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Aerobic stability of whole plant corn silage inoculated with a bacterial inoculant in three maturity stages

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ABSTRACT

Maturity stage (MS) at harvesting is the most important factor that determines the nutritive value and productivity of corn silage. Once opened, the silage for supplyis exposed to oxygen deterioration due to aerobic microbial activity, which could negatively influence the quantity and quality of silage. There are bacterial inoculants containing microorganisms which improve the process of fermentation and the aerobic stability of silage. The aim was to evaluate the effect on aerobic stability produced by a bacterial inoculant in whole plant corn silage at different maturity stages (MS). The experimental design was factorial (n=3). The factors were: inoculated and state of maturity; the first with two levels: control and inoculated and the second with three levels: 25, 35 and 45% MS. The data were analyzed by ANOVA and the comparison of means by the Tukey test ($p<0.05$). Maize crop was harvested: 25, 35 and 45% dry matter (DM). Chopped material was ensiled in 20 L buckets (six silos/MS), three of them were Inoculated (I) and three were not Control. The interactions between I*MS were not significant ($P>0.05$) for all variables. To the evaluated variables, there was no difference ($P>0.05$) among Inoculated vs. Control treatments. There was a difference ($P<0.05$) between MS, where 25% DM had higher water-soluble carbohydrates and acetic acid, which allowed greater aerobic stability than with 35 and 45% DM. DM losses with 45% DM treatment had the highest ($P<0.05$) loss compared to 25 and 35% DM. Results suggested there was no effect of bacterial inoculation but there were effect maturity stages on the aerobic stability of silage. As the harvest is delayed the silage has lower aerobic stability.

Keywords: aerobic deterioration, pH, ammonia nitrogen, fermentation products, dry matter losses.

RESUMEN

La etapa de madurez (EM) en la cosecha es el factor más importante que determina el valor nutritivo y la productividad del ensilaje de maíz. Una vez abierto el silo para el suministro, se expone al deterioro del oxígeno debido a la actividad aeróbica microbiana, que podría influir negativamente en la cantidad y calidad del ensilaje. Existen inoculantes bacterianos que contienen microorganismos que mejoran el proceso de fermentación y la estabilidad aeróbica del ensilaje. El objetivo fue evaluar el efecto sobre la estabilidad aeróbica de un inoculante bacteriano en ensilaje de maíz de plantas enteras en diferentes etapas de madurez. El diseño experimental fue un diseño factorial (n=3). Los factores fueron: inoculado y estado de madurez; el primero con dos niveles: control e inoculado y el segundo con tres niveles: 25, 35 y 45% MS. Los datos se analizaron

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*mediante ANVA y la comparación de medias por la prueba de Tukey (p<0.05). La cosecha de maíz fue con: 25, 35 y 45% de materia seca (MS). El material picado se ensiló en cubos de 20 l (seis silos/EM), tres de ellos fueron Inoculados (I) y tres fueron Control. Las interacciones entre I*EM no fueron significativas (P>0.05) para todas las variables. Para las variables evaluadas, no hubo diferencia (P>0.05) entre los tratamientos Inoculado vs. Control. Hubo diferencias (P<0.05) entre la EM, donde el ensilaje con 25% MS tenía más carbohidratos solubles en agua y ácido acético, lo que permitió una mayor estabilidad aeróbica que con un 35 y un 45% MS. La pérdida en el ensilaje con 45% MS fue más alta (P<0.05) en comparación con 25 y 35% MS. Los resultados sugirieron que no hubo efecto de la inoculación, pero sí la etapa de madurez afectó la estabilidad aeróbica del ensilaje. A medida que se retrasa la cosecha, el ensilaje tiene menor estabilidad aeróbica.*

Palabras clave: *deterioro aeróbico, pH, nitrógeno amoniacal, productos de la fermentación, pérdidas de materia seca.*

INTRODUCTION

In livestock production regions that have seasonal variations in climate, the production of forages also varies throughout the year depending on rainfall, temperature and day length (Wilkinson and Rinne, 2018). The conservation forages as silage is an important source of nutrients for livestock nutrition because it enables crops to be available for use either throughout the year or in periods of restricted seasonal availability of pasture for the grazing animal (Wilkinson and Davies, 2012). The maize for silage is one of the crops more used because it has the ability to yield relatively high quantities of starch and dry matter per hectare to low-cost (Wilkinson and Rinnie, 2018).

