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Corn silage quality under delayed sealing and microbial inoculant use

Abstract – The objective of this work was to determine the effects of sealing delay for 12 hours and of the use of microbial inoculant with heterofermentative bacteria on whole-plant corn (*Zea mays*) silage. The experimental design was completely randomized in a 2×2 factorial arrangement (with or without inoculant × with or without sealing delay). Silage was evaluated for: chemical composition, in vitro dry matter digestibility (IVDMD), fermentative parameters, losses, aerobic stability, and microbiological profile. The heating of the silage caused by respiration increased the contents of neutral detergent insoluble protein and of acid detergent insoluble protein by 77 and 27.3%, respectively. Soluble carbohydrate losses increased the contents of neutral detergent fiber corrected for ash and protein by 9.1% and of acid detergent fiber by 5.1%, but decreased the content of nonfibrous carbohydrates by 11.2%. IVDMD did not differ between treatments. Silages with delayed sealing and the inoculant showed higher pH and contents of acetic acid, propionic acid, and ammoniacal nitrogen, but a lower content of lactic acid. Delayed sealing reduces the nutritional value, increases the fibrous fractions, and decreases the soluble carbohydrates, whereas the use of inoculant does not improve the nutritional value and aerobic stability of the silages.

Index terms: Lactobacillus, feed evaluation, fermentation, forage conservation, microbiology.

Qualidade da silagem de milho submetida a atraso na vedação e uso de inoculantes microbianos

Resumo – O objetivo deste trabalho foi determinar os efeitos do atraso de vedação por 12 horas e do uso de inoculante com bactérias heterofermentativas na silagem de plantas inteiras de milho (*Zea mays*). O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 2×2 (com ou sem inoculante × com ou sem atraso de vedação). Avaliou-se a silagem quanto a: composição química, digestibilidade in vitro de matéria seca (DIVMS), parâmetros fermentativos, perdas, estabilidade aeróbica e perfil microbiológico. O aquecimento da silagem causado pela respiração aumentou o teor de proteína insolúvel em detergente neutro e o de proteína insolúvel em detergente ácido em 77 e 27,3%, respectivamente. As perdas de carboidratos solúveis aumentaram o teor de fibras em detergente neutro corrigidas para cinzas e proteínas em 9,1% e de fibras em detergente ácido em 5,1%, mas diminuíram o teor de carboidratos não fibrosos em 11,2%. A DIVMS não diferiu entre os tratamentos. As silagens com vedação atrasada e inoculadas apresentaram maiores pH e teores de ácido acético, ácido propiônico e nitrogênio amoniacal, mas menor teor de ácido láctico. A vedação atrasada reduz o valor nutricional, aumenta as frações fibrosas e diminui os carboidratos solúveis, enquanto o uso de inoculante não melhora o valor nutricional e a estabilidade aeróbica das silagens.

Termos para indexação: Lactobacillus, avaliação alimentar, fermentação, conservação de forragem, microbiologia.

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Introduction

Although silage commercialization and transport are common activities in farms in Brazil, Israel, and other countries for several reasons (Kim & Adesogan, 2006; Chen & Weinberg, 2014; Michel et al., 2016), the decision between purchasing already prepared silage or in natura material to ensilage at the farms is still controversial. When silage is purchased, it is exposed to air, which can reactivate aerobic microorganisms that can cause aerobic deterioration and reduce feed nutritional value (Michel et al., 2016; Anjos et al., 2018). When in natura material is purchased, there is a greater air exposure because of delayed sealing before ensilage due to the transport between farms and ensiling at the farm, which increases plant cell respiration time and can decrease soluble carbohydrate content, impairing the fermentative processes, increasing the fibrous fractions, and decreasing the nutritional value of silages (Brüning et al., 2018).

