R. Bras. Zootec., 53:e20230123, 2024
https://doi.org/10.37496/rbz5320230123

Forage crops
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Maturity stage at harvest on the chemical composition, fermentation losses, and starch and NDF digestibility of whole-plant corn silages

Queila Gouveia Tavares[1]*, Denise Volpi[1], Natália Nunes de Melo[1], Lucelia de Moura Pereira[1], Gabriela Letícia Delai Vigne[1], Maity Zopollatto[1], Patrick Schmidt[1]

[1] Universidade Federal do Paraná, Departamento de Zootecnia, Curitiba, PR, Brasil.

***Corresponding author:**
queila.tavares13@hotmail.com

Received: September 26, 2023
Accepted: June 4, 2024

How to cite: Tavares, Q. G.; Volpi, D.; Melo, N. N.; Pereira, L. M.; Vigne, G. L. D.; Zopollatto, M. and Schmidt, P. 2024. Maturity stage at harvest on the chemical composition, fermentation losses, and starch and NDF digestibility of whole-plant corn silages. Revista Brasileira de Zootecnia 53:e20230123.
https://doi.org/10.37496/rbz5320230123

Editors:
Gustavo José Braga
Luiz Felipe Ferraretto

ABSTRACT - This study aimed to evaluate the effects of two maturity stages of whole-plant corn at harvest (32.1 or 42.5% dry matter (DM)) and a commercial microbial inoculant composed of *L. buchneri* and *L. plantarum* at concentration of 110,000 CFU/g of fresh forage (1.1 × 10^5 CFU/g FF) on fermentation losses, aerobic stability, chemical composition, and digestibility of starch and neutral detergent fiber (NDF). A factorial and randomized design was used (two DM contents, both with or without inoculant), with five replicates per treatment. Dry matter at harvest affected most variables, except lignin, NDF digestibility, ethanol, and lactic and acetic acids. Drier silages differed in total DM losses (−1.7%) and lactic acid bacteria (LAB) population (+1.2 log CFU/g). The use of the inoculant affected the levels of ether extract (+0.27% DM), starch (+2.9% DM), and lignin (−0.17% DM). The LAB (+1.6 log CFU/g) and yeast (−2.82% log CFU/g) populations were also influenced, as well as aerobic stability at six days. The inoculant × DM interaction was observed in the water-soluble carbohydrates content, being higher in silages with 32.1% DM and in those not inoculated for both DM. Crude protein was also higher in these silages, whether inoculated or not. Wetter silages were more prone to gas losses when inoculated (+2.5% DM) and lost more effluent when not inoculated (+4.82 kg/t FF). However, total DM losses during aerobic stability were on average 10.58% DM lower in these silages, with inoculation being preferred (6.72% DM vs 11.60% DM (control)). Under these conditions, harvesting corn for silage at 42.5% DM is indicated to obtain a more energetic silage, as noted both in the increased starch content and the reduced losses associated with fermentation.

Keywords: additive, aerobic stability, harvesting time, *L. buchneri*, maize silage

1. Introduction

The corn plant is considered the most suitable crop for silage production due to characteristics such as adequate dry matter (DM) content, water-soluble carbohydrates (WCS), and low buffering capacity (Nussio et al., 2001). These favor lactic acid-based fermentation, facilitate the reduction of pH, and provide a suitable ambient for the growth of lactic acid bacteria (LAB), resulting in high-quality silage (Borém et al., 2017; Neumann et al., 2017).

The maturity stage of the plants at harvest is an important factor, considering its effect on the nutritional composition of the silage and its fermentation quality (Wang et al., 2015). Whole-plant corn harvested at traditional DM content (30–35%) can provide a favorable environment for the growth of desirable LAB, preserving its quality (Kung et al., 2000). Corn harvested at a higher DM content

(> 35% DM) tends to have both lower fiber quality and digestibility, compromising forage packing and leading to increased oxygen presence within the silo (Nussio et al., 2001). On the other hand, harvesting corn with higher DM content increases net energy due to the greater participation of grains in the mass, despite its lower starch degradability (Ferraretto and Shaver, 2012). Moreover, the protein content of the silage is reduced due to the lower participation of stem and leaves (Horst et al., 2020a). Thus, the moment of harvesting corn for silage is crucial and must consider the nutritional composition and potential intake of the feed, beyond logistics.