Although there are several factors that determine the nutritive value of corn silage, the maturity stage at harvesting is the major (Johnson *et al*., 2003). Johnson *et al*. (2002) found that this moment is when the dry matter is 35%. This time to harvest lasts a few days although there are differences between maize hybrids (Camarasa *et al*., 2013). Silage should not be performed in the optimum moment for different causes such as weather conditions, wet soil and contractor delay.

The main goal in silage is to maintain the original quality of the preserved crop, as much as possible (Wilkinson and Davies, 2012) and for this reason, the absence of oxygen and acidification of the material is needed (McDonald *et al*., 1991). It is now recognized that there are changes which may occur during the feed-out phase when the silo feed face is open and the material is exposed to air (Wilkinson and Davies, 2012). These changes are called aerobic deterioration beginning when air penetrates silage (Pahlow and Muck, 2009), and it has negative effects on nutritional value (Woolford, 1990) because yeasts assimilate lactic acid, causing the pH to increase. Thus, the microorganisms that were inhibited by low pH begin to proliferate and spoil the silage (Tabacco *et al*., 2011a). As a result, its nutritional value is reduced owing to loss of fermentation products that are potentially digestible substrates (Whitlock *et al*., 2000).

Bacterial inoculants containing homofermentative bacteria, such as *Lactobacillus plantarum* and *Enterococcus faecium*, have been developed with the purpose of producing lactic acid and reducing the pH quickly (Wilkinson and Davies, 2012). However, these bacteria have also been responsible for reducing aerobic stability in corn silage due to low production of volatile fatty acids that inhibit the fungal activity (Muck and Kung, 1997). For these purposes, bacterial inoculants containing heterofermentative bacteria, such as *Lactobacillus buchneri*, have been developed by improving the aerobic stability of silage (Tabacco *et al*., 2011b; Wilkinson and Davies, 2012), through the fermentation of lactic acid to acetic acid and inhibition of yeast (Driehuis *et al*., 2001) and clostridial growth (Tabacco *et al*., 2011b). Therefore, the aim of this study was to analyze the effects of the use of a bacterial inoculant, with both bacterial types, in whole plant corn silage at different maturity stages on fermentation parameters and on aerobic stability.

MATERIALS AND METHODS

The study was carried out at the Pergamino Experimental Station of the National Institute of Agricultural Technology (INTA), Buenos Aires, Argentina (33° 560S, 60° 340W; elevation 66 m a.s.l.).

Maize (*Zea Mays*; simple corn hybrid AX 882 HCL/MG) crop and it was harvested at three stages: 25, 35 and 45% dry matter (DM). The DM percentage was performed cut every week from the dough stage, 5 plants were dried for 48 hours at 60°C. During the harvest, 80 plants were collected in each moment and were chopped with a Cibus F Wintersteiger machine. Half of this material was inoculated at a dose of 2 g of product per ton of fresh material with 166.7 ml of distilled water (1.5×10^5 cfu/g of fresh forage) and untreated (the same amount of water was applied to the treatment administered as placebo). The product (Commercial product: SiloSolve® AS, CHR Hansen) that was used to inoculate was a multi-strain inoculant, containing both homofermentative and heterofermentative microor-

ganisms, with a minimum content of not less than 7.5 x 10^{10} cfu that was composed of *Lactobacillus buchneri, Enterococcus faecium* and *Lactobacillus plantarum*. Chopped material was ensiled in three 20 L buckets (six silos for maturity stage) and sealed. Treatments were constituted: 1) Untreated 25% DM (without additive); 2) Treated 25% DM (with additive); 3) Untreated 35% DM; 4) Treated 35% DM; 5) Untreated 45% DM; 6) Treated 45% DM. Four repetitions were performed at each maturity stage.

The silos were opened after 80 d and three samples were taken to perform: chemical analysis, fermentation quality and aerobic stability. To chemical analysis: a sample of each repetition was taken and a pool was provided for their analysis: DM content was determined by oven drying for 48 h at 60ºC, neutral detergent fiber (NDF). Acid detergent fiber (ADF) were analyzed with a modified procedure using sulphite and amylase (Van Soest *et al*., 1991). Crude protein (CP) was determined by the Kjeldahl method (AOAC 1990) and apparent *in vitro* DM digestibility (IVDMD) by incubation for 36 h in daisy^{II} equipment (Van Soest, 1994). The organic acids, water-soluble carbohydrates (WSC), pH and N-NH_3 were analyzed with approximately 250 g of fresh silage at the beginning and when the micro-silos were opened and then at the end of the experiment (after breaking aerobic stability or when the temperature of the silage was 2ºC above ambient temperature). The organic acids analyzed were lactic, acetic, propionic and butyric acid. The methodology used was by Gas Chromatography, with orthophosphoric acid 25% in 0.5M sulfuric acid, at 0.5 ml each 2 ml of sample and then centrifuged for 10 minutes at 10.000 rpm (Friggens *et al*., 1998). The equipment used was Konik 5000B with Robokrom GC auto-sampler.