The negative effects of delayed sealing are proportional to air-exposure time. According to Kim & Adesogan (2006), exposures longer than 10 hours, as frequently observed in Brazil (Anjos et al., 2018), may impair the fermentation process by reducing soluble carbohydrate content. In addition, there may be a greater contamination by yeasts, which can decrease aerobic stability after silo opening (Ruxton & McDonald, 1974) and impair the fermentation process due to the growth of enterobacteria, clostridia, and fungi.

The growth of anaerobic microorganisms may, however, be mitigated by inoculants with heterofermentative strains through the production of acetic and propionic acids, which reduce mold and yeast growth and can increase silage aerobic stability (McDonald et al., 1991; Muck et al., 2018). The use of inoculants, therefore, can reduce the post-opening losses and maintain the nutritional value of silages.

Although the effects of re-ensiling and microbial inoculant use on silage quality are well known in Brazil (Michel et al., 2016; Anjos et al., 2018; Coelho et al., 2018; Santos et al., 2021), those of delayed sealing due to green forage transport still have not been described.

The objective of this work was to determine the effects of sealing delay for 12 hours and of the use of microbial inoculant with heterofermentative bacteria on whole-plant corn silage.

Materials and Methods

The BM 3063 PRO 2 corn hybrid was planted in November 2018, spaced at 70 cm, in an experimental farm located in the municipality of Igarapé, in the state of Minas Gerais, Brazil (20°4'24.79"S, 44°21'18.90"W, at 849 m altitude). Fertilization was applied both at planting, using 350 kg ha^{-1} 04-30-10 (N-P_2O_5-K_2O) + 35 kg ha^{-1} urea (48% N), and at 36 days after planting as topdressing, using 400 kg ha^{-1} nitrate + 22 kg ha^{-1} micronutrient fertilizer + 86 kg ha^{-1} potassium chloride.

The experiment was carried out in a completely randomized design, in a 2×2 factorial arrangement, with or without microbial inoculant × with or without sealing delay for 12 hours, with five replicates. The used inoculants were the following heterofermentative strains: *Lactobacillus buchneri* NCIMB 40788 and *Propionibacterium acidipropionici* CNCM I-4661 (Lallemand Animal Nutrition, Milwaukee, WI, USA), at an approximate concentration of 4.0×10^4 and 6.0×10^4 colony-forming units (CFU) per gram, respectively.

The material for ensilage was harvested with 30.2% dry matter (DM), chopped to a theoretical length of 1.0 to 2.0 cm using the JF C120 AT conventional silage harvester (JF Máquinas Agrícolas, Itapira, SP, Brazil), and divided into four fractions – two not exposed and two exposed to air. The first and second fractions that were not exposed to air received, respectively, bacterial inoculant and mineral water, both at a proportion of 2.0 mL kg^{-1} fresh forage. Before being ensiled, the other two fractions were exposed to air for 12 hours, in a shed, at ambient temperature, with or without inoculant, which was diluted in mineral water and then sprayed evenly through a backpack pump. This 12-hour delay was chosen because it is considered the minimum time for the silage commercialization process in Brazil (Anjos et al., 2018). During the exposure period, air temperature ranged from 19.5 to 26.5°C and relative humidity from 67 to 93% according to data obtained from the automatic weather station of Instituto Brasileiro de Meteorologia, located 24 km from the shed.

The temperature of the chopped corn forage was measured using the TAE-110 mercury thermometer (Equitherm, Curitiba, PR, Brazil), inserted 10 cm deep in the silage mass center. A total of 11.7±1.3 kg whole-plant corn silage were then ensiled and compacted manually in silos – 20 L buckets with Bunsen valve and lid to allow gas removal, with a cotton fabric bag

containing 2.0 kg dry sand placed at the bottom of each to allow the measurement of effluents (Pedroso et al., 2008).

The silos were opened after 333 days of ensiling for analyses of silage chemical composition, in vitro dry matter digestibility (IVDMD), fermentative parameters – pH, ammoniacal nitrogen (NH_3-N), and lactic, acetic, propionic, butyric, and valeric acids –, losses in gas effluent, total DM, aerobic stability, and microbiological profile.

