

Effects of commercial inoculants on tropical corn silage, rehydrated grain, and snaplage

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ABSTRACT - Three experiments were carried out to evaluate the effects of the following commercial inoculants: *Lactiplantibacillus plantarum*, *Lactocaseibacillus rhamnosus*, and *L. buchneri* (LP+LB) at 2.5×10^5 CFU g⁻¹; as well as *L. rhamnosus*, *L. buchneri*, and *L. diolivorans* (LB+LD) at 2.5×10^5 CFU g⁻¹; and the same inoculants at double dosage (2LB+2LD) at 5.0×10^5 CFU g⁻¹. In Exp. 1, corn silage was uninoculated and inoculated with LP+LB and LB+LD and stored in 5-L jars for 15, 30, 45, and 90 d. In Exp. 2, reconstituted corn grain and, in Exp. 3, whole-ear corn silage (snaplage) were uninoculated and inoculated with LP+LB, LB+LD, and 2LB+2LD and stored for 60 d. The fermentation products, microbial counts, and aerobic stability (AS) were assessed. In Exp. 1, the LP+LB inoculant reduced ($P = 0.03$) the pH at 30 d. The LP+LB inoculant resulted in a higher ($P < 0.001$) LAB count at 90 d. The lactic acid (LA) concentration was higher at 45 and 90 d ($P < 0.001$). The LB+LD inoculant resulted in higher acetic acid (AA) and propionic acid (PA) content at 45 and 90 d ($P < 0.001$). Inoculation reduced ($P = 0.02$) losses. In Exp. 2, LA was higher ($P = 0.001$) in silages inoculated with LP+LB and LB+LD than in the other treatments. Higher ($P < 0.001$) AA and PA were observed with the 2LB+2LD inoculant compared with the other silages. In Exp. 3, lower pH ($P = 0.001$) and higher LA ($P = 0.001$) were observed with the LP+LB inoculant than in the other treatments. Inoculation with 2LB+2LD resulted in higher ($P < 0.001$) AA and PA than the other treatments. In corn silage, inoculation with LB+LD enhanced fermentation from day 45, promoting higher acetic and propionic acid concentrations and greater AS. The AS of rehydrated corn grain and snaplage was improved by LB+LD, but only when the double dose was applied.

Keywords: aerobic stability, *Lentilactobacillus buchneri*, *Lentilactobacillus diolivorans*

1. Introduction

Corn silage is susceptible to deterioration processes, leading to high dry matter losses (DML), especially in tropical regions due to the high lactic acid concentration and climate conditions (Borreani et al., 2018). Due to their high yeast counts and lower acetic acid concentrations, reconstituted corn grain silage (RCGS) and whole-ear corn silage (snaplage; SNAP) may deteriorate even more rapidly than whole-plant corn silage (WPCS) (Silva et al., 2023). Muck (1996) identified the bacterium *Lentilactobacillus buchneri*, which is able to improve the aerobic stability (AS) of silages (Silva et al., 2023). Furthermore, Krooneman et al. (2002) identified the bacterium *Lentilactobacillus diolivorans*, capable of converting 1,2-propanediol into propionic acid (PA), which is effective in controlling the

growth of spoilage microorganisms in silages (Borreani et al., 2018). In contrast, *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus* are both homolactic bacteria commonly used to improve acidification during silage storage (Muck et al., 2018).

Previous studies have reported the use of inoculation with *L. buchneri* and *L. diolivorans*. Saylor et al. (2022) and Diepersloot et al. (2021) evaluated corn grain silage (CGS) and whole-plant sorghum silage, respectively, in temperate climate regions and observed an increase in the acetic acid (AA) concentration without changes in the PA concentration. However, the characteristics of the ensiled material can influence the activity and efficacy of the inoculated strains, especially in tropical regions. Our hypothesis was that the same bacterial inoculant can exert different effects in different feedstuffs. Therefore, the aim of this study was to evaluate the effect of *L. buchneri* and *L. diolivorans* on the fermentation profile, microbial counts, fermentation end products, and AS of WPCS, RCGS, and SNAP under warm conditions.