Silage inoculants based on selected bacteria can improve fermentation during the ensiling process (Muck et al., 2018). The inoculants limit the proliferation of undesirable microorganisms, such as yeasts and molds, maintaining the silage quality and improving aerobic stability (Schmidt et al., 2014). However, the plant DM content at harvest affects the performance of the inoculants (Silva et al., 2022), and this effect is still poorly understood. Hence, it is hypothesized that inoculating silages produced from the same hybrid at the same field will yield varied silage outcomes, when the only source of variation is the DM content at the time of ensiling. This study aimed to evaluate corn silages with or without microbial inoculant under two DM contents (32.1 and 42.5%) over the chemical and microbiological composition of the silages, as well as their fermentation losses and aerobic stability.

2. Material and Methods

2.1. Sowing and harvesting the crop

The experiment was carried out in southern Brazil, in the city of Pinhais, Paraná (latitude 25°23'19.6" S and longitude 49°07'44.4" W). The climate is CFb according to the Köppen classification. The area receives approximately 1,630 mm of annual precipitation (SIMEPAR, 2020).

The corn hybrid (Pioneer P4285VYHR®) was sown on October 30, 2019, and was established in a 120 m^2 area, with 0.5 m row spacing, and 4 cm depth. Four seeds were sown per linear meter, targeting 80,000 plants/ha.

The recommendations of the Manual de adubação e calagem para o estado do Paraná ("Fertilization and Liming Manual of the State of Paraná") were followed to determine the fertilization values (Pavinato et al., 2017). The base fertilization used 650 kg/ha of NPK 8-20-20 formulated fertilizer. No pesticides were applied during the entire crop cycle to abide by the environmental legislation of the area. Cultivation, such as herbs control and sidedress fertilizing, were manually done.

To ensure different DM contents, two manual harvests at 10 cm soil height were done on the same plot: the first with 32.1% DM, 133 days after sowing, and the second with 42.5% DM, 145 days after sowing. The DM contents were monitored using the microwave oven method (Oliveira et al., 2015). At each harvest, the plants were immediately processed in a stationary forage machine (Menta, model Super 15 T) adjusted for particles of 10 mm theoretical cutting length.

2.2. Ensiling

Plastic buckets of 20 L were used as experimental silos, equipped with Bunsen valves to allow fermentation gases to escape and prevent air from entering. Two kilograms of oven-dried sand were placed at the bottom of each silo to absorb possible effluents, separated from the forage by a 1-mm plastic netting and a cheesecloth. All empty silos (plastic bucket + sand + netting + cheesecloth) were weighed before ensiling for gravimetric estimation of losses.

The treatments were prepared simultaneously in two sites, 4 m apart according to recommendation of Melo et al. (2023), with previously sanitized tarps covering the floor. The inoculant (Pioneer® 11C33) consisting of *Lentilactobacillus buchneri* and *Lactiplantibacillus plantarum* was diluted in distilled water (2 L/t) and manually sprayed onto 73 kg of fresh forage (FF) (Table 1) at a rate of 110,000 CFU/g FF, according to the manufacturer's recommendations. The control treatment received the same dosage

of distilled water. After homogenization the forage was individually weighed to standardize the mass in all experimental units (11.8 kg/silo) and was quickly packed into silos, resulting in a bulk density of 590 kg FF/m^3. Finally, every silo was sealed and stored at room temperature for 74 days. Two openings were carried out to keep the same fermentation period for both silages.

Table 1 - Chemical and microbiological characteristics of corn plants at the time of ensiling

Variable	DM content (%)		Mean
	32.1	42.5	
pH	5.9	5.7	5.8
DM_{CORR} (%)	34.6	45.3	39.9
Ash (% DM)	3.2	3.1	3.1
Neutral detergent fiber (% DM)	46.1	45.2	45.6
Acid detergent fiber (% DM)	26.6	26.9	26.7
Crude protein (% DM)	7.7	6.9	7.3
Ether extract (% DM)	3.3	3.2	3.1
Water-soluble carbohydrates (% DM)	12.7	8.1	10.4
Lignin (% DM)	44.8	49.7	47.2
Starch (% DM)	27.9	31.8	29.8
Lactic acid bacteria (log CFU/g)	4.30	4.50	4.40
Yeasts (log CFU/g)	5.10	4.30	4.70
Filamentous fungi (log CFU/g)	4.60	3.50	4.05

DM - dry matter; DM_{CORR} - dry matter corrected to 105 °C; CFU - colony-forming units.