For the analyses, silage samples were pre-dried in a forced-ventilation oven, at 55°C, for 72 hours and ground to 1.0 mm in the Thomas Model 4 Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). The following contents were obtained by methods 934.01, 954.01, 942.05, and 920.39 (Helrich, 1990), respectively: DM in an oven at 105°C, crude protein (CP) by the Kjeldahl method, ash in muffle at 600°C for 4 hours, and ether extract (EE) by the Soxhlet extractor, as well as organic matter by the difference between DM and ash. The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were determined by the sequential method described by Van Soest et al. (1991), using the Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY, USA). Residues from the NDF and ADF analyses were subjected to ash and CP determination to obtain the values of neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP). These values were used to correct NDF and ADF for ash and protein (NDFap and ADFap, respectively).

The nonfibrous carbohydrate (NFC) content was estimated according to National Research Council (NRC, 2001) by: NFC = 100 - (% NDF +% CP +% EE +% Ash). IVDMD was obtained as in Tilley & Terry (1963) and adapted by Holden (1999), using the Daisy II rumen simulator (Ankom Technology, Macedon, NY, USA). Ruminal fluid was collected from a fistulated bovine that was fed a diet composed of 10 kg (DM) corn silage and 2.5 kg (DM) commercial feed with 24% CP.

To determine pH and NH_3-N content, silage juice was extracted using a hydraulic press (2.5 kgf cm^{-3}). pH was measured with the HI 221 digital potentiometer (Hanna Instruments, Woonsocket, RI, USA). The ratio of the NH_3-N content in total N (NH_3-N/TN) was obtained by Kjeldahl distillation without the digestion step (Latimer, 2012), using magnesium oxide and calcium chloride as neutralizing medium for ammonia evaporation, with boric acid as the receptor solution and 0.1 mol L^{-1} hydrochloric acid as a titrant.

The contents of acetic, propionic, butyric, and valeric short-chain fatty acids (SCFAs) were determined by gas chromatography based on the external calibration curve made with the following chromatographic standards of Chem Service, Inc. (West Chester, PA, USA): 995, 990, 987, and 990 mL L^{-1} for the acetic (CAS 64-19-97), propionic (CAS 9.4.79), butyric (CAS 107-92-6), and valeric (CAS 109-52-4) acids, respectively. Lactic acid content was obtained in the 6405 UV/VIS spectrophotometer (Jenway, Staffordshire, UK) according to the method adapted from Pryce (1969). The lactic:acetic acid ratio was calculated by dividing lactic acid by acetic acid content.

The experimental silos were weighed at different times: before ensiling, when they were empty, except for the dry sandbag in them; after being filled with compacted forage and sealed with an adhesive tape; before being opened, to determine gas losses; and after being opened, without the silage but with the lid and sandbag, to determine effluent losses. The total DM loss was estimated by the difference between the initial and final dry mass weight of the experimental silos in relation to the amount of dry mass ensiled, without the weight of the silo + lid + sandbag set at ensiling and opening time (Jobim et al., 2007).

For the aerobic stability test, two plastic buckets with 2.0 kg of silage in each were covered with aluminum foil and placed in an air-conditioned room maintained at 25±1°C. At sampling time, silage was homogenized and 200 g from each bucket were sampled. In the first bucket, silage temperatures were recorded at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, 36, 402, 54, 68, 80, 92, 104, 116, 128, 140, 152, 164, 176, 188, 200, 212, 224, and 240 hours after silo opening using the TAE-110 mercury thermometer (Equitherm, Curitiba, PR, Brazil), which was inserted 10 cm deep in the silage mass center. Aerobic stability was calculated as the time, in hours, for silages to show an increase of 2°C in relation to ambient temperature after silo opening (Ranjit & Kung Jr, 2000). In the second bucket, the silages were sampled at 0, 48, 72, 120, 168, and 240 hours after silo opening for changes in the pH and NH_3-N values to be evaluated as described previously.