2. Material and methods

2.1. General Information

Three experiments were conducted at the Universidade Federal de Lavras, Brazil (21°13'49" S, 44°58'10" W), evaluating three different feeds: Exp. 1 = WPCS (31.6 ± 0.05% dry matter (DM)); Exp. 2 = RCGS (moisture content = 33.05 ± 0.02%); Exp. 3 = SNAP (63.0 ± 0.83% DM). In Exp. 1 and 3, the corn was harvested with a self-propelled harvester at a theoretical length of cut of 14 mm and 8 mm. In Exp. 2, corn grain (moisture = 12.5%) was obtained commercially and grounded in a feed mill with a 5 mm sieve. The following commercial inoculants were used in the three treatments: *L. plantarum* DSM 12837, *L. rhamnosus* NCIMB 30121, and *L. buchneri* DSM 12856 (LP+LB) at a dosage of 2.5×10^5 CFU g⁻¹ fresh forage; as well as *L. rhamnosus* NCIMB 30121, *L. buchneri* DSM 12856, and *L. diolivorans* DSM 32074 (LB+LD) at 2.5×10^5 CFU g⁻¹ fresh forage; and the same inoculants at double dosage (2LB+2LD) at 5.0×10^5 CFU g⁻¹ fresh forage. In all experiments, the compaction (by stomping) and inoculation (using a hand sprayer) processes were performed manually, and 5-L plastic buckets were used as experimental silos and stored in a closed room.

2.2. Experiment 1

The following treatments were used: WPCS without inoculant (WPCS-1), WPCS with LP+LB (WPCS-2), and WPCS with LB+LD (WPCS-3), each stored for 15, 30, 45, and 90 d. Prior to inoculation, 0.9 mL of the inoculants pre-diluted (600 mL H₂O/100 g of inoculant) was mixed in 2 L of water and added to the forage. The same amount of water (without inoculant) was added to the control treatment. A total of 2.9 ± 0.03 kg (density = 575 ± 6.8 kg m⁻³) was compacted per replicate (four replicates per treatment). Weight losses during storage were calculated by the difference between the DM weight at ensiling and the DM weight at opening (Tabacco et al., 2009). For the AS test, 2 kg of silage was placed in polystyrene boxes (25.5 × 17.5 cm) for 10 d at 26 ± 1.56 °C in a closed room with a heater. The room and silage temperatures were recorded every 15 min using dataloggers (Pro 2.07.09, Escort Console, SP, Brazil). The AS was considered the period during which the silage remained stable until reaching 2 °C above the ambient temperature (Ranjit and Kung, 2000).

2.3. Sample analysis and preparation

The silage samples were pre-dried in a laboratory oven at 55 °C for 72 h and then dried at 105 °C for 16 h to determine the DM concentration. Microbial counts of lactic acid bacteria (LAB), yeasts, and molds were carried out through serial dilution, using an aqueous extract (30 g of fresh silage and 270 mL of distilled water). The LAB were enumerated on MRS agar (Merck, Whitehouse Station, NY, USA) by incubating plates at 30 °C for 3 days under anaerobic conditions. Yeasts and molds were counted using the pour plate technique on Yeast Extract Glucose Chloramphenicol Agar (DIFCO, West Molesey, Surrey, UK) after incubation at 25 °C for 3 and 5 days, respectively. The colonies were counted according to

Spoelstra et al. (1988). The silage pH was measured according to Bernardes et al. (2019). Organic acids and alcohols were determined by high-performance liquid chromatography (Bernardes et al., 2015).

2.4. Experiment 2

The following treatments were used: RCGS without inoculant (RCGS-1), RCGS inoculated with LP+LB (RCGS-2), RCGS inoculated with LB+LD (RCGS-3), and RCGS inoculated with 2LB+2LD (RCGS-4), each stored for 60 d. The inoculation process was carried out as described in Exp. 1. Inoculation was performed simultaneously with rehydration (400 L H₂O/ton of ground corn grain). The corn grain was rehydrated until reaching $33.05 \pm 0.02\%$ moisture content, and 5.353 ± 0.117 kg (density = $1,000 \pm 6.80$ kg m⁻³) was compacted per replicate (seven replicates per treatment). The ensiling process, AS test, microbiological and fermentative analyses, and calculation of losses were performed as described in Exp. 1.