Aerobic stability is defined as the number of hours that silages maintain temperature before increasing more than 2°C above ambient temperature (Taylor and Kung Jr, 2002; Ranjit and Kung, 2000). The aerobic stability was performed with the methodology proposed by Basso *et al*. (2012). Three kilograms of fresh silage material was placed in a plastic tray 40 cm long and 30 cm wide and 10 cm high. They were incubated in a room at 20°C. The maximum aerobic stability was performed in 12 consecutive days in which the temperature was measured (Digital Thermometer Thermo; -50 to 70°C ± 1°C) every 6 hours (3 am, 9 am, 15 pm and 21 pm) in the center of the silage mass and ambient temperature near the trays.

Statistical analysis

Silage data were analyzed in a completely randomized design using InfoStat (Di Rienzo *et al*., 2018). Significant differences among treatments were calculated by the use of pairwise comparisons with Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

The DM percentages of the different silages were within the range goal for each maturity stage (table 1). With respect to chemical analysis, NDF and ADF values were on average 39.5 ± 4.4% (mean ± standard deviation) and 21.6 ± 3.0% respectively, whereas IVDMD averaged 65.4 ± 1.0%.

For all variables, interaction inoculated * maturity stage was not significant (table 2). The treatments inoculated did not have significant differences in none of the variables analyzed before and after rupture of aerobic stability of corn silage (table 2). However, significant differences were observed in some of the variables due to the effect of the maturity stage. The high concentration of acetic acid observed in the untreated treatment probably explains the absence of the effect of inoculant. This agrees with the observation made by Wilkinson and Davies (2012)in which the silages that contain a greater concentration of this acid possess greater aerobic stability. Like none of the variables of the fermentative quality was affected by the treated treatment, mainly, the discussion is going to be about the effect of the maturity stage upon the variables analyzed in the silages.

The concentration of N-NH_3 was similar (table 2) for silage with different maturity stages and shows that the degree of protein breakdown during the fermentation process was low (Wilkinson, 2005). The possible reason for this can be the low temperature reached by the silages (Borreani *et al*., 2018). Low levels indicate well preserved silages (McDonald *et al*., 2002). According to Ojeda *et al*. (1991), in well preserved silage the optimal concentration is less than 0.07% of N-NH_3.

Treatments	Maturity Stage, DM	DM	NDF	ADF	CP	IVDMD
Control	**25%**	25.1	43.8	24.8	7.3	65.9
	35%	35.4	35.1	19.2	6.9	66.5
	45%	43.2	41.2	21.9	6.6	64.1
Inoculated	**25%**	25.1	42.9	24.3	7.4	65.5
	35%	35.4	40.9	22.4	6.4	64.4
	45%	43.3	33.1	17.1	6.9	65.9

Table 1. Content of dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and in vitro dry matter digestibility (IVDMD) of corn silage with or without inoculant and three maturity stages.

The WSC were different in the maturity stages, greater in the silage with 25% DM with respect to 35 and 45% DM, possibly due to a translocation into the grain and subsequent conversion into starch. According to Vieira da Cunha (2009), to achieve well-preserved silages, this must have a concentration of 6-8% WSC. Before the rupture of the aerobic stability, the difference was 98%, whereas after the rupture of the aerobic stability was 153%, between 25 vs 35 and 45% DM.

The concentration of acetic acid was different between the maturity stages before the rupture of aerobic stability (table 2), but it did not have significant differences after the rupture of aerobic stability (0.07 ± 0.04 %). Silages with 25% DM had the highest concentration of acetic acid, and this difference was 58% compared to 35% DM. In turn, these last showed higher concentration compared to 45% DM, the difference being 44%. According to Filya (2003), corn silage in early maturity stages generates more acetic acid which has antifungal activity. