated for 30 h at 39 °C (Holden, 1999). A blank filter bag was used to account for bacterial contamination. Post-incubation, filter bags were washed in a washing machine (3 cycles), dried at 55 °C for 48 h, and weighed. The IVDMD was calculated according to the difference between their weight before and after the incubation.

2.3. Fermentation characteristics, effluent losses, and DM recovery rate

The silages were evaluated for pH (Bernardes et al., 2019), ammonia nitrogen content (AOAC Official Method 941.04; AOAC, 1990), and the content of organic acids and alcohols (Tabacco et al., 2009).

Concentrations of organic acids (lactic, acetic, propionic, and butyric) and alcohols (ethanol and 1,2-propanediol) were determined using high-performance liquid chromatography (HPLC). The aqueous extract was acidified with 50% (v/v) sulfuric acid, filtered, and analyzed using a Shimadzu chromatograph, model LC-10Ai (Shimadzu Corporation-Tokyo, Japan), equipped with a dual detection system comprising an ultraviolet radiation detector (UV) (UV-Vis/SPD-10 Ai) and a refractive index detector (RID 10A). An ion exclusion column (Shim-pack SCR-101H, 7.9 mm × 30 cm) operated at 50 °C was used for the chromatographic separation of acids. The mobile phase was a perchloric acid solution with a pH of 2.1 and a flow rate of 0.6 mL min^{-1} was used. The alcohols were detected using the refractive index, and organic acids were detected using UV radiation at a wavelength of 210 nm.

The effluent losses (kg t^{-1}) were quantified according to the weights of the bucket (Tb), the bucket + sand + NWF (PV_i) before filling the silos, the forage mass (FM_i), and the empty bucket + sand + NWF (PV_f) after the silage was removed from the silo. Effluent losses (E) were calculated using the equation, according to Jobim et al. (2007):

$$E\ (kg\ t^{-1}) = [(PV_f - Tb) - (PV_i - Tb)]/FM_i \times 1000 \quad (2)$$

The weights of initial forage mass (FM_i), final silage mass (SM_f), initial forage DM content (DM_i), and final silage DM content (DM_f) were recorded. Dry matter recovery (DMR) from silage was determined using the equation according to Jobim et al. (2007):

$$DMR\ (\%) = (SM_f \times DM_f)/(FM_i \times DM_i) \times 1000 \quad (3)$$

Dry matter losses (DML) were calculated by subtracting DMR from 100, in which:

$$DML\ (\%) = 100 - DMR \quad (4)$$

2.4. Aerobic stability

To evaluate aerobic stability, 1.5 kg of silage was used in plastic buckets (9 L), which was covered with aluminum foil to prevent excessive moisture loss from the silage and contamination by external elements, in the aerobic stability assay (Tabacco et al., 2009). The silages were exposed to air in an air-conditioned room at 22 °C for seven days. The temperature of the room and silage were recorded every 30 min using dataloggers placed in the center of the buckets. Aerobic stability was defined as the time (h) the silage remained stable before its temperature exceeded the ambient temperature by 2 °C (Moran et al., 1996). The silage average temperature (T; in °C) and maximum temperature (MT; in °C) of each sample and the time to reach the maximum temperature (TMT; in h) were recorded and analyzed as dependent variables in this study.

2.5. Statistical analysis

The assumptions of normality of errors and homogeneity of variance were tested using the Cramer–von Mises and Brown–Forsythe tests, respectively. The results underwent analysis of variance using the

SAS program (Statistical Analysis System, version 9.4) through the PROC MIXED procedure. All results are reported as the least-square means. The means were separated by SLICE option when a significant F-test was detected for the interaction of the factors. Means were compared using Tukey's test at a 5% significance level. The variables relating to silage stored for 120 days, and not exposed to air were analyzed using statistical model I. The variables related to silage with or without inoculant, exposed or not to air at different times, and relocated or NR were analyzed using statistical model II.

$$\text{Model I: } y_{ij} = \mu + MI_i + e_{ij} \quad (5)$$

with y_{ij} being the value of each observation, μ representing the overall average, MI_i the fixed effect of the microbial inoculant, and e_{ij} the residue.

$$\text{Model II: } y_{ij} = \mu + MI_i + ET_j + (ET \times MI)_{ij} + e_{ij} \quad (6)$$

with y_{ij} representing the value of each observation, μ denoting the overall average, MI_i the fixed effect of the microbial inoculant (WI, LPPA, LPLB, and LB), ET_j the fixed effect of air exposure time (0, 12, 24, 48, and 60 h), $(ET \times MI)_{ij}$ the interaction between exposure time × microbial inoculant, and e_{ij} the standard error.

3. Results

3.1. Characterization of the whole-plant corn

At harvest, the chemical composition of the fresh whole-plant corn showed DM and OM contents of 344.5 and 957.4 g kg^{-1}, respectively. The CP, EE, NDF, and NFC contents were 57.5, 33.5, 508.1, and 358.3 g kg^{-1} DM, respectively, and the IVDMD was 638.6 g kg^{-1} DM.

3.2. Whole-plant corn silage with or without microbial inoculant, after 120 days of fermentation (ET 0)

Inoculation significantly altered (P<0.05) the chemical composition of the silages (Table 1). Silages from the WI group had a lower (P<0.01) DM content compared with those from inoculated groups. The CP concentration was significantly higher (P<0.01) in the LPPA silage than in the other silages. Without inoculant, LPLB, and LPPA silages exhibited lower (P<0.01) NDF concentrations than the LB silage. The LB silage had a significantly lower (P<0.01) NFC concentration compared with the other silages. Both LPPA and LB silages experienced significantly lower (P<0.01) DM losses and produced less (P<0.01) effluent than the WI and LPLB silages.

Table 1 - Chemical composition (g kg^{-1} DM), dry matter losses (g kg^{-1}), and effluent losses (g kg^{-1}) of WPCS with or without microbial inoculant after 120 days of fermentation (ET 0)

Variable	Silage[1]				P-value
	WI	LPLB	LPPA	LB	
DM (g kg^{-1})	290.6b	301.3a	296.9a	300.9a	<0.01
CP	61.8b	63.5b	65.8a	57.8b	<0.01
EE	24.3	30.7	31.2	33.1	0.19
NDF	477.0b	479.0b	485.0b	528.0a	<0.01
NFC	390.3a	379.1b	372.1a	335.4b	<0.01
DML	64.2a	55.3b	32.3c	28.4c	<0.01
Effluents	4.02a	3.10a	1.07b	0.51b	<0.01

DM - dry matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; NFC - non-fiber carbohydrate; DML - dry matter losses.
[1] WI - without inoculant; LPLB - *L. plantarum* + *L. buchneri*; LPPA - *L. plantarum* + *P. acidipropionici*; LB - *L. buchneri*.
Means in the same row followed by different letters are different by Tukey's test (P<0.05).