2.5. Experiment 3

The following treatments were used: SNAP without inoculant (SNAP-1), SNAP inoculated with LP+LB (SNAP-2), SNAP inoculated LB+LD (SNAP-3), and SNAP inoculated with 2LB+2LD (SNAP-4), each stored for 60 d. The pre-dilution, dosages, and inoculation processes were performed as described in Experiments 1 and 2, and 3.268 ± 0.09 kg (density = 616.5 ± 18 kg m⁻³) was compacted per replicate (five replicates per treatment). The ensiling process, AS test, microbiological and fermentative analyses, and calculation of losses were performed as described in Exp. 1.

2.6. Experimental design and statistical analysis

In Exp. 1, the data were analyzed in a completely randomized design in a 3×4 factorial arrangement, with the fixed effects of inoculant, storage time and their interactions (model 1). In Experiments 2 and 3, the fixed effect of inoculant was applied (model 2).

$$y_{ijk} = \mu + I_i + T_j + (I \times T)_{ij} + e_{ijk}, \quad (1)$$

in which y_{ijk} = dependent variable, μ = overall mean of all observations, I_i = fixed effect of inoculant, T_j = fixed effect of storage time, $(I \times T)_{ij}$ = interaction between inoculant $\times$ storage time, e_{ijk} = random residual effect.

$$y_{ij} = \mu + I_i + e_{ij}, \quad (2)$$

in which y_{ij} = dependent variable, μ = overall mean of all observations, I_i = fixed effect of inoculant, e_{ij} = random residual effect.

The data were analyzed using the PROC MIXED procedure of the SAS statistical software (Statistical Analysis System – SAS Institute, 2001), and the means were estimated using “LSMEANS”. In all experiments, comparisons among data were performed using Tukey’s test, with statistical significance defined as $P \leq 0.05$.

3. Results

3.1. Experiment 1

There were significant effects of the inoculation $\times$ storage time interaction on pH ($P = 0.01$), LAB counts ($P < 0.001$), PA ($P = 0.004$), ethanol ($P < 0.001$), 1,2-propanediol ($P < 0.001$) concentrations, and AS ($P < 0.001$; Table 1). Lower pH was observed when WPCS-1 was stored for 90 d in comparison to WPCS-1 stored for 15 and 30 d. Likewise, WPCS-2 was stored for 30, 45, and 90 d compared with WPCS-2 at 15 d, and in WPCS-3 stored for 90 d compared with WPCS-3 stored for 15, 30, and 45 d. The silage WPCS-2 stored for 45 and 90 d had higher pH than WPCS-1 and WPCS-3 at the same storage time (Table 1).

At 15 d, silage WPCS-3 had a higher LAB count than WPCS-1, whereas it did not differ from WPCS-2 (Table 1). At 45 d, WPCS-2 and WPCS-3 had similar LAB counts and both were higher than WPCS-1 silage (Table 1). At 90 d, the LAB count was highest for WPCS-2 compared with the other silages at the same storage time. Higher LAB counts were observed when silage WPCS-1 was stored for 15 and 30 d, when silage WPCS-2 was stored for 90 d, and when silage WPCS-3 was stored for 45 and 90 d, compared with the respective silages stored at the other storage times (Table 1).

The WPCS-3 silage had a higher PA concentration at 45 and 90 d compared with WPCS-3 stored for 15 and 30 d (Table 1). The PA concentrations were similar among the silages at 15 d. Silages WPCS-3 had higher PA concentrations at 45 and 90 d than silages WPCS-1 and WPCS-2 at the same storage time (Table 1). Lower ethanol concentration was observed for WPCS-1 silage stored for 15 d compared with the same silage stored for 45 and 90 d, and when WPCS-2 and WPCS-3 were stored for 30, 45, and 90 d compared with the respective silages stored for 15 d (Table 1). The ethanol concentrations were similar for WPCS-2 and WPCS-3 silages, but higher compared with WPCS-1 at 15 d. At 45 and 90 d, ethanol concentrations were similar in WPCS-2 and WPCS-3 silages, but they were lower compared with WPCS-1 (Table 1).

The 1,2-propanediol concentration of WPCS-1 did not change with the different storage times, and this concentration was always lower than that observed in the inoculated silages (Table 1). The WPCS-3 had a lower 1,2-propanediol concentration than WPCS-2 after 90 d. At 45 and 90 d of storage, WPCS-1 had higher AS compared with the same silage stored for 15 and 30 d (Table 1). The AS was higher for the inoculated silages stored for 90 d in comparison to 15, 30, and 45 d. The AS was higher in WPCS-2 and WPCS-3 from 30 to 90 d of storage in comparison to WPCS-1 at the same storage time (Table 1).

The DM concentration decreased ($P < 0.001$) as storage time increased, with the lowest value at 90 d (Table 1). Storage time ($P = 0.01$) and inoculation ($P = 0.01$) affected yeast counts, which were higher at 15 d, and lower in WPCS-2 and WPCS-3, respectively (Table 1). Inoculated silages had lower ($P = 0.001$) mold counts. The lactic acid (LA) concentration was affected ($P < 0.001$) by storage time, with higher values at 45 and 90 d. Inoculation ($P < 0.001$) and storage time ($P < 0.001$) affected the AA concentration, which was higher in WPCS-3 and when silages were stored for 45 and 90 d, respectively (Table 1). The DML were affected ($P = 0.02$) by inoculation during aerobic exposure, with lower DML in WPCS-2 compared with WPCS-1, while WPCS-3 did not differ from either (Table 1).

Table 1 - Dry matter, fermentation profile, microbial count, losses and aerobic stability of whole-plant corn silage (WPCS), with or without inoculant, at different storage times

Variable	Storage time	Silage ¹			Mean	SEM	P-value ²		
		WPCS-1	WPCS-2	WPCS-3			I	T	I × T
DM (g kg ⁻¹ FM)	15	320.0	324.5	321.7	322.0A	0.308	0.937	<0.001	0.080
	30	333.2	338.0	336.0	335.7A				
	45	306.0	306.7	301.5	304.7B				
	90	302.2	290.0	299.2	297.1C				
	Mean	315.3	314.8	314.6					
pH	15	3.70aA	3.72aA	3.73aA	3.71	0.008	0.034	<0.001	0.010
	30	3.66aB	3.65aB	3.66aB	3.66				
	45	3.65bBC	3.67aB	3.65bB	3.66				
	90	3.63bC	3.66aB	3.62bC	3.64				
	Mean	3.66	3.67	3.66					
LAB (log CFU g ⁻¹)	15	7.26bA	7.45abC	7.56aB	7.42	0.092	<0.001	<0.001	<0.001
	30	7.00aA	7.11aD	7.17aC	7.10				
	45	6.48bB	7.85aB	7.92aA	7.41				
	90	5.09cC	8.21aA	8.00bA	7.10				
	Mean	6.46	7.66	7.66					

Continues...

Table 1 (Continued)

Variable	Storage time	Silage ¹			Mean	SEM	P-value ²		
		WPCS-1	WPCS-2	WPCS-3			I	T	I × T
Yeast (log CFU g ⁻¹)	15	4.23	3.98	3.41	3.88A	0.424	0.001	0.010	0.643
	30	3.22	2.44	2.21	2.62B				
	45	3.43	2.49	2.59	2.83B				
	90	3.65	2.25	1.50	2.46B				
	Mean	3.63a	2.79b	2.43b					
Mold (log CFU g ⁻¹)	15	2.01	1.75	1.39	1.70	0.471	0.001	0.602	0.857
	30	2.40	1.32	1.29	1.67				
	45	2.99	1.38	1.40	1.92				
	90	3.09	1.67	1.64	2.13				
	Mean	2.62a	1.53b	1.42b					
Lactic acid (g kg ⁻¹ DM)	15	53.6	51.6	54.2	53.1B	0.244	0.184	<0.001	0.132
	30	58.6	53.3	58.0	56.6B				
	45	60.3	63.1	67.4	63.6A				
	90	59.2	67.3	64.6	63.7A				
	Mean	57.9	58.8	61.1					
Acetic acid (g kg ⁻¹ DM)	15	13.8	13.3	14.5	13.9B	0.166	<0.001	<0.001	0.069
	30	14.3	14.5	18.7	15.8B				
	45	15.0	19.1	23.0	19.0A				
	90	15.0	20.4	27.0	20.8A				
	Mean	14.5c	16.8b	20.8a					
Propionic acid (g kg ⁻¹ DM)	15	2.32aA	2.20aB	2.33aB	2.28	0.043	<0.001	<0.001	0.004
	30	2.20bA	2.70aAB	3.05aB	2.65				
	45	2.76bA	2.82bAB	4.90aA	3.49				
	90	2.10cA	3.50bA	5.87aA	3.82				
	Mean	2.34	2.80	4.04					
Ethanol (g kg ⁻¹ DM)	15	1.17bB	2.95aA	1.60aA	1.90	0.011	<0.001	<0.001	<0.001
	30	1.45aAB	1.25aB	1.16aB	1.28				
	45	1.67aA	1.0 bB	1.30bB	1.34				
	90	1.67aA	1.52bB	1.35bB	1.51				
	Mean	1.49	1.69	1.35					
1,2-propanediol (g kg ⁻¹ DM)	15	0.45bA	4.10aD	4.15aC	2.90	0.028	<0.001	<0.001	<0.001
	30	0.32bA	5.60aC	5.40aB	3.77				
	45	0.40bA	8.40aB	8.33aA	5.71				
	90	0.40cA	9.22aA	7.57bA	5.73				
	Mean	0.39	6.83	6.36					
Aerobic stability (h)	15	28aB	27aD	32aD	29	4.8	<0.001	<0.001	<0.001
	30	40bB	63aC	63aC	55				
	45	57bA	82aB	94aB	78				
	90	60bA	175aA	175aA	137				
	Mean	46	87	91					
DM losses (g kg ⁻¹ DM)	15	13.7	8.7	9.3	10.6	0.072	0.024	0.546	0.126
	30	10.8	8.4	13.6	10.9				
	45	10.0	9.3	11.7	10.4				
	90	13.3	11.1	11.2	11.8				
	Mean	11.9a	9.4b	11.4ab					

DM - dry matter; FM - fresh matter; SEM - standard error of the mean.

¹ WPCS-1 - WPCS without inoculant; WPCS-2 - WPCS inoculated with LP+LB; WPCS-3 - WPCS inoculated with LB+LD.² I - inoculation; T - storage time; I × T - inoculation × storage time interaction.

The same lowercase letters in the row and uppercase letters in the column do not differ from each other by Student's t-test at 5% significance.

3.2. Experiment 2

Higher pH ($P < 0.001$) was observed in RCGS-4. Higher LAB count ($P < 0.001$) was observed in RCGS-4 (Table 2). Silage RCGS-4 had lower ($P < 0.001$) yeast count. The LA concentration was higher ($P = 0.001$) in RCGS-2 compared with the other RCGS (Table 2). Higher AA concentration ($P < 0.001$) was observed in RCGS-4 (Table 2). Silage RCGS-4 had the highest PA ($P < 0.001$) and ethanol ($P < 0.001$) concentrations, and lower ($P < 0.001$) 1,2-propanediol concentration (Table 2). The greatest AS ($P < 0.001$), and the lowest DML ($P < 0.001$) was observed in RCGS-4 silage (Table 2).

3.3. Experiment 3

Silage SNAP-2 had higher ($P = 0.001$) DM concentration compared with SNAP-1 and SNAP-4 (Table 2). Lower pH ($P = 0.001$) was observed in SNAP-2. Silage SNAP-4 had higher ($P < 0.001$) LAB counts (Table 2). Silage SNAP-3 and SNAP-4 had lower ($P = 0.006$) yeast counts compared with SNAP-1. The LA concentration was higher ($P = 0.001$) in SNAP-2 compared with SNAP-3 and SNAP-4. Silage SNAP-4 had higher AA ($P < 0.001$) and PA ($P < 0.001$) concentrations compared with the other SNAP silages (Table 2).

Table 2 - Dry matter, fermentation profile, microbial count, losses and aerobic stability of inoculated reconstituted corn grain silage (RCGS) and snaplage (SNAP)

Variable	RCGS ¹				Mean	SEM	P-value
	RCGS-1	RCGS-2	RCGS-3	RCGS-4			
DM (g kg ⁻¹ FM)	642.1	652.3	644.7	644.8	645.9	0.994	0.887
pH	3.81b	3.78b	3.80b	3.87a	3.81	0.010	<0.001
Lactic acid bacteria (log CFU g ⁻¹)	5.16d	5.91c	6.67b	7.52a	6.31	0.157	<0.001
Yeast (log CFU g ⁻¹)	3.06a	2.95a	3.13a	1.04b	2.54	0.129	<0.001
Mold (log CFU g ⁻¹)	1.80	1.95	2.09	1.46	1.82	0.173	0.091
Lactic acid (g kg ⁻¹ DM)	47.1bc	51.5a	50.7ab	45.9c	48.8	0.094	0.001
Acetic acid (g kg ⁻¹ DM)	6.64c	7.54bc	9.79b	19.4a	10.8	0.058	<0.001
Propionic acid (g kg ⁻¹ DM)	0.20b	0.20b	0.20b	0.99a	0.39	0.004	<0.001
Ethanol (g kg ⁻¹ DM)	13.0d	24.5b	20.6c	31.9a	22.5	0.087	<0.001
1,2-propanediol (g kg ⁻¹ DM)	0.12b	0.14b	1.76a	0.81b	0.70	0.020	<0.001
Aerobic stability (h)	61b	52b	73b	168a	88	13.8	<0.001
DM losses (g kg ⁻¹ DM)	69.1a	74.5a	49.2a	12.5b	51.3	0.868	<0.001
Variable	SNAP ²				Mean	SEM	P-value
	SNAP-1	SNAP-2	SNAP-3	SNAP-4			
DM (g kg ⁻¹ FM)	608.8b	625.8a	618.6ab	613.0b	616.5	0.252	0.001
pH	3.82a	3.76b	3.83a	3.86a	3.82	0.013	0.001
Lactic acid bacteria (log CFU g ⁻¹)	5.04c	6.64b	6.54b	7.42a	6.41	0.172	<0.001
Yeast (log CFU g ⁻¹)	3.19a	2.64ab	1.75b	1.78b	2.34	0.285	0.006
Mold (log CFU g ⁻¹)	2.57	2.43	2.38	1.66	2.26	0.297	0.170
Lactic acid (g kg ⁻¹ DM)	55.3ab	62.0a	31.7c	41.7bc	47.7	0.458	0.001
Acetic acid (g kg ⁻¹ DM)	10.0b	7.9b	10.6b	18.6a	11.8	0.094	<0.001
Propionic acid (g kg ⁻¹ DM)	-	-	0.18b	0.40a	0.14	0.003	<0.001
Ethanol (g kg ⁻¹ DM)	16.8a	4.52b	5.40b	6.02b	8.19	0.247	0.008
1,2-propanediol (g kg ⁻¹ DM)	0.52b	-	0.68b	1.24a	0.61	0.007	<0.001
Aerobic stability (h)	52b	52b	47b	102a	63	3.8	<0.001
DM losses (g kg ⁻¹ DM)	64.2ab	76.6a	54.2b	20.9c	53.9	0.450	<0.001

DM - dry matter; FM - fresh matter; SEM - standard error of the mean.

¹ RCGS-1 - RCGS without inoculant; RCGS-2 - RCGS inoculated with LP+LB; RCGS-3 - RCGS inoculated with LB+LD; RCGS-4 - RCGS inoculated with 2LB+2LD.

² SNAP-1 - SNAP without inoculant; SNAP-2 - SNAP inoculated with LP+LB; SNAP-3 - SNAP inoculated with LB+LD; SNAP-4 - SNAP inoculated with 2LB+2LD.

The same letters in the row do not differ from each other by Tukey's test at 5% significance.

The PA was not detected for SNAP-1 and SNAP-2 silages. Lower ($P = 0.008$) ethanol concentration was observed in the inoculated silages. Silage SNAP-4 had higher 1,2-propanediol concentration ($P < 0.001$), greater AS ($P < 0.001$), and lower DML ($P < 0.001$; Table 2).

4. Discussion

The WPCS and RCGS silages did not show changes in DM concentration with inoculation. In contrast, higher DM concentration in SNAP-2 may reflect the activity of homolactic LAB, due to their greater capacity to preserve DM during storage (Muck et al., 2018), suggesting that the type of ensiled material and substrate available for fermentation may have a greater influence on the effectiveness of inoculation in preserving DM (Zopollatto et al., 2009).

The silages in all the experiments evaluated exhibited pH values lower than 4.0, indicating a good fermentation profile (Kung Jr. et al., 2018), due to the presence of LA. According to Pahlow et al. (2003), a period of 2 to 6 weeks is necessary for the fermentation profile to stabilize inside the silo. In WPCS-1 and WPCS-3, pH stabilization occurred between 30 and 45 d. A longer storage time associated with bacterial inoculation leads to a higher concentration of LA at 45 d, explaining the lower pH values of the silages (Kung Jr. et al., 2018). In RCGS and SNAP, however, the presence of *L. buchneri* strains may have contributed to higher pH values (Danner et al., 2003), probably due to the acetic acid production by the anaerobic fermentation pathway of LA (Oude Elferink et al., 2001), which also explains the reduction in LA concentration and the increase in AA concentration.

The higher LAB counts in the silages evaluated are related to inoculation and they also explain the higher concentrations of LA in RCGS-2 and SNAP-2 silages, and AA in WPCS-3, RCGS-4 and SNAP-4 silages. Once again, this highlights that the same inoculant can bring about different responses depending on the type of feed and the dosage used. At the beginning of ensiling, the presence of residual oxygen within the silo is common. In addition, a short storage period may not be long enough to ensure sufficient production of organic acids to control yeast growth, probably due to compete successfully with the epiphytic microflora during the primary fermentation phase but became predominant during the storage phase (Driehuis et al., 1999; Oude Elferink et al., 2001). The higher yeast count in WPCS stored for 15 and 30 d may be related to residual oxygen and not to the AA content in those periods. In RCGS and SNAP silages, the presence of the heterofermentative LAB possibly controlled yeast growth, due to the antifungal properties of AA (Muck et al., 2018).

In WPCS-2 and WPCS-3, the increase of 1,2-propanediol concentration can be explained due to the presence of *L. buchneri* in inoculant composition, which is capable of converting LA in AA and 1,2-propanediol (Oude Elferink et al., 2001) throughout the storage time. In addition, the higher PA concentration in WPCS-3 at 45 and 90 d can be explained by the activity of the bacterium *L. diolivorans*, which converts 1,2-propanediol into 1-propanol and PA (Krooneman et al., 2002). The mechanism of *L. buchneri* and *L. diolivorans* bacteria regarding 1,2-propanediol and PA production described before also explains their concentrations in RCGS and SNAP silages with bacterial inoculation.

It is important to highlight that, in WPCS, the effects of inoculation on 1,2-propanediol and PA concentrations were observed at the standard inoculant dosage (manufacturer recommendation). In RCGS, the standard dosage was sufficient to increase the 1,2-propanediol concentration, but the increase in PA only occurred when the doubled dosage was used. In SNAP silage, these effects on 1,2-propanediol and PA concentration only occurred when doubled dosage was used.

Regarding ethanol concentration, the antifungal properties of AA may have contributed to better control of yeast growth (Kung Jr. et al., 2018), resulting in lower ethanol production in WPCS inoculated and associated with the increase in storage time. Similarly, the same antifungal properties of AA also explain the lower ethanol concentration in RCGS-3 and SNAP inoculated silages.

In both experiments, the higher AS and lower DML can be attributed to the increase in AA and PA concentrations, which inhibit the growth of spoilage microorganisms (Borreani et al., 2018). It is noteworthy that the effects observed in AS and DML in WPCS occurred when the silage was inoculated

with the standard dosage, whereas the same effects in RCGS and SNAP only appeared when the doubled dosage was used. This finding supports the hypothesis that the same inoculant can elicit different responses in different silages.

5. Conclusions

The same inoculant led to different effects when different silages were inoculated. Furthermore, the association between *L. buchneri* and *L. diolivorans* intensified fermentation and contributed to increasing AA and PA concentrations and AS, however, these effects on depends on the dosage used. A standard dosage for WPCS is recommended, although a double dose was required at ensiling of RCGS and SNAP silages.

Data availability

All data are available in the manuscript.

Author contributions

Conceptualization: Bernardes, T. F. **Data curation:** Cardoso, M. V. S. B.; Costa, G. F. and Bernardes, T. F. **Formal analysis:** Cardoso, M. V. S. B.; Costa, G. F.; Rêgo, A. C. and Bernardes, T. F. **Investigation:** Cardoso, M. V. S. B.; Costa, G. F.; Rêgo, A. C. and Bernardes, T. F. **Methodology:** Bernardes, T. F. **Project administration:** Bernardes, T. F. **Writing – original draft:** Cardoso, M. V. S. B.; Costa, G. F.; Rêgo, A. C. and Bernardes, T. F. **Writing – review & editing:** Cardoso, M. V. S. B.; Costa, G. F.; Rêgo, A. C. and Bernardes, T. F.

Conflict of interest

The authors declare no conflict of interest.

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