2.3. Sampling and analysis

Silos were weighed before and after the storage to estimate losses by effluent, gases, and total dry matter losses (TDML) (Jobim et al., 2007). Samples were collected during ensiling (n = 2) and after opening the silos from each replicate. When opening the silos, the top 5 cm of silage were discarded to avoid micro-aeration tampering. The remaining silage from each silo was transferred to a sterile plastic bag and homogenized for sampling.

For DM content and chemical analysis, 300 g samples of forage and silages were collected in duplicate. In fresh samples, pH was determined through 1:10 water extract (Kung et al., 1984), and DM content in a forced-ventilation oven at 65 °C for 72 h (AOAC, 1995). Subsequently, the dried samples were ground in a Wiley mill through a 1-mm mesh sieve. The samples were then packed in plastic bags and shipped to a commercial laboratory for analysis of ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, ether extract (EE), lignin, *in situ* starch digestibility in 24 h, and 48 h-NDF digestibility assessment. Analyses were performed by near-infrared reflectance spectroscopy (NIRS) applying a calibration curve from the Rock River Laboratory, USA.

Other wet samples of silages were subjected to 1:10 water extraction in a blender for 60 s, and double-filtered and frozen for the measurement of volatile fatty acids (VFA) by high-performance liquid chromatography (HPLC), using a Shimadzu (Kyoto, Japan) chromatograph. The HPLC columns used in the analysis included Rezex RHM 300 × 7.8 (Phenomenex, USA) and the analytical parameters recommended by the manufacturer (Mobile Phase: H2SO4 5.0 mmol L^{-1}; flow rate: 0.6 mL^{-1} min; column temperature: 65 °C).

For microbiological analysis, 25 g of FF and 25 g of silage were collected and diluted aseptically in 225 mL of saline solution, which was autoclaved the day before and prepared with sodium chloride (NaCl), potassium chloride (KCl), calcium chloride hexahydrate ($CaCl_2.6H_2O$), and sodium carbonate ($NaHCO_3$), according to Kung and Ranjit (2001). Then, the samples were homogenized in a paddle blender (MA440/CF, Marconi®) for 4 min at 150 rpm, filtered through three layers of gauze, and subjected to

serial dilution. The dilution of LAB culture was performed in MRS broth (ACC – Rogosa and Sharpe, Merck®). The samples were plated on Petrifilm plates (AC, 3M®), placed in an anaerobic jar, transferred to an incubator at 30 °C for 48 h, and then counted. For the filamentous fungi (molds) and yeasts analysis, samples were diluted in a saline solution, plated on Petrifilm plates (YM, 3M®), and placed in an incubator at 23.5 °C. The colonies were counted after 72 h of incubation for yeasts and 120 h for molds, on the same plate. A lab stereoscope was used for visually distinguish between molds and yeasts.

Aerobic stability was assessed following the methodology described by Kung et al. (2000). Samples (4 kg) from each replicate were placed in open buckets, and a data logger thermometer (Lascar Electronics/UK, model EL-USB-1) was placed in the geometric center of the mass to record the temperature every 5 min for 10 days, in a controlled temperature room (23.5 ± 1 °C). Another bucket (~750 g) from each replicate was used to evaluate pH every two days throughout the aerobic exposure period.

2.4. Statistical analysis

A completely randomized experimental design was used in a 2 × 2 factorial scheme (two DM contents [32.1 and 42.5%], with or without microbial inoculant) with five replicates per treatment, totalizing 20 experimental units (silos).

The CFU count from the raw microbiology data was converted to a logarithmic scale before applying the following general mathematical statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

in which μ = general average associated with all observations; α_i = effect of the i-th level of the DM factor; β_j = effect of the j-th level of the microbial inoculant factor; $(\alpha\beta)_{ij}$ = effect of the interaction between the i-th level of the DM factor and the j-th level of the microbial inoculant factor; ε_{ijk} = experimental error associated with each observation.

The variance analysis was utilized. If significant differences were found, the means were compared by the F test, both at 5% significance ($P<0.05$). These analyses were performed using the SISVAR® software (System of Analysis of Variance for Balanced Data; Ferreira, 2011).

3. Results

The DM content at harvest influenced all the chemical parameters but lignin content and the acids concentration (Table 2). The first harvested silages (32.1% DM) presented greater contents of ash, NDF, ADF, WSC, and CP, as well as the estimated starch digestibility. The 42.5% DM silages presented greater pH, EE, and starch ($P<0.05$). Inoculation of the silages significantly increased ($P<0.05$) the EE and starch contents, while WSC and lignin decreased. Trends of lactic acid decrease ($P = 0.052$) and acetic acid increase ($P = 0.084$) were also detected (Table 2).

There were interactions between inoculant and DM content for the CP ($P = 0.045$) and WSC ($P = 0.0001$) contents (Table 3). Crude protein content in silages with 42.5% DM was slightly lower than in silages with 32.1% DM. Applying inoculant in corn silages with higher DM content increased by 2.5% the CP content compared with the control. The inoculated silages presented a lower residual WSC content, and this value was slightly higher for 32.1% DM silages (Table 3).

The use of inoculant did not affect the estimates of fermentative losses (Table 4), although an interaction between the factors was detected for gas losses and effluent production ($P = 0.0306$ and $P = 0.0346$, respectively). The 42.5% DM silages presented lower values of total DM losses, gas losses, and effluents than the 32.1% DM silages.

Gas losses were five times higher in inoculated silages at 32.1% DM than in inoculated silages at 42.5% DM ($P = 0.0306$; Table 5). Effluent production was twice as high in 32.1% DM control silages compared with the 42.5% DM control ones. Adding inoculant to the 32.1% DM silages decreased the effluent losses by 28%.

Table 2 - Chemical composition and volatile organic acids concentration of corn silages, with or without inoculant, under two dry matter (DM) contents

Variable	Additive[1]		DM content (%)		SEM	Effect		
	Control	Inoculant	32.1	42.5		Inoculant (I)	DM	I × DM
pH	3.8	3.8	3.7	3.87	0.01	0.3486	0.0001	0.408
DM (%)	38.7	38.9	33.0	44.7	0.22	0.4672	<0.0001	0.251
Ash (% DM)	3.1	3.1	3.2	3.00	0.06	0.7438	0.0109	0.671
NDF (% DM)	45.4	43.0	45.4	43.0	0.79	0.0507	0.0460	0.409
ADF (% DM)	26.07	24.6	26.3	24.4	0.54	0.0728	0.0242	0.410
WSC (% DM)	6.32	5.40	5.87	4.93	0.07	<0.0001	<0.0001	0.0001
CP (% DM)	7.6	7.65	7.9	7.3	0.04	0.3357	<0.0001	0.045
EE (% DM)	3.03	3.3	3.09	3.2	0.04	0.0007	0.0331	0.715
Lignin (% DM)	4.2	4.03	4.1	4.1	0.05	0.0203	0.5468	0.712
Starch (% DM)	26.1	29.0	24.5	30.7	0.93	0.0434	0.0002	0.457
24 h starch digestibility (% starch)	89.4	89.7	92.01	87.08	0.52	0.6911	<0.0001	0.353
48 h NDF digestibility (% NDF)	69.26	70.00	70.09	69.2	0.33	0.1373	0.0668	0.890
Lactic acid (% DM)	6.9	6.5	6.4	6.8	0.32	0.052	0.933	0.826
Acetic acid (% DM)	1.3	1.6	1.4	1.5	0.09	0.084	0.676	0.445
Butyric acid (% DM)	ND	ND	ND	ND	-	-	-	-
Ethanol (% DM)	0.56	0.50	0.50	0.56	0.03	0.278	0.230	0.157

NDF - neutral detergent fiber; ADF - acid detergent fiber; WSC - water soluble carbohydrates; CP - crude protein; EE - ether extract; SEM - standard error of the mean; ND - not detected.
[1] Control: without microbial inoculant; Inoculant: composted of *L. buchneri* and *L. plantarum* bacteria (110,000 CFU/g fresh forage).

Table 3 - Crude protein and soluble carbohydrates according to inoculant use and dry matter (DM) content

DM content (%)	Additive[1]		SEM
	Control	Inoculant	
Crude protein (% DM)			
32.1	7.99A	7.92A	0.06
42.5	7.21Bb	7.39Ba	0.06
Water soluble carbohydrates (% DM)			
32.1	6.43Aa	5.87Ab	0.06
42.5	6.21Aa	4.93Bb	0.06

SEM - standard error of the mean.
[1] Control: without microbial inoculant; Inoculant: composted of *L. buchneri* and *L. plantarum* bacteria (110,000 CFU/g fresh forage).
Means followed by different letters, lowercase in the row and uppercase in the column, differ statistically by the F test (P<0.05).

Table 4 - Fermentation losses in corn silages with and without inoculant, under two dry matter (DM) contents

Variable	Additive[1]		DM content (%)		SEM	Effect		
	Control	Inoculant	32.1	42.5		Inoculant (I)	DM	I × DM
TDML (% DM)	2.3	2.2	3.1	1.4	0.34	0.9002	0.0040	0.212
GL (% DM)	1.6	1.8	2.4	0.9	0.33	0.5992	0.0050	0.0306
EL (kg/t DM)	7.2	5.9	7.5	5.6	0.87	0.1361	0.0274	0.0346

TDML - total dry matter losses; GL - gas losses; EL - effluent losses; SEM - standard error of the mean.
[1] Control: without microbial inoculant; Inoculant: composted of *L. buchneri* and *L. plantarum* bacteria (110,000 CFU/g fresh forage).

Table 5 - Gas and effluent losses according to inoculant use and dry matter (DM) content

DM content (%)	Additive[1]		SEM
	Control	Inoculant	
Gas losses (% DM)			
32.1	1.78	3.14A	0.53
42.5	0.37	0.64B	0.53
Effluent losses (kg/t FF)			
32.1	9.59Aa	6.89b	0.92
42.5	4.77B	6.42	0.92

FF - fresh forage; SEM - standard error of the mean.
[1] Control: without microbial inoculant; Inoculant: composted of *L. buchneri* and *L. plantarum* bacteria (110,000 CFU/g fresh forage).
Means followed by different letters, lowercase in the row and uppercase in the column, differ statistically by the F test (P<0.05).

The LAB counts (Table 6) varied according to inoculant and DM content, presenting higher counts in inoculated (+37.2%) and high DM (+26.7%) silages. The yeast count was negatively affected by inoculant addition (P = 0.0021), which halved the number of colonies.

After opening the silos, the inoculated silages kept lower pH values compared with the control silages at both DM contents, throughout the ten days of aerobic exposure (Figure 1). Similarly, the aerobic stability (time for increasing 2 °C above the room temperature) was improved (+140.3 h) due to the inoculation (Table 7).

An interaction between inoculant and DM content was detected for total DM losses after aerobic exposure (DMLae; P = 0.006; Table 8). Unlike losses associated with fermentation, DMLae were greater for 42.5% DM silages regardless of inoculant addition. For the 32.1% DM silages, the use of inoculant halved the DMLae.

Table 6 - Microbial count (log CFU/g) of corn silages with or without inoculant, under two dry matter (DM) contents

Variable	Additive[1]		DM content (%)		SEM	Effect		
	Control	Inoculant	32.1	42.5		Inoculant (I)	DM	I × DM
LAB (log CFU/g)	4.3	5.9	4.5	5.7	0.33	0.0047	0.0216	0.12
Yeasts (log CFU/g)	5.02	2.5	4.2	3.3	0.49	0.0021	0.2291	0.166
Molds (log CFU/g)	2.1	3.2	3.3	2.03	0.61	0.2176	0.1512	0.118

LAB - lactic acid bacteria; SEM - standard error of the mean.
[1] Control: without microbial inoculant; Inoculant: composted of *L. buchneri* and *L. plantarum* bacteria (110,000 CFU/g fresh forage).

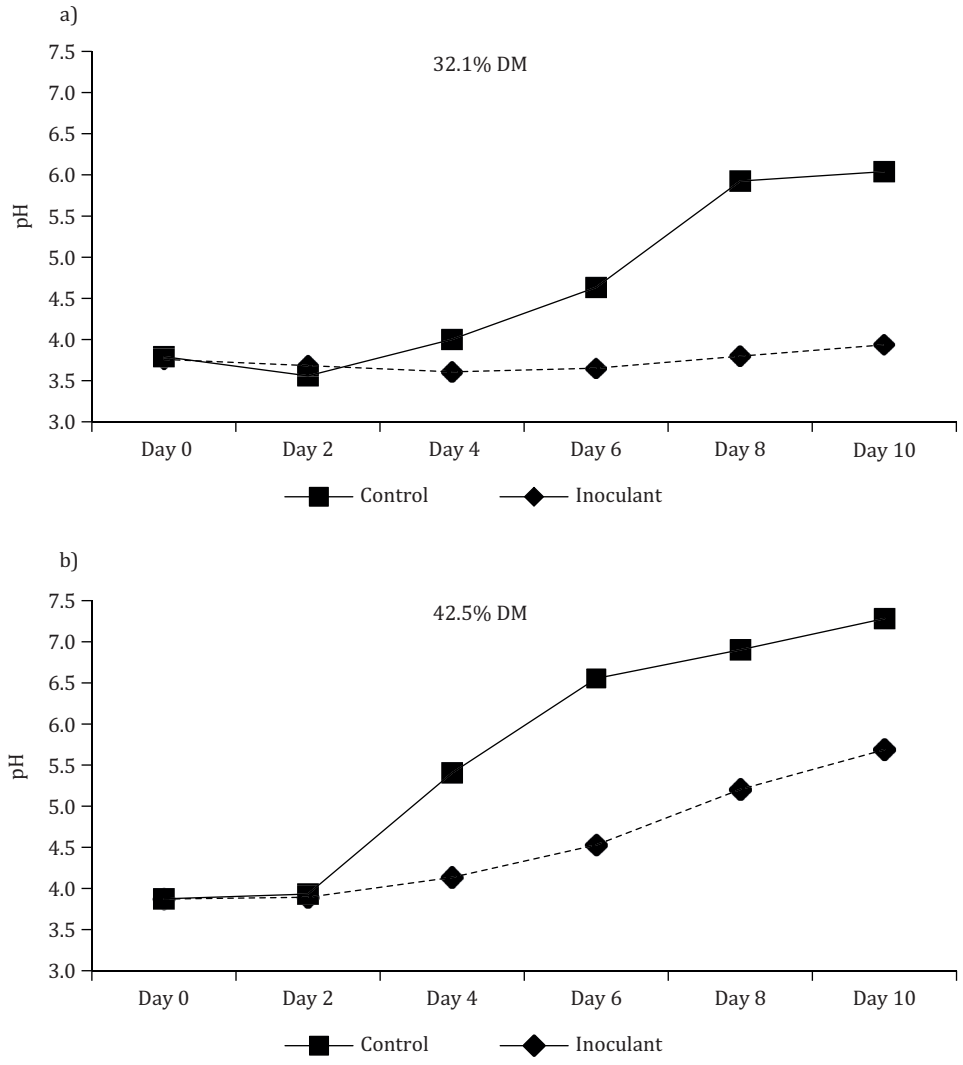

Figure 1 - Characterization of the pasture and supplementation of the three phases of the experiment.

Table 7 - Aerobic stability (AS) of corn silages with or without inoculant, under two dry matter (DM) contents

Variable	Additive[1]		DM content (%)		SEM	Effect		
	Control	Inoculant	32.1	42.5		Inoculant (I)	DM	I × DM
AS (h)	56.5	196.8	123.7	129.6	11.59	<0.0001	0.7227	0.159
MaxT (°C)	34.5	27.3	28.4	33.4	2.91	0.0978	0.2434	0.085
DMLae (% DM)	16.05	12.8	9.1	19.7	0.34	0.9002	0.0040	0.006

MaxT - maximum temperature; DMLae - dry matter losses after aerobic exposure; SEM - standard error of the mean.
[1] Control: without microbial inoculant; Inoculant: composted of *L. buchneri* and *L. plantarum* bacteria (110,000 CFU/g fresh forage).

Table 8 - Dry matter losses after aerobic exposure (DMLae) according to inoculant use and dry matter (DM) content

DM content (%)	Additive[1]		SEM
	Control	Inoculant	
32.1	11.60Ba	6.72Bb	0.53
42.5	20.50A	18.98A	0.53

SEM - standard error of the mean.
[1] Control: without microbial inoculant; Inoculant: composted of *L. buchneri* and *L. plantarum* bacteria (110,000 CFU/g fresh forage).
Means followed by lowercase letters in the row and uppercase letters in the column differ statistically by the F test (P<0.05).

4. Discussion

4.1. Chemical composition and fermentation products

Twelve days were spent between the first and the second harvest. Beyond the intentional 10 percentual units increase of the DM content, the advanced phenological stage of the corn plant deeply changed the chemical composition of the whole plant corn (Table 1) as well as their silages (Table 2). The pH range, around 3.8, was adequate for all the silages (McDonald et al., 1991). Lower DM content silages presented lower pH, possibly due to a higher water activity and its higher WSC content (Table 1). According to McDonald et al. (1991), acid production is hindered due to the increased osmotic pressure, meaning high DM silages stabilize fermentation at a greater pH. However, the analyzed acids concentration was similar for both the DM contents (Table 2).

Harvesting at 42.5% DM led to an expressive increase of the starch (25.3%) and EE (3.4%) content, while a slight decrease in starch and NDF digestibility was detected (5.3 and 1.3%, respectively). Those data suggest an improvement in the nutritional quality of the silages when postponing harvesting.

Plant proteins are generally abundant in the leaves. The protein concentration of silages from the second harvest was lower than from the first. Despite the phenological stage, the leaves stilled green at the second harvest (not evaluated). It is important to emphasize that the starch content increase causes a relative dilution in the centesimal composition of other nutrients, such as CP (Seleiman et al., 2017).

The lower starch digestibility for 42.5% DM silages is possibly due to the protein matrix of the kernel. The matrix is composed of proteins with high proline concentration, a hydrophobic amino acid that hinders rumen bacteria from accessing starch, since this environment is constituted mostly by liquids (Larson and Hoffman, 2008; Lage et al., 2017). However, the magnitude of starch digestibility decrease due to late harvest seems to be small when compared with the increase of the starch content. In fact, 42.5% DM silages presented 119.5 g of digestible starch per kg (wet basis), while 32.1% DM silages presented 74.3 g of digestible starch/kg. According to Owens and Balasan (2013) the starch digestibility in whole-plant corn silage is approximately 90%.

The NDF digestibility was also decreased at the second harvest. This variable is related to the chewing capacity and voluntary intake of ruminants (Van Soest, 1994). To estimate the impact of this variable over the intake and production of theoretical cows consuming the silages of this trial, our data was applied to the Milk 2006 model (University of Wisconsin), using corn productivity data for R5 and R5.5 stage (Horst et al., 2020b). The 32.1%DM silages allow 1,386 kg of milk/ton silage and 34,481 kg of milk/ha; the 42.5% DM silages allow 1,463 kg of milk/ton silage and 38,041 kg of milk/ha.

The inoculation was effective in preserving nutrients through the fermentation, which led to a greater starch and EE content, while the residual WSC contents of the silages were lower for the inoculated ones. Those effects may be related to the higher efficiency of the inoculated LAB for producing acids from carbohydrates (Muck et al., 2018), mainly WSC. Trends of lactic acid decrease and acetic acid increase due to the inoculant were detected, probably related to the conversion of lactic into acetic acid by the inoculated *L. buchneri* (Muck, 2010).

4.2. Fermentation losses

The fermentation losses were low for all the treatments (Table 4), related to a typical homolactic fermentation (McDonald et al., 1991). Nevertheless, the 42.5% DM treatments presented lower gas, effluent, and, consequently, TDML, probably due to a slightly better fermentation that probably comes from their higher starch content.

Most of the silage gas is produced in the first days of fermentation (Bueno et al., 2020), driven by the respiration of residual air in the pores of the vegetal material. Thus, higher DM silages tend to produce more gas due to packing issues. In this trial, we applied the same wet basis bulk density for both the DM silages, and the gas production was not increased by the increase in the DM content at harvest (Table 5).

Higher moisture silages tend to produce more effluent due to cell extrusion and cytoplasmic content release caused by the compression during the ensiling process (Macêdo and Santos, 2019). Rabelo et al. (2012) showed 13 kg of effluents per ton of FF in 30% DM corn silages with or without microbial inoculants. At this trial, the 32.1% DM was adequate, and effluents seem not to be a concern.

The inoculation with LAB can reduce final pH and effluent losses by improving homolactic fermentation and decreasing DM losses (Gandra et al., 2016). Interestingly, the inoculation decreased effluent production only in 32.1% DM silages (Table 5). However, this interaction was not detected for lactic acid production.

4.3. Silage microbial population

Lactic acid bacteria (LAB) count differed for both the inoculant and DM contents, without interactions between them (Table 6). As expected, adding strains that prevailed during fermentation resulted in higher LAB counts in inoculated treatments. Providing sufficient substrate is one of the required conditions for adequate LAB growth (McDonald et al., 1991; Lin et al., 1992), as observed in 42.5% DM silages probably related to their higher starch content. The metabolism of LAB populations can vary based on the fermentation substrate and water activity, especially when introduced via inoculants (Rabelo et al., 2014; Muck et al., 2018).

Lower yeast population in inoculated silages may be related to other *L. buchneri* operation mechanisms, given that the inoculant did not increase the acetic acid content. These mechanisms refer to the formation of other antifungal compounds, such as 1.2 propanediol, benzoic acid, catechol, hydrocinnamic acid, salicylic acid, 3-phenylactic acid, 3-hydroxydecanoic acid, and 4-hydroxybenzoic acid (Muck et al., 2018; Arriola et al., 2021). The synergistic impacts of these compounds harm the yeast metabolism while enhancing the aerobic stability of the inoculated silages (Muck et al., 2018).

4.4. Aerobic stability

Inoculation led to an expressive increase of 140 h in aerobic stability of the silages (Table 7). This effect is related to the lower yeast population in inoculated silages, probably due to the fermentative metabolism of *L. buchneri*. This bacterium is associated with increased aerobic stability by yeast growth inhibition (Muck et al., 2018; Melo et al., 2023).

Arriola et al. (2021) performed a refined meta-analysis of 158 research articles to evaluate the effects of *L. buchneri*-based inoculants in silages. Authors concluded that *L. buchneri* inoculation increases aerobic stability due to greater acetate concentration and lower yeast counts for all inoculant combinations, rates of inoculation, and forage types except tropical forages.

Silages with 42.5% DM showed greater DM losses during exposure to air than the 32.1% DM ones (Table 8). It seems to be a multifactorial effect related to the greater content of substrates remaining in these silages, such as starch and EE, which contribute to the development of aerobic microorganisms and, consequently, to the increase in pH (Chen and Weinberg, 2014; Lima et al., 2016). Additionally, dryer silages tend to be more porous, which could lead to more air entrance into the silage after opening the silo; however, we did not evaluate that. Yeasts constitute the main group of microorganisms that starts the degradation process shortly after opening the silos (McDonald et al., 1991), providing the ideal environment for the subsequent development of aerobic bacteria and molds.

The inoculation only decreased the DMLae for 32.1% DM silages, likely because these silages presented higher amounts of fermentation products other than acetic acid (Muck et al., 2018). Considering the correlation between DM losses and pH increase as an outcome of acids degradation by yeasts, the inoculated silages presented greater resistance to degradation. This effect was more evident in the 32.1% DM silages (Figure 1a) than in the 42.5% DM silages (Figure 1b).

Our data showed that, for the conditions of this trial, delaying harvest by 12 days to reach 42.5% DM content was beneficial, given the increased silage nutritive level (mainly starch), without compromising other quality parameters (except 24 h starch and 48 h NDF digestibility) or causing losses associated with fermentation.

The findings presented here cannot be extrapolated to other corn hybrids or field conditions. At last, farmers need to pay more attention to silo feed-out when using high DM silages, since drier silages are prone to aerobic deterioration regardless of inoculant use.

5. Conclusions

Harvesting 42.5% DM corn silages led to higher energy levels, considering the increase in ether extract and starch contents, despite lower estimates of starch and DM digestibility. All the silages presented adequate fermentation patterns and small DM losses as gas and effluents. The inoculant strongly increased the aerobic stability, although drier silages were less stable when exposed to air.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Tavares, Q. G.; Volpi, D.; Melo, N. N. and Zopollatto, M. **Formal analysis:** Tavares, Q. G. and Schmidt, P. **Funding acquisition:** Schmidt, P. **Investigation:** Tavares, Q. G.; Volpi, D.; Pereira, L. M.; Vigne, G. L. D.; Zopollatto, M. and Schmidt, P. **Methodology:** Tavares, Q. G.; Volpi, D.; Melo, N. N.; Pereira, L. M.; Vigne, G. L. D.; Zopollatto, M. and Schmidt, P. **Project administration:** Tavares, Q. G. **Resources:** Schmidt, P. **Supervision:** Volpi, D.; Zopollatto, M. and Schmidt, P. **Validation:** Schmidt, P. **Writing – original draft:** Volpi, D.; Zopollatto, M. and Schmidt, P. **Writing – review & editing:** Tavares, Q. G.; Zopollatto, M. and Schmidt, P.

Acknowledgments

The authors gratefully acknowledge Pioneer for funding this research. The authors would also like to thank the Centro de Assessoria de Publicação Acadêmica (CAPA) of the Universidade Federal do Paraná (UFPR) for assistance with English language translation and developmental editing.

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