3.3. Whole-plant corn silage with 120 days of fermentation, with or without microbial inoculant, before (ET 0 h) and after different times of aerobic exposure

There was a significant interaction (P<0.01) between ET × MI on yeast counts (Table 2). Yeast counts increased with ET across all treatments. At 60 h, the WI and LPLB silages exhibited higher yeast counts compared with other time points. In LPPA silage, yeast counts were lower at 0, 12, and 24 h but increased at 48 and 60 h. The LB silage showed lower yeast counts at 0, 12, and 24 h, with an increase after 48 h, although counts at 60 h were not significantly different. The WI silage presented higher yeast counts than the LPLB, LPPA, and LB silages after 120 days of storage. The LB silage had the lowest yeast counts at 12, 24, 48, and 60 h when compared with the other silages for the corresponding periods.

An interaction (P<0.01) between ET × MI was observed in mold counts (Table 2). Mold counts were lower in the WI silage at 48 and 60 h and in the LPLB silage at 24, 48, and 60 h compared with other time points. The LPPA silage exhibited lower counts at 48 and 60 h, with no significant difference from counts at 12 and 24 h. The LB silage had the lowest mold count at 60 h of exposure, which was significantly lower than at 0 and 12 h, but not different from 24 and 48 h. The LPLB silage had a lower count of molds at 24 h compared with the other silages at the same ET.

An interaction (P<0.01) between ET × MI was observed in NH_3-N levels (Table 2). Silages inoculated with LPLB, LPPA, and LB and exposed for 0 and 12 h exhibited lower NH_3-N levels compared with the

Table 2 - Microbiological counts (CFU g^{-1}) and fermentative characteristics (g kg^{-1} DM) of WPCS with or without inoculant, before (0 h) and after exposed to air (12, 24, 48, and 60 h)

ET (h)	Silage[1]	Yeasts	Molds	pH	NH_3-N (%TN)	T (°C)	DM	LA	AA	PA	Ethanol	1,2-propaneidol
0[2]	WI	3.50Ac	2.00Aa	3.78	5.94Aa	27.7	290.2c	79.7	32.9	5.43	7.42Aa	3.01
	LPLB	2.50Bd	2.20Aa	3.85	2.36B	27.5	300.0b	73.3	31.7	6.65	4.67Ba	0.91
	LPPA	2.60Bd	1.70Ba	3.83	2.11B	27.7	296.1c	77.5	30.8	6.87	4.43Ba	1.43
	LB	2.60Bc	2.10Aa	3.78	2.55B	27.3	300.8c	87.6	27.8	3.95	3.85Ba	2.04
12	WI	3.60Ac	2.05Aa	3.90	5.32Aa	27.7	339.9b	75.5	29.9	6.68A	2.06Ab	5.28
	LPLB	3.05Bc	1.60Bb	4.08	2.38B	27.7	354.4a	69.4	31.7	7.72A	2.62Ab	1.28
	LPPA	2.90Bb	1.50Bab	3.98	2.11B	27.5	339.5b	69.2	30.8	7.32A	1.94Ab	2.01
	LB	2.50Cc	1.77Bab	3.96	2.55B	27.2	373.0ab	80.3	27.8	3.22B	0.96Bb	0.83
24	WI	3.50Ac	1.65Ab	3.98	4.86Aa	27.6	346.4b	73.6	31.7	9.52A	2.13Ab	3.08
	LPLB	3.30Bc	1.00Cc	4.08	2.06B	27.8	358.6a	76.7	26.4	5.32B	1.19Bb	1.19
	LPPA	3.15Cb	1.40Bab	4.08	2.30B	27.0	337.8b	68.4	28.9	7.87A	3.30Aab	2.01
	LB	2.20Dc	1.45Bbc	3.96	4.11A	27.8	340.8b	75.4	24.5	3.25B	2.47Aab	2.29
48	WI	3.90Bb	1.25Bc	4.08	2.91Ab	28.6	345.9b	63.8	26.7	8.32	1.42b	2.69
	LPLB	3.70Bb	1.00Bc	4.10	1.86B	28.8	363.0a	67.6	26.7	6.67	1.19b	1.06
	LPPA	4.70Aa	1.00Bb	4.10	2.32AB	27.9	369.2a	67.8	24.4	5.22	1.01c	0.73
	LB	3.35Cb	1.40Abc	3.96	4.17A	28.7	369.7ab	64.5	23.9	4.42	0.96b	1.65
60	WI	4.90Aa	1.15c	4.08	2.65Bb	34.2	397.7Aa	57.7	17.6	5.85	0.63b	4.48
	LPLB	4.65Aa	1.00c	4.10	2.01B	30.1	363.4Ba	68.9	25.6	6.50	1.19b	1.56
	LPPA	4.80Aa	1.00b	4.10	2.95B	30.2	388.2Aa	60.2	17.6	4.32	0.93c	0.70
	LB	3.90Ba	1.00c	3.96	4.15A	31.4	383.3Aa	64.5	20.3	4.50	0.96b	1.43
SEM		0.19	0.11	0.06	0.21	0.27	0.73	0.34	0.16	0.08	0.36	0.06
Effects												
MI		<0.01	<0.01	<0.01	<0.01	0.98	0.07	0.02	<0.01	<0.01	<0.01	<0.01
ET		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.24	<0.01	0.40
ET × MI		<0.01	<0.01	0.96	<0.01	0.59	<0.01	0.12	0.08	<0.01	<0.01	0.30

NH_3-N (%NT) - ammonia nitrogen as percentage of total nitrogen; T - temperature; DM - dry matter; LA - lactic acid; AA - acetic acid; PA - propionic acid; MI - microbial inoculant; ET - exposure time; ET × MI - interaction of factors.
[1] WI - without inoculant; LPLB - *L. plantarum* + *L. buchneri*; LPPA - *L. plantarum* + *P. acidipropionici*; LB - *L. buchneri*.
[2] Silage after 120 days of fermentation; not exposed to air.
Uppercase letters compare microbial inoculants within each time and lowercase letters compare times within each inoculant, in which means followed by different letters are different by Tukey's test (P<0.05).

WI silage at corresponding ET. The inoculant did not influence NH_3-N levels in LPLB, LPPA, and LB silages; however, it reduced NH_3-N in the WI silage at the longest ET. The NH_3-N content in the WI silage was lower after 48 and 60 h of exposure than at 0, 12, and 24 h.