The aerobic microorganisms were counted immediately after silo opening and after the loss of

aerobic stability. For aerobic microorganism count (yeast, mold, and aerobic bacteria), the spread plate technique in 10^{-1} dilution was used. A sample of 25 g was weighed and added to flasks containing 225 mL of 0.1% peptone solution sterilized in an autoclave, at 121°C, for 15 min and stirred manually for 2 min. From the obtained extract, after 1 min rest, decimal dilutions (10^{-2} to 10^{-5}) were prepared in test tubes containing 9.0 mL sterile 0.1% peptone solution. An amount of 0.1 mL of the dilutions was spread over disposable Petri dishes, and the inoculum was spread over agar surface with the Drigalski spatula.

Total yeast and total mold were counted, respectively, on a plate containing tryptone glucose yeast extract agar after aerobic incubation for four days at 30±1°C and on a plate containing dichloran Rose-Bengal chloramphenicol after aerobic incubation for five days at 25±1°C. Total bacterial counts were performed on plate count agar after aerobic incubation for 48 hours at 35°C (Pitt & Hocking, 2009). Microbial counts were determined at silo opening and after the loss of aerobic stability, with values expressed as CFU per gram of silage, and were transformed into log10 to obtain a normal distribution.

Silage chemical composition, IVDMD, fermentative parameters, losses, aerobic stability and aerobic microorganism count were analyzed. Shapiro-Wilk's and Bartlett's tests were used to verify the statistical assumptions of normality and homogeneity of variances, respectively. The analysis of variance was performed using the following statistical model:

$$Y_{ijk} = \mu + E_i + I_j + EI_{ij} + e_{ijk},$$

where Y_{ijk} represents the observed response values; μ is the overall average; E_i is the delayed sealing effect (i = 0 or 12 hours); I_j is the inoculant use effect (j = with or without inoculant use); EI_{ij} is the effect of the interaction between delayed sealing i and inoculant use j; and e_{ijk} represents the random error associated with observation with delayed sealing i, inoculant use j, and replicate k, being $e_{ijk} \sim N(\mu, \delta^2)$.

The main factor means were compared by Fisher's test, at 5% probability. When there was an interaction between factors, means were compared by Tukey's test, at 5% probability. Orthogonal polynomials were used to determine whether the exposure time for aerobic stability assessment resulted in a linear or quadratic effect on the NH_3-N/TN ratio and pH values. Statistical difference was admitted when the error rate was lower than 5% probability. All analyzes were performed using the R statistical software (R Core Team, 2019).

Results and Discussion

DM content showed a significant interaction (p=0.04) between sealing delay and inoculant use (Table 1), being 4.3% higher in silages that were immediately sealed and inoculated. Conversely, Kim & Adesogan (2006) observed an increase in the DM content of silages subjected to a sealing delay of 3 hours and stored after ensiling at 40°C, but not at 20°C. According to these authors, there was a decrease in DM losses depending on ensiling temperature.

The delay in sealing for 12 hours increased the contents of CP by 4.5%, NDIP by 77%, and ADIP by 27.3% (Table1). Kim & Adesogan (2006) found a CP increase of 24.3% in whole-plant corn silage under a 3-hour delayed sealing compared with immediate sealing. The changes observed in the present study are attributed to the process of carbohydrate oxidation that releases heat and increases NDIP and ADIP contents through the Maillard reaction, which can reduce protein availability (Krizsan & Randby, 2007). Therefore, it is important to evaluate low availability fractions and NH_3-N in silages under delayed sealing, since, although CP content may not change, its quality and availability may be lower.

The content of NFC decreased by 11.2% and that of NDFap increased by 9.1% with delayed sealing (Table 1). The loss of soluble carbohydrates by the respiratory process possibly explains the increase in NDF. Brüning et al. (2018) reported an increase in NDF content and a decrease in NFC content when sealing was delayed for two days. However, Kim & Adesogan (2006), when simulating transport conditions in Florida, USA, did not observe any changes in silages exposed to air for 3 hours. Under Brazilian conditions, where the ensiling process takes an average of 12 hours (Anjos et al., 2018), the results of the present study allowed concluding that a longer exposure time decreases NFC and increases NDFap. Furthermore, the increase in fibrous fractions and decrease in soluble fractions did not reduce IVDMD. These results are possibly related to silage quality, showing the importance of carefully planning and

executing silage stages, especially when there is a need to delay silo sealing.

For gas losses, there was a significant interaction between delayed sealing and inoculant use (Table 1). Under delayed sealing, gas losses were 51.1% lower in the silage with the inoculant and 40.3% lower compared with immediate sealing. Total DM losses also showed a significant interaction between delayed sealing and inoculant use, being 36.8% lower in the silage under immediate sealing and with the inoculant. The silages with delayed sealing, however, did not differ significantly for total DM losses, which is indicative of qualitative and not quantitative losses.

pH values showed a significant interaction between delayed sealing and inoculant use (Table 1). Under delayed sealing, pH increased 10.6% in the silages with the inoculant due to its heterofermentative pathway; despite this, the pH was still considered adequate for the conservation process of silages (Kung Jr. et al., 2018). The lactic acid content was also indicative of a significant interaction between delayed sealing and inoculant use, being 1.62 times higher in the silage with delayed sealing and without the inoculant. In the silage with immediate sealing and the inoculant, the production of lactic acid was probably due to the first out of two fermentative pathways of *L. buchneri* (McDonald et al., 1991). The first converts glucose and fructose into lactic acid, acetic acid, mannitol, CO_2, and H_2O, whereas the second converts lactic acid into acetic acid, 1,2 propanediol, and small ethanol amounts (Oude Elferink et al., 2001).

The acetic acid content was influenced by inoculant use (Table 1), being 2.13 times higher in inoculated silages. In addition, delayed sealing and inoculant had an isolated effect on the lactic:acetic acid ratio, which was 2.78 times higher in silages that were not

Table 1. Chemical composition and fermentative profile parameters of corn silage under the treatments immediate sealing (SIL) or delayed sealing for 12 hours (EXP) with or without (control) inoculant[1].

Parameter	Control		Inoculant[2]		SEM	p-value		
	SIL	EXP	SIL	EXP		I	E	I x E
Chemical composition								
DM (g kg^{-1} FM)	277	286	290	284	1.57	ns	ns	*
Ash (g kg^{-1} DM)	36.7	40.9	31.6	38.1	1.65	ns	ns	ns
Crude protein (g kg^{-1} DM)	85.0	89.1	85.2	88.8	0.59	ns	**	ns
NDIP (g kg^{-1} DM)	8.40	15.3	8.40	14.4	0.31	ns	**	ns
ADIP (g kg^{-1} DM)	6.20	8.40	6.60	8.00	0.26	ns	**	ns
Ether extract (g kg^{-1} DM)	35.4	35.3	27.8	38.7	1.66	ns	ns	ns
NDFap (g kg^{-1} DM)	398	444	403	429	3.48	ns	**	ns
ADFap (g kg^{-1} DM)	207	216	205	217	2.67	ns	ns	ns
ADL (g kg^{-1} DM)	29.5	30.1	33.5	31.4	0.91	ns	ns	ns
NFC (g kg^{-1} DM)	444	389	452	406	4.64	ns	**	ns
IVDMD (g kg^{-1} DM)	615	613	619	612	7.29	ns	ns	ns
Fermentative profile parameter								
pH	3.52	3.49	3.43	3.87	0.01	**	**	**
Lactic acid	100	92.5	100.5	56.9	0.65	**	**	**
Acetic acid	12.4	17.5	33.6	29.9	1.08	**	ns	ns
Lactic:acetic acid ratio	8.42	5.51	3.06	1.95	0.26	**	**	ns
Propionic acid	0.87	0.98	0.74	1.91	0.02	*	**	**
Butyric acid	7.31	5.92	1.25	10.2	0.51	ns	*	**
Valeric acid	0.40	0.24	0.21	0.78	0.05	ns	ns	**
Gas loss (% DM)	9.63	5.90	4.71	7.56	0.58	ns	ns	**
Effluent loss (g kg^{-1} FM)	353	229	357	194	6.43	ns	**	ns
Total DM loss (% DM)	12.9	8.08	8.15	9.40	0.58	ns	ns	*

[1]DM, dry matter; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; NDFap, neutral detergent fiber corrected for ash and protein; ADFap, acid detergent fiber corrected for ash and protein; ADL, acid detergent lignin; NFC, nonfibrous carbohydrate; IVDMD, in vitro DM digestibility; FM, fresh matter; SEM, standard error of mean; I, inoculant effect; E, delayed sealing effect; and I × E, interaction effect. [2]Mixture of *Lactobacillus buchneri* and *Propionobacterium acidipropionici* at the concentrations of 4.0×10^4 and 6.0×10^4 CFU g^{-1}, respectively. * and **Significant at 5 and 1% probability, respectively. [ns]Nonsignificant.

inoculated and 35% lower in silages under delayed sealing. In a meta-analysis of whole-corn plant silage, Kleinschmit & Kung Jr. (2006) found that the lactic:acetic acid ratios were close to 3:1 in silages that were not inoculated and were 2.3:1 and 1.3:1 in silages inoculated with low and high rates (≤100,000 CFU g^{-1}, >100,000 CFU g^{-1}) of *L. buchneri*. These values are similar to those observed in the present study for silages with delayed sealing, but are much lower than that of 8.42 for silages with immediate sealing and without the inoculant.

Propionic acid content showed a significant interaction between delayed sealing and inoculant use (Table 1). The content of this acid was 1.95 times higher in silages with delayed sealing and the inoculant, but lower in silages with immediate sealing and the inoculant since *P. acidipropionici* does not perform well in acid medium (Ávila & Carvalho, 2020). Therefore, due to their low pH, good quality silages inoculated with *P. acidipropionici* may not show any benefits.

Under immediate sealing, butyric acid content decreased 82.8% in the silages with the inoculant (Table 1), whereas, under delayed sealing, valerate content was 3.3 times higher in the silages with the inoculant. According to Kung Jr. et al. (2018), the values obtained here for butyric acid are high. In the present study, silages with a higher butyric acid content also showed a higher NH3-N/TN ratio, which indicates a greater proteolysis during storage and a higher pH.

During air exposure after silo opening, there was a significant effect of the interaction between inoculant use and delayed sealing on the NH3-N/TN ratio at 0, 72, and 240 hours (Figure 1). At these hours, under immediate sealing, inoculant use reduced the NH3-N/TN ratio in the silages by 12.9, 10.8, and 13.8%, respectively. Under delayed sealing, the inoculated silages showed NH3-N/TN ratios 14.5 and 20.1% higher at 0 and 240 hours than the non-inoculated silages. The greater air exposure after silo opening, therefore, caused a linear increase (R^2 = 0.916) in the NH3-N/TN ratio, which was of 0.051 g kg^{-1} in each hour of exposure. Oliveira et al. (2017) highlighted that this ratio reflects protein degradation intensity in silages.

At all air exposure times, silages with delayed sealing and the inoculant showed a higher pH (Figure 2) and, consequently, a good quality and conservation.

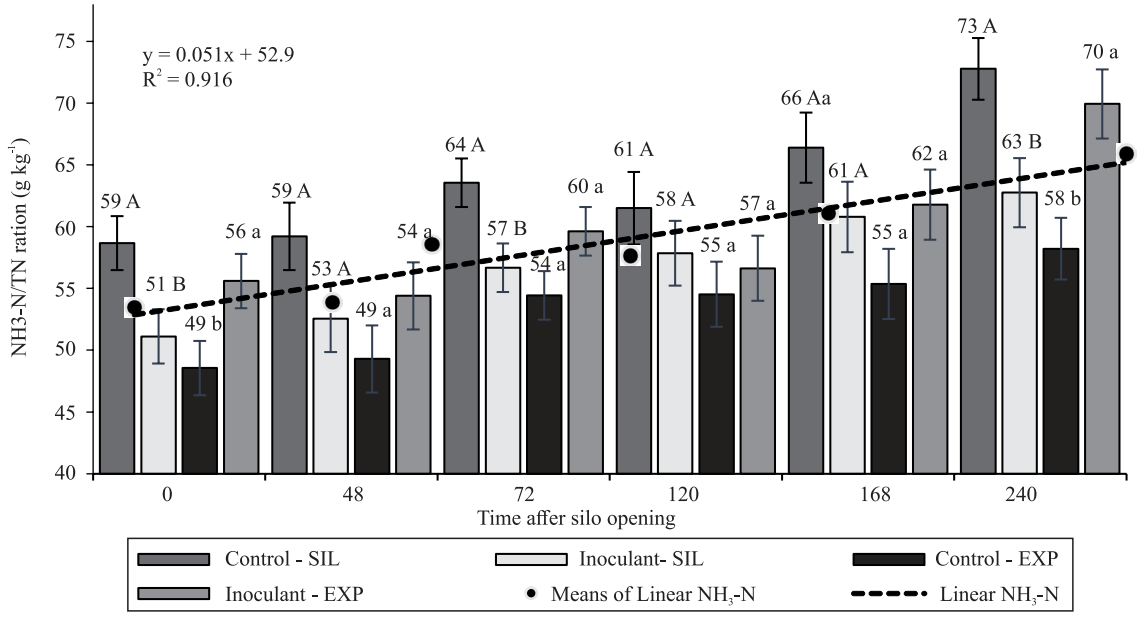

Figure 1. Means of the ammoniacal nitrogen/total nitrogen (NH_3-N/TN) ratio during aerobic stability assessment at 0, 48, 72, 120, and 240 hours of the exposure to air of silages under the treatments immediate sealing (SIL) or delayed sealing for 12 hours (EXP) with or without inoculant. Means followed by equal letters, uppercase to compare the SIL treatments and lowercase to compare the EXP treatments, do not differ by Fisher's test, at 5% probability. SEM, standard error of mean: 1.1, 1.36, 0.98, 1.35, 1.42, and 1.32 for 0, 48, 72, 120, 168, 1.42, and 240 hours, respectively.

Therefore, pH was effective for silage conservation even under delayed sealing (Kung Jr. et al., 2018).

There was no difference in aerobic stability between silages (Table 2). Mold counts were two times higher in silages with delayed sealing and 43% lower in inoculated silages compared, respectively, with immediately sealed and non-inoculated silages. Aerobic bacteria count was 1.12 times higher in inoculated silage under delayed sealing. According to Wilkinson & Davies (2013), silages with delayed sealing may present a greater contamination by yeasts that can remain inactive during the stable phase and reactivate after

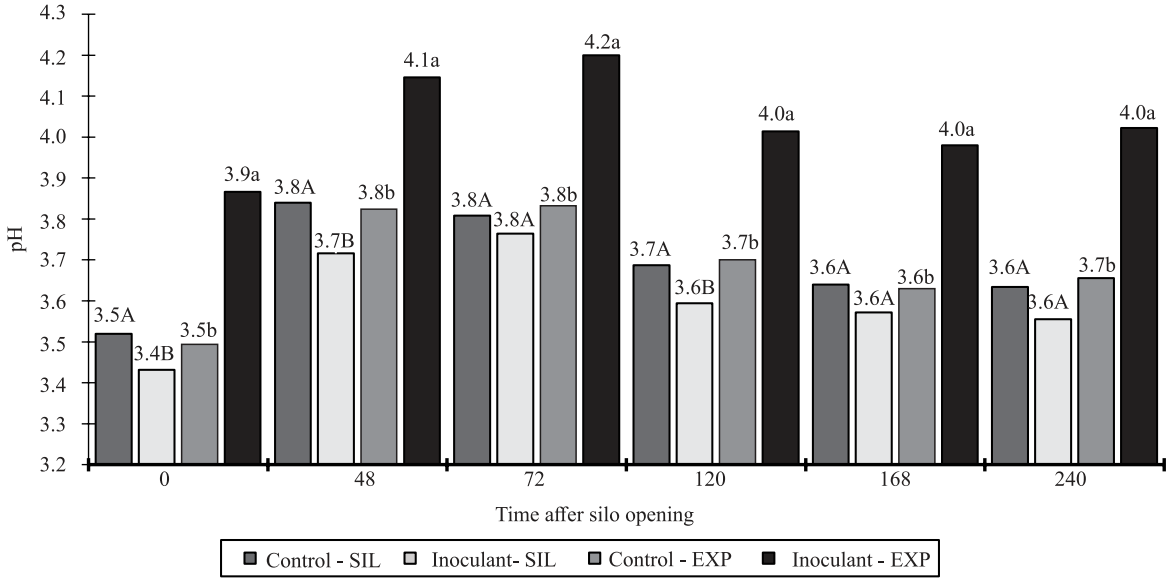

Figure 2. Means of pH during aerobic stability assessment at 0, 48, 72, 120, and 240 hours of exposure to air of silage under the treatments immediate sealing (SIL) or delayed sealing for 12 hours (EXP) with or without inoculant. Means followed by equal letters, uppercase to compare the SIL treatments and lowercase to compare the EXP treatments, do not differ by Fisher's test, at 5% probability. SEM, standard error of mean: 1.1, 1.36, 0.98, 1.35, 1.42, and 1.32 for 0, 48, 72, 120, 168, 1.42, and 240 hours, respectively.

Table 2. Parameters of aerobic stability and total microbial count of silages under the treatments immediate sealing (SIL) or delayed sealing for 12 hours (EXP) with or without (control) inoculant[(1)].

Parameter	Control		Inoculant[(2)]		SEM	p-value		
	SIL	EXP	SIL	EXP		I	E	I x E
Aerobic stability (hours)	240	230	216	220	8.43	ns	ns	ns
Microbial count at silo opening:								
Bacteria (log_{10} CFU g^{-1})	5.63	5.63	5.46	6.32	0.09	ns	*	*
Yeast (log_{10} CFU g^{-1})	5.14	4.90	4.88	5.22	0.12	ns	ns	ns
Mold (log_{10} CFU g^{-1})	2.94	3.81	0.60	3.25	0.34	*	*	ns
Counts after aerobic stability loss:								
Bacteria (log_{10} CFU g^{-1})	5.70	5.55	5.22	6.64	0.24	ns	ns	ns
Yeast (log_{10} CFU g^{-1})	5.73	5.91	5.5	6.42	0.14	ns	ns	ns
Mold (log_{10} CFU g^{-1})	4.84	6.1	5.34	5.55	0.35	ns	ns	ns

[(1)]CFU, colony-forming units; SEM, standard error of the mean; I, inoculant effect; E, delayed sealing effect; and I × E, interaction effect. [(2)]Mixture of *Lactobacillus buchneri* and *Propionobacterium acidipropionici* at the concentrations of 4.0×10^4 e 6.0×10^4 CFU g^{-1}, respectively.* and **Significant at 5 and 1% probability, respectively. [ns]Nonsignificant.

silo opening and air exposure; however, this increase did not occur in the present study. Brüning et al. (2018) reported a lower aerobic stability in whole-plant corn silage due to delayed sealing, but under an air exposure time four to eight times longer than that in the present work, which explains the observed differences. The 12-hour delay in sealing, therefore, does not increase silage contamination with spoilage microorganisms enough to reduce aerobic stability.

Conclusions

1. Delayed sealing for 12 hours reduces the nutritional value, increases the fibrous fractions, and decreases the soluble carbohydrates of silages.
2. The use of microbial inoculant with heterofermentative bacteria does not improve the nutritional value and aerobic stability of silages.

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