An interaction (P<0.01) between ET × MI was observed in DM levels (Table 2). Silage inoculated with LPLB and exposed to air for 60 h exhibited lower DM content compared with that of the other treatments at the same ET. The inoculant did not influence the DM content of silages opened after 120 d and exposed for 12, 24, and 48 h; however, ET increased the DM content of the silages. Starting from 12 h of exposure, the DM content stabilized over time for silages inoculated with LPLB. The LPPA silage demonstrated higher DM content at 48 and 60 h relative to other time points. The LB silage presented a greater DM at 60 h compared to 0, 12, and 24 h, yet it was comparable to that at the 48 h time point. The WI silage showed a higher DM content after 60 h of exposure than at other time intervals.

There was an interaction (P<0.01) between ET × MI in propionic acid levels (Table 2). The LB silage exhibited lower propionic acid content after 12 h of exposure compared with the other treatments at the same duration. Furthermore, at 24 h, LB silage also showed a reduced concentration of propionic acid relative to WI and LPPA silages, yet it was comparable to LPLB silage. A similar significant interaction (P<0.01) was observed for ethanol levels (Table 2). Inoculation did not influence ethanol concentration in silages after 48 and 60 h of exposure; however, it decreased with prolonged ET. The LB silage had the lowest ethanol concentration at 12 h compared with the other silages at the same interval. Without inoculant and LPLB silages exhibited lower ethanol concentrations at 12, 24, 48, and 60 h, whereas LPPA silage had reduced ethanol content at 48 and 60 h compared with other intervals. The minimal ethanol concentration in LB silage was noted at 12, 48, and 60 h of exposure, showing no significant difference from that of the 24 h interval.

Exposure time significantly affected (P<0.01) the pH values of the silages (Table 2). The pH increased from 3.78 to 3.98 as ET extended from 0 to 12 h, which was not significantly different from subsequent times (24 h = 4.08; 48 h = 4.05; 60 h = 4.05). Inoculation also significantly influenced (P<0.01) silage pH (Table 2), with WI and LB silages exhibiting the lowest pH values (3.98 and 3.90, respectively), in contrast with the higher pH values of the other inoculated silages (LPLB = 4.04; LPPA = 4.02). Silage temperature was significantly affected (P<0.01) by ET alone (Table 2). As exposure progressed, silage temperature initially remained stable at 27.4 °C for 0, 12, and 24 h, but increased at 48 h (28.5 °C) and 60 h (31.5 °C).

Exposure time significantly affected the concentrations of lactic acid (P<0.01) and acetic acid (P<0.01; Table 2), with both decreasing as ET increased. Lactic acid concentration decreased from 79.5 g kg^{-1} DM at the time of opening to 65.9 g kg^{-1} DM at 48 h and 62.8 g kg^{-1} DM at 60 h, with no significant difference between the 12 h (73.6 g kg^{-1} DM) and 24 h (73.4 g kg^{-1} DM) measurements. Similarly, acetic acid concentration was 30.8 g kg^{-1} DM at the time of opening and decreased to 25.4 g kg^{-1} DM at 48 h and 20.3 g kg^{-1} DM at 60 h, with the 12 h (30.0 g kg^{-1} DM) and 24 h (27.9 g kg^{-1} DM) values not differing significantly. Inoculation also significantly affected the levels of lactic acid (P<0.01) and acetic acid (P<0.01; Table 2). The LB silage had the highest lactic acid concentration (74.4 g kg^{-1} DM), followed by LPLB (71.1 g kg^{-1} DM), with no significant difference from the WI (70.0 g kg^{-1} DM) and LPPA (68.6 g kg^{-1} DM) silages. For acetic acid, the LB silage had the lowest content (24.7 g kg^{-1} DM) compared with the WI (27.7 g kg^{-1} DM), LPLB (28.4 g kg^{-1} DM), and LPPA (26.5 g kg^{-1} DM) silages.

The microbial inoculation significantly affected (P<0.01) the 1,2-propanediol content (Table 2). The highest concentration of 1,2-propanediol was observed in the WI silage (4.40 g kg^{-1} DM), compared with the silages LPLB (1.20 g kg^{-1} DM), LPPA (1.20 g kg^{-1} DM), and LB (1.70 g kg^{-1} DM).

3.4. Whole-plant corn silage was relocated or not, with or without MI, after 210 days of fermentation

The LAB count, pH, and lactic acid content of the silages were unaffected (P>0.05) by MI, ET, or their interaction (Table 3). However, there was a significant interaction (P<0.01) between ET × MI for mold

counts (Table 3). Mold counts varied over the exposure period only in silages inoculated with LPPA, with lower counts at NR, 12, and 24 h compared with 48 and 60 h. When opened after 210 days and exposed for 12 and 24 h, the WI silage exhibited higher mold counts than the silages inoculated at the corresponding times. Conversely, the WI silage did not differ from the LPPA silage at 48 and 60 h, in which both exhibited higher mold counts compared with the LPLB and LB silages during the same period.

There was a significant interaction (P<0.01) between ET × MI on the NH_3-N content (Table 3). Inoculation with LPLB and LB did not affect the NH_3-N content over time compared with the other treatments. At NR, 12, and 24 h, the WI and LB silages exhibited higher NH_3-N content than the LPLB and LPPA silages at corresponding times. The highest NH_3-N content was observed in the LPPA and LB silages after 48 h of exposure and in the LPPA silage alone at 60 h when compared with the other treatments at the same intervals. The WI silage presented lower NH_3-N content at 48 and 60 h of exposure, which was not significantly different from the content at 24 h. Inoculation with LPPA resulted in an increase in NH_3-N content over time, peaking at 60 h, although this was not significantly different from the levels at NR, 12, and 48 h.

An interaction (P<0.01) between ET × MI was observed in the propionic acid content (Table 3). When relocated and exposed for 12 h, the LB silage exhibited a lower propionic acid content compared with that of the other silages at the same ET. Both relocated LPLB and LB silages demonstrated decreased

Table 3 - Microbiological counts (CFU g^{-1}) and fermentative characteristics (g kg^{-1} DM) of WPCS with or without inoculant, non-relocated (NR) and relocated (after exposed to air of 12, 24, 48 or 60 h)

ET (h)	Silage[1]	LAB	Yeasts	Molds	pH	NH_3-N (%TN)	LA	AA	PA	Ethanol	1,2-propanediol
NR[2]	WI	2.99	2.50	4.02A	3.92	2.70Aa	74.9	32.9	5.08Bb	7.36Aa	6.42Aa
	LPLB	5.66	2.20	3.63B	3.82	1.62B	60.4	32.7	6.41Aab	3.63Ac	0.61B
	LPPA	4.75	2.87	3.29Bb	3.82	2.09Bab	70.5	32.0	4.76Bb	5.26Ba	0.91Bb
	LB	9.97	2.33	3.35B	3.87	3.50A	73.1	26.7	5.20Ba	1.89Da	2.27Ba
12	WI	3.31	2.00	4.06A	3.79	2.73Aa	77.2	29.5	6.13Aa	1.57Ab	3.4Ab
	LPLB	4.40	2.00	3.63B	3.82	1.68B	77.5	29.3	7.29Aa	0.69Bb	0.61B
	LPPA	4.20	2.97	3.66Bb	3.82	2.25Bab	72.5	30.0	6.30Aa	0.68Bc	0.42Bb
	LB	3.57	2.40	3.69B	3.76	2.90A	83.7	28.5	3.98Bb	0.93Bb	2.12Aa
24	WI	3.66	2.20	4.12A	3.82	2.42Aab	73.0	34.1	8.18Aa	0.69c	2.33Abc
	LPLB	3.67	2.08	3.81B	3.74	1.61B	76.4	25.1	7.00Aa	0.74b	1.43A
	LPPA	4.36	3.08	3.70Bb	3.76	1.65Bb	76.3	30.2	6.19Aa	0.72c	0.27Bb
	LB	3.76	2.28	3.50B	3.78	3.17A	66.0	27.0	3.93Bb	0.89b	1.62Ab
48	WI	4.12	2.50	4.44A	3.84	2.06Bb	72.3	26.8	7.69Aa	0.67c	2.21b
	LPLB	4.52	2.93	3.82B	3.93	2.00B	64.3	27.9	4.21Bc	0.69b	1.60
	LPPA	4.46	2.93	4.71Aa	3.79	2.19Aab	65.1	26.2	5.26Ba	0.83c	0.36b
	LB	3.94	2.75	3.32B	3.82	2.33A	74.6	23.0	3.51Bb	0.68c	1.74b
60	WI	5.12	2.00	4.60A	3.94	2.05Bb	66.1	26.0	7.32Aa	0.66Bc	0.60Cc
	LPLB	3.87	2.29	3.24B	3.85	1.76B	69.3	24.8	5.49Ab	0.64Bb	1.36B
	LPPA	4.58	2.86	4.46Aa	3.97	3.51Aa	73.1	26.7	3.52Bb	1.91Ab	2.82Aa
	LB	3.77	2.95	3.24B	3.83	2.61B	69.7	19.8	3.95Bb	0.85Bb	0.34Cc
SEM		0.68	0.22	0.16	0.04	0.17	0.24	0.15	0.56	0.13	0.04
Effects											
MI		0.16	<0.01	<0.01	0.68	<0.01	0.45	<0.01	<0.01	<0.01	<0.01
ET		0.41	0.05	<0.01	0.34	0.01	0.06	<0.01	<0.01	<0.01	<0.01
ET × MI		0.28	0.34	<0.01	0.96	<0.01	0.16	0.16	<0.01	<0.01	<0.01

LAB - lactic acid bacteria; NH_3-N (%NT) - ammonia nitrogen as percentage of total nitrogen; LA - lactic acid; AA - acetic acid; PA - propionic acid; MI - microbial inoculant; ET - exposure time; ET × MI - interaction of factors.
[1] WI - without inoculant; LPLB - *L. plantarum* + *L. buchneri*; LPPA - *L. plantarum* + *P. acidipropionici*; LB - *L. buchneri.*
[2] Silage after 210 days of fermentation.
Uppercase letters compare microbial inoculants within each time and lowercase letters compare times within each inoculant, in which means followed by different letters are different by Tukey's test (P<0.05).

levels of propionic acid after 24 h of exposure, in contrast with the other silages at equivalent exposure durations. Neither inoculated nor relocated silages displayed an increase in propionic acid content from the opening time until 12 h of exposure, with no significant difference from subsequent times. The LPLB silage, once relocated, showed a reduction in propionic acid content after 48 h of exposure when compared with other durations. The LPPA silage experienced an increase in propionic acid from the time of opening until 12 h, which then stabilized until 48 h, followed by a decrease at 60 h of exposure; this pattern did not significantly differ from the levels in the opening at 210 days. The propionic acid content in silage inoculated with LB decreased as ET increased, with the highest levels observed at the time of opening, which differed from subsequent times.

An interaction ($P<0.01$) between ET × MI was observed regarding ethanol content (Table 3). Neither 24 nor 48 h of exposure altered the ethanol content in silages with or without inoculation and/or relocation. Silage inoculated with LB and NR exhibited lower ethanol content compared with that of the other silages at the same time point. Conversely, relocated silages inoculated with LPLB, LPPA, and LB showed no significant differences in ethanol content after 12 h of exposure. Relocating after 60 h resulted in a higher ethanol content in the LPPA silage relative to other silages at the same ET. Ethanol content was highest at the opening time for all silages compared with other time points. The ethanol content of relocated and inoculated LPLB and LPPA silages remained consistent across 12, 24, 48, and 60 h of exposure. The WI silage exhibited lower ethanol content at 24, 48, and 60 h compared with NR and 12 h. The LPPA silage showed a reduced ethanol content at 12, 24, and 48 h of exposure relative to other time points.

An interaction ($P<0.01$) between ET × MI was observed in the 1,2-propanediol content (Table 3). The 1,2-propanediol content in the silages was unaffected at 48 h exposure. However, relocating the silages after 24 h of exposure resulted in those inoculated with LPPA having a lower 1,2-propanediol content compared with that of the others at the same point. Conversely, both WI and LB silages exhibited lower 1,2-propanediol content regardless of whether they were relocated after 60 h of air exposure. The 1,2-propanediol content in silages inoculated with LPLB remained unchanged with increased ET. Without inoculant silages demonstrated lower 1,2-propanediol content at both 24 and 60 h of exposure, whereas LPPA silage had a higher content at 60 h. After 60 h, the LB silage presented a reduced 1,2-propanediol content.

Yeast counts were significantly altered ($P = 0.05$) by ET (Table 3). An exposure of 48 h resulted in higher yeast counts in silages (2.78 log CFU g^{-1}) compared with other durations (NR = 2.47; 12 h = 2.35; 24 h = 2.42; 60 h = 2.55 log CFU g^{-1}). Inoculation significantly affected ($P<0.01$) yeast counts (Table 3). Silages inoculated with LPPA (2.96 log CFU g^{-1}), LPLB (2.30 log CFU g^{-1}), and LB (2.56 log CFU g^{-1}) exhibited higher yeast counts than the WI silage (2.24 log CFU g^{-1}).

The acetic acid content was significantly affected ($P<0.01$) by ET (Table 3). As ET increased, the acetic acid content decreased to 31.1 g kg^{-1} DM initially, which was comparable to the levels at 12 (29.3 g kg^{-1} DM) and 24 h (29.1 g kg^{-1} DM). This content further declined to 25.9 g kg^{-1} DM at 48 h and to 24.3 g kg^{-1} DM after 60 h of exposure. Additionally, inoculation significantly altered ($P<0.01$) the acetic acid content in the silages (Table 3). The LB silage exhibited a lower acetic acid content (24.9 g kg^{-1} DM) in comparison with the other silages, with the WI at 29.9 g kg^{-1} DM, LPLB at 27.9 g kg^{-1} DM, and LPPA at 29.0 g kg^{-1} DM.

The NDF content of the silages was unaffected by the inoculant, ET, or their interactions ($P>0.05$) (Table 4). However, the interaction between ET × MI significantly influenced the DM content of the silages ($P = 0.04$) (Table 4). Specifically, the LB silage at 48 h and the WI silage at 60 h exhibited higher and lower DM content, respectively, compared with other treatments at equivalent ET. The DM content in the WI, LPLB, and LB silages was reduced at NR, 12, and 24 h relative to other times. Inoculation did not affect the DM content of silages at NR, 12, and 24 h of exposure.

The CP content changed significantly ($P = 0.02$) due to the interaction between ET × MI (Table 4). The LB silage exhibited the lowest CP content initially and after 60 h of exposure. In contrast, the LPLB silage showed a higher CP content at 12, 24, and 48 h relative to the other silages at corresponding ET. Inoculation with LPLB resulted in reduced CP at 60 h of exposure compared with other time points.

Exposure time × microbial inoculant significantly affected the NFC content of the silages (P = 0.01) (Table 4). As ET increased, NFC concentration in LB silage varied; NR, 48, and 60 h ET were similar, exhibiting lower NFC content than at 12 and 24 h. Furthermore, the LB silage exposed for 24 h had a higher NFC content than the other silages at the same exposure duration.

Exposure time and microbial inoculant interaction significantly affected IVDMD in silages (P<0.01; Table 4). Extended ET during relocation only altered the IVDMD of LB silages, diminishing their digestibility, with the lowest values observed at 48 and 60 h compared with shorter ET. Microbial inoculation resulted in higher IVDMD for LB silages at the time of silo opening, surpassing the digestibility of other silages upon opening.

The interaction between ET × MI significantly affected the DML of silages (P<0.01; Table 4). While extending the ET alone did not alter the DML of silages, either with or without the MI, the inoculant did influence DML when silages were exposed for 60 h. Inoculated silages (LPLB, LPPA, LB) exhibited lower DML compared with the WI silage.

The MT of the silages was significantly altered (P<0.01) by the interaction between ET × MI (Table 4). Extending the ET did not influence the MT of silages inoculated with LPPA, nor did inoculation with LPPA affect the MT of silages exposed for 12, 48, and 60 h. The LB silage and the WI silage exhibited higher and lower MT, respectively, when NR and exposed for 24 h, compared with the other silages at the same ET. The interaction between ET × MI significantly affected the TMT (P<0.01) of the silages (Table 4). The TMT of the silages was altered only when they were inoculated or not and NR, with the

Table 4 - Chemical composition (g kg^{-1} DM), IVDMD, DML, and aerobic stability of WPCS with or without inoculant, non-relocated (NR) and relocated (after exposed to air of 12, 24, 48, or 60 h)

ET (h)	Silage[1]	DM	CP	NDF	NFC	IVDMD	DML	AS (h)	MT (°C)	TMT (h)
NR[2]	WI	303.8b	65.4B	452.2	410.0	646.4B	46.6	122	28.9Ba	58Bb
	LPLB	308.6	75.0Aa	411.2	444.7	577.2C	41.3	105	28.3Ba	132A
	LPPA	316.6b	62.4B	425.4	459.7	598.9C	58.7	70	28.2B	124A
	LB	312.2b	55.3C	449.9	441.5b	674.7Aa	48.4	71	33.0Aa	99Ab
12	WI	309.1b	65.5B	448.9	409.1	641.0	102.6	137	26.3ab	153a
	LPLB	316.9	74.6Aa	427.2	432.9	616.8	96.4	166	24.9a	157
	LPPA	322.0b	64.8B	423.5	456.5	607.9	68.3	149	27.0	147
	LB	320.0b	64.1B	407.0	473.9a	640.3b	72.4	94	30.3ab	134a
24	WI	316.1b	60.8B	450.9	412.7B	649.7	96.7	168	22.7Bb	164a
	LPLB	321.1	74.4Aa	422.8	439.3B	590.7	98.7	142	26.3Aa	151
	LPPA	325.8b	60.4B	457.6	412.4B	545.3	45.8	143	25.2A	162
	LB	326.5b	62.5B	387.1	489.0Aa	624.1b	50.9	97	29.3Aab	131a
48	WI	334.9Ba	59.9B	435.8	432.0	599.3	81.5	168	25.0b	146a
	LPLB	318.3B	72.6Aa	464.8	404.7	631.9	93.7	168	23.0b	108
	LPPA	335.5Ba	60.9A	456.3	421.1	685.8	60.8	160	24.8	165
	LB	346.7A	58.7B	444.1	437.1b	588.4c	61.5	103	25.5b	164a
60	WI	335.2Ba	60.8B	407.9	459.2	570.6	126.7A	165	23.5b	162a
	LPLB	344.1A	64.3Ab	418.1	459.8	591.4	62.5B	168	22.9b	153
	LPPA	350.7Aa	60.1B	447.8	429.4	627.5	31.3B	157	23.7	161
	LB	363.6Aa	56.3C	423.2	454.4b	592.6c	93.6B	123	27.1b	160a
SEM		0.50	0.17	1.42	1.41	1.98	0.44	9.29	0.96	14.9
Effects										
MI		<0.01	<0.01	0.12	<0.01	0.12	<0.01	<0.01	<0.01	0.23
ET		<0.01	<0.01	0.14	0.07	0.07	<0.01	<0.01	<0.01	<0.01
ET × MI		0.04	0.02	0.06	0.01	<0.01	<0.01	0.08	<0.01	<0.01

DM - dry matter; CP - crude protein; NDF - neutral detergent fiber; NFC - non-fibrous carbohydrates; IVDMD - *in vitro* dry matter digestibility (g kg^{-1} DM); DML - dry matter losses (g kg^{-1} DM); AS - aerobic stability; MT - maximum temperature; TMT - time to reach maximum temperature; MI - microbial inoculant; ET - exposure time; ET × MI - interaction of factors.
[1] WI - without inoculant; LPLB - *L. plantarum* + *L. buchneri*; LPPA - *L. plantarum* + *P. acidipropionici*; LB - *L. buchneri*;
[2] Silage after 210 days of fermentation.
Uppercase letters compare microbial inoculants within each time and lowercase letters compare times within each inoculant, in which means followed by different letters are different by Tukey's test (P<0.05).

TMT being lower for the WI silage compared with the others at the same ET. The TMT of the WI and LB silages was affected by advancing ET. The TMT was lower for the NR silage compared with other ET for both silages.

Aerobic stability was significantly affected (P<0.01) by ET (Table 4). As ET increased, so did the aerobic stability of the silages, with the lowest stability at NR (92 h) compared with subsequent times (12 h = 136 h; 24 h = 137 h; 48 h = 150 h; 60 h = 153 h), which did not significantly differ from one another. The MI also significantly altered (P<0.01) the aerobic stability of the silages (Table 4). Inoculation with LB resulted in silages with 97 h of stability, which was less than that of the other inoculated silages (WI = 152 h; LPLB = 150 h; LPPA = 136 h). The WI and LPLB silages exhibited the longest stability times.

4. Discussion

4.1. Whole-plant corn silage with or without microbial inoculant, after 120 days of fermentation (ET 0)

Although corn plants present desirable characteristics (DM, fermentable sugars, and low buffering capacity) for ensilage process, many factors can affect the fermentation profile that occurs in the silo. Among them, the types and population of epiphytic microorganisms on the plant have can affect silage characteristics (Knief et al., 2010; Vogel et al., 2016). In the current study, the population of epiphytic microorganisms on the whole-plant corn was probably composed by heterofermentative microorganisms, resulting in a production of several end-products fermentation, especially in WI silages. Heterofermentative LAB can convert glucose into lactic acid, ethanol, and carbon dioxide, in addition to producing other metabolites such as acetic acid (Muck et al., 2018; Benjamim da Silva et al., 2021). However, the inoculation with LB showed an unexpected result with lower amino acid concentration; however, a possible explanation about that is unclear with the data from the present study.

Occasional DML can be observed during forage storage in silages (Borreani et al., 2018). Both microbial metabolism and plant characteristics at harvest can contribute to increased DML, consequently reducing DM content. Regarding microbial metabolism, some microorganisms are capable of degrading carbohydrates (e.g., glucose) and produce organic acids and CO_2 (e.g., heterolactic pathway), which reduce DM concentration (Borreani et al., 2018). Regarding plant characteristics, a high humidity concentration at the plant harvesting can affect effluent production, increasing DML during storage time (Pahlow et al., 2003; Borreani et al., 2018; Ferraretto et al., 2018). Both CO_2 production by microorganisms and effluent production can affect DM concentration in silages.

However, LAB inoculation is a technology used globally in ensilage to minimize the negative impacts of fermentation on the quality of ensiled feed by reducing DML. This explains the lower DML and higher DM concentration in the current study, which alters nutrient content, similar to the results observed by Oliveira et al. (2017). Effluent production during storage is directly associated with the DM content of silages and the fermentation profile. The improved DM recovery in silages inoculated with LPPA and LB likely explains the reduced effluent production observed in these silages. Furthermore, the LPPA and LB inoculated silages exhibited low pH values (< 4.0), which may have diminished the extent of cell wall rupture due to decreased enzymatic activity in an acidic environment (Madigan et al., 2016). Microbial inoculant promoted the production of antifungal acids during fermentation. The *P. acidipropionici* synthesizes propionic acid from glucose (Ranaei et al., 2020), which inhibits microbial growth, particularly that of yeast (Moon, 1983).

4.2. Whole-plant corn silage with 120 days of fermentation, with or without microbial inoculant, after different times of aerobic exposure

Aerobic exposure and the duration of silage exposure outside the silo can significantly impact the quality of ensiled material before the relocation process begins. A primary issue affecting WPCS is

its susceptibility to deterioration upon contact with atmospheric air (Woolford, 1990; Borreani et al., 2018). As aerobic microorganisms, yeasts are chiefly responsible for WPCS spoilage and tend to proliferate during exposure, particularly in hot climates (Pahlow et al., 2003; Bernardes et al., 2018). This proliferation accounts for the increased yeast counts observed in this study. Moreover, yeasts metabolize lactic acid (Rooke and Hatfield, 2003), leading to its decreased concentration. Our findings indicated that extended aerobic exposure reduced the lactic acid content in the silages, likely due to yeast metabolism.

Another consequence of yeast growth is the elevation of silage temperature, stemming from the heat released during their metabolic activity in the presence of oxygen (Kung et al., 2018). As aerobic ET increased, so did silage temperature, which can be attributed to the heightened presence and metabolic activity of yeasts in the air-exposed mass. The tropical location of the WPCS in this study may have further stimulated yeast growth, as higher temperatures can expedite yeast metabolism and proliferation (Van Uden, 1984).

The use of heterolactic LAB inoculants can reduce yeast metabolic activity due to the antifungal action of acetic acid formed during storage (Rooke and Hatfield, 2003). Generally, inoculation with heterofermentative LAB increases acetic acid concentration. However, silages inoculated with LB in this study had lower acetic acid content upon silo opening; however, the real explanation of this effect is unclear based on the present data in the study. During aerobic exposure, the decrease in acetic acid is likely related to compound volatilization over time (Weiss and Auerbach, 2012). Despite this, the yeast population was lower in the inoculated silages, which can be probably explained by the higher proliferation of LAB, evidenced by the concentrations of lactic acid and acetic acid in the ET 0 h. During the fermentation process the LAB compete for substrate with the yeasts (Jonsson and Pahlow, 1984), and this competition may have reduced the initial yeast population, influencing the proliferation of these microorganisms during aerobic exposure, especially in silages with LB. Additionally, the presence of acetic acid (10.0–30.0 g kg^{-1} DM) with an antifungal effect may have inhibited yeast growth in the silages in the ET 0 (Kung et al., 2018).

Similarly, filamentous fungi contribute to the degradation process in WPCS but are regarded as secondary microorganisms. Unlike yeast, they do not metabolize lactic acid as a substrate (Woolford, 1990; Rooke and Hatfield, 2003). However, the population of filamentous fungi decreased over time in contrast to that of yeast. This reduction may be attributed to the acetic acid presence and the acidic conditions within the silages, which, when combined, can inhibit their growth more effectively, even in the presence of oxygen.

The pH of silages remained low during aerobic exposure due to the significant lactic acid content (~6%), thus preserving the acidic characteristic of the feed. The pH is directly related to the presence of lactic acid and typically increases as the concentration of the acid diminishes (Kung et al., 2018). Nevertheless, the silages in this study sustained an acidic pH, even with decreased lactic acid levels. This suggests that the lactic acid concentration was adequate to keep the pH within the optimal range (3.6–4.2) for WPCS (Kung et al., 2018), even during oxygen exposure. Notably, silages WI and those inoculated with LB exhibited lower pH values. Moreover, LB silage had a higher lactic acid content. The elevated lactic acid concentration in LB silages during exposure likely resulted from its higher initial LAB levels when the silos were opened. Additionally, the presence of antifungals may have inhibited yeast growth, thereby preserving the lactic acid in the silage throughout the exposure and, as a result, promoting lower pH values in LB silages.

In WPCS, the presence of NH_3-N is related to the ensiling process, in which the mechanical processing of corn grains breaks the pericarp and disrupts the starch-protein matrix, thus facilitating proteolytic activity and increasing starch digestibility (Ferraretto et al., 2018). Proteolysis during storage can result from microbial activity corn kernel enzymes and fermentation acid solubilization (Junges et al., 2017). This response is intensified with prolonged storage (Kung et al., 2018). Creating an anaerobic environment and rapidly and effectively lowering pH values are key to diminishing the metabolic activity of these microorganisms during storage (Pahlow et al., 2003). The application of LAB-based

inoculants can facilitate a swift pH decline, thereby more quickly inhibiting the activity of Bacillus and Clostridium during storage, which may account for the reduced NH_3-N levels observed when the silos were opened. As with other organic compounds, NH_3-N is highly volatile at elevated temperatures (Weiss and Auerbach, 2012; Kung et al., 2018), which could explain the decreased NH_3-N levels observed throughout exposure in WI silages.

Silage is also subject to uncontrollable climate-related factors, such as temperature, when exposed to air. Prolonged exposure allows oxygen to permeate the silage for extended periods and subjects it to ambient temperature. This likely accounts for the observed rise in silage DM content during aerobic exposure, attributable to moisture loss from water evaporation within the silage. Propionic acid formation in WPCS can result from the metabolism of propionic acid bacteria (Rooke and Hatfield, 2003). However, these bacteria, particularly Propionibacterium species, are typically found in low concentrations due to their limited tolerance for acidic environments. The consistent concentration of propionic acid in silages upon opening may be attributed to the inefficient production of this organic compound during ensiling, even when WPCS is inoculated with LPPA. This inefficiency could be due to the inhibited growth of Propionibacterium, caused by the rapid pH decrease in the initial days of ensiling (Filya et al., 2006), which is particularly pronounced when *L. plantarum* is present. Notably, silages exposed for 12 h had higher propionic acid levels in the WI, LPLB, and LPPA treatments, whereas those exposed for 24 h exhibited lower levels in the LPLB and LB treatments.

Propionibacterium bacteria are aerotolerant and primarily produce propionic acid through the Wood–Werkman cycle fermentation pathway, utilizing glucose as a substrate (Ahmadi et al., 2017). Temperature is a crucial factor that can influence the entire fermentation process (Gonzalez-Garcia et al., 2017). Various genera of Propionibacterium have been examined, with each requiring an optimal temperature range that can vary between 14 and 40 °C or 30 and 40 °C (Ranaei et al., 2020). Although no interaction effect for silage temperature was observed in the current study, it is likely that different genera of Propionibacterium present in the silage were affected by the temperature during aerobic exposure. However, further studies are necessary to identify and assess the growth of such microorganisms in these silages.

Ethanol production in WPCS is associated with the metabolism of various microorganisms, particularly yeast and *L. buchneri* (Oude Elferink et al., 2001; Rooke and Hatfield, 2003). Inoculation with LAB tends to decrease ethanol production by inhibiting yeast growth during storage and feed-out (Narendranath et al., 1997). Ethanol, a highly volatile alcohol, can evaporate more rapidly under certain environmental conditions (Weiss and Auerback, 2012; Muck et al., 2018), likely accounting for the observed decrease in ethanol levels over time. Although yeast counts increase with air exposure, suggesting potential for higher ethanol production, the observed reduction in ethanol content with extended ET is probably due to its enhanced volatilization rather than a decrease in microbial production.

A variety of microorganisms can produce 1,2-propanediol (Tao et al., 2021). Bacteria of the genus *L. buchneri* can perform the anaerobic conversion of lactic acid into acetic acid and 1,2-propanediol (Oude-Elferink et al., 2001). However, no difference was observed between silages with and without inoculant at the time of opening (ET 0 h) to 120 days of storage. Similarly, Tabacco et al. (2011) evaluated the effect of two LB strains and found that the concentrations of acetic acid and 1,2-propanediol, indicative of LB activity, did not differ between treated and untreated silages. Conversely, microorganisms of the species *Lactobacillus diolivorans* can degrade 1,2-propanediol into propionic acid, thereby reducing its concentration in silages (Krooneman et al., 2002).

In the WI silage, non-inoculation likely resulted in a higher content of 1,2-propanediol due to the metabolic activity of other microorganisms, such epiphytic LB population and the storage time, suggesting that different strains of LB can produce different metabolites, but were not quantified in the present study. These findings corroborated with those of Oude-Elferink et al. (2001), who detected this alcohol in untreated corn silages stored for extended periods (over 120 d), attributing it to heightened LB activity.

4.3. Whole-plant corn silage, relocated or not, with or without MI, after 210 days of fermentation

The characteristics of the relocated silages were influenced by the quality of the fresh silage and the ET to air, unlike the NR silages WI. The LAB count, pH of the silages, and lactic acid concentration are variables directly related to the fermentation profile. Lactic acid formation from LAB metabolism reduces the silage pH (Pahlow et al., 2003). The relocated silages displayed pH values optimal for WPCS, indicating an acidic feed. It is important to note that LAB requires available substrates, such as glucose, for metabolism and tends to halt growth in acidic environments (Rooke and Hatfield, 2003). The concentration of soluble carbohydrates decreases with time of exposure (Gerlach et al., 2013). Therefore, although the relocated silages were exposed to air, this process did not alter the LAB population in the silages, and consequently did not alter the lactic acid concentration and pH. Thus, it is believed that residual soluble carbohydrates may have been sufficient to maintain the existing LAB population, but this variable was not determined in this study.

Unlike the case with lactic acid, acetic acid production was altered in silages even after relocation. The disparity between silages may be directly linked to variations in acetic acid levels following exposure and subsequent relocation. The formation of acetic acid in these silages could be tied to the anaerobic conversion of lactic acid into acetic acid by *L. buchneri*. Although LB was inoculated into only one silage, *L. buchneri* species are commonly present in the epiphytic population of WPCS (Lin et al., 1992; Santos et al., 2019; Peng et al., 2021). The higher acetic acid concentration in the NR and WI silage might be attributed to the absence of relocation, which prevented acetic acid volatilization during exposure. Despite not being inoculated, the elevated acetic acid levels in these silages could also stem from the accumulation of this organic compound during fermentation. The increased exposure of silages before relocation likely led to more significant volatilization of acetic acid, resulting in lower concentrations in the relocated silages.

The increased yeast count in silages relocated after 48 h of exposure can be attributed to the microbial population of these organisms present at the time of relocation. Moreover, the diminished concentration of acetic acid in silages exposed for 48 h may have led to reduced inhibition of microbial growth (Muck et al., 2018). In contrast, the lower mold count in silages inoculated with LPPA and NR, and exposed from 24 h could be due to the presence of antifungal acids. However, the mold count in relocated silages was significantly higher than in the silage before relocation, suggesting that relocation does not positively impact mold count.

The increased formation of NH_3-N in WI and LB silages NR and silages after 12, and 24 h of exposure likely resulted from the microbial degradation, corn kernel enzymes, and fermentation acid solubilization (Junges et al., 2017). Lower propionic acid content was observed in silages inoculated with LPPA, which may be attributed to the inhibitory effect of pH on *P. acidipropionici* growth, which is not adapted to acidic conditions (<4). Therefore, silage pH below 4 may have inhibited the growth of the bacteria and, consequently, the propionic acid production (Michel et al., 2017). The presence of this acid in other silages could be due to the activity of different microorganisms capable of producing propionic acid (Krooneman et al., 2002). The decrease in ethanol concentration in silages is likely due to the suppression of yeast growth during fermentation, facilitated by acetic acid and the acidic environment within the silages (Pahlow et al., 2003). Similarly, the 1, 2-propanediol content in silages exposed to air could result from the metabolic processes of *L. buchneri* and other alcohol-producing microbes (Oude-Elferink et al., 2001; Tao et al., 2021). The elevated levels of ethanol and 1,2-propanediol in NR and WI silages might be linked to the absence of aerobic exposure, preventing volatilization of these compounds before relocation and leading to their accumulation in these silages.

It is possible to observe that the DM content of silages presented lower values than those obtained at the time of relocation. Whether through conventional ensilage using fresh forage or relocation from silage, the conservation process causes fermentation losses (Borreani et al., 2018). However, no differences in DML were observed in silages up to 48 h of exposure. Unexpectedly, higher DM and DML values were observed in silages with 60 h of exposure. Therefore, it can be assumed that the

DM concentration in silages after relocation is linked to the DM content at the time of relocation. The higher DML values in the WI silage may be related to the higher mold count. Furthermore, acetic acid production by heterofermentative LAB, which uses the pentose metabolic pathway, results in CO2 as one of the fermentation products, leading to an increase in DML (Muck et al., 2018). Silages relocated and inoculated with LB could maintain higher DM content after 48 h, likely due to the control of undesirable microorganisms during storage.

Proteolytic activity in corn silages is the result of several mechanisms, including grain proteases, microorganisms and solubilization by fermentation acids (Simpson, 2001; Junges et al., 2017). Saylor et al. (2020) reported increased proteolysis during storage due to higher concentrations of soluble protein and NH_3-N in silages. Additionally, *Lactobacillus* bacteria may contribute to the reduction of CP concentration in silages. These microorganisms can utilize alternative substrates, such as nitrogen compounds, in the absence of soluble carbohydrates for fermentation (Pahlow et al., 2003), which may account for the lower CP content in silages inoculated with LB. The levels of other nutritional components in the feed influenced the NFC content. Despite no changes in NDF content, the increased NFC concentration in silages inoculated with LB and relocated after 12 and 24 h of exposure could be attributed to the reduced CP levels in these silages. It was observed that the use of inoculant led to a decrease in NFC, a highly digestible organic compound, after 48 h of exposure, resulting in decline in IVDMD, like that observed by Anjos et al. (2018). The action of molds and yeasts reduces soluble carbohydrates, which can reduce NFC and proportionally increase fibrous fractions (Michel et al., 2017). However, in this study, no change in NDF was observed after 48 h of exposure. The highest IVDMD in LB silages and NR, is associated with the digestible components of the NDF fraction of the plant. Although LB silages exhibited higher NDF content upon silo opening after 120 days of storage, the NDF may have contained more digestible fractions, potentially enhancing the IVDMD of the silages after 90 days of relocation.

Yeasts, microorganisms responsible for silage degradation in aerobic environments, can have their growth controlled by acetic acid (Muck et al., 2018). Interestingly, silages were more stable after 12 h of exposure, likely due to reduced yeast counts and the presence of acetic acid. However, inoculation with LB, which promotes acetic acid formation, unexpectedly resulted in silages with lower stability. This may be attributed to a lower acetic acid concentration in these silages, leading to increased activity of spoilage microorganisms. This outcome may also relate to the MT recorded during the stability test. The rise in temperature is directly linked to the heat released from microbial metabolism during aerobic exposure, which could explain the higher MT in LB silages at the initial ET. In contrast, the lower MT in silages inoculated and then relocated after exposure suggests diminished activity of spoilage microorganisms, such as yeasts. The TMT was also elevated in silages relocated after 48 and 60 h of exposure, likely due to the reduced intensity of deterioration observed during the aerobic stability assay.

The relocated corn silages had greater stability compared with the non-relocated silages. This can be explained by the fact that non-relocated silages may have higher concentrations of substrates, such as residual soluble carbohydrates and lactic acid, which are used by heat-producing spoilage microorganisms, promoting a faster increase in temperature (Queiroz et al., 2021). In the relocated silages, on the other hand, these substrates may have been partially consumed in aerobic exposure during relocation, resulting in lower availability for these microorganisms and, consequently, greater stability.

5. Conclusions

Corn silages inoculated with the strains *L. plantarum* (DSM3676; DSM3677) + *L. buchneri* (DSM13573), *L. plantarum* + *P. acidipropionici*, or *L. buchneri* (DSM13573) exhibit better preservation of chemical composition and improved fermentative characteristics compared with non-inoculated corn silages. The duration of air exposure influences the fermentative characteristics of corn silages. Silages treated with *L. buchneri* (DSM13573) demonstrate enhanced control of yeast growth during 60 h of

air exposure; however, they lose this antifungal effect after undergoing a new fermentation process (relocation). Relocated corn silages maintain greater stability than non-relocated silages.

Data availability

The entire dataset supporting the results of this study is available upon reasonable request to the corresponding author.

Author contributions

Conceptualization: Rego, A. C. **Data curation:** Mendonça, R. C. A. **Investigation:** Mendonça, R. C. A.; Queiroz, A. M. and Cardoso, M. V. S. B. **Project administration:** Rego, A. C. **Resources:** Domingues, F. N.; Faturi, C. and Silva, T. C. **Supervision:** Bernardes, T. F.; Domingues, F. N.; Rondina, D.; Faturi, C. and Rego, A. C. **Validation:** Bernardes, T. F.; Domingues, F. N.; Rondina, D. and Faturi, C. **Visualization:** Mendonça, R. C. A.; Silva, T. C. and Rego, A. C. **Writing – original draft:** Mendonça, R. C. A.; Silva, T. C. and Rego, A. C. **Writing – review & editing:** Mendonça, R. C. A.; Queiroz, A. M.; Cardoso, M. V. S. B. and Rego, A. C.

Conflict of interest

The authors declare no conflict of interest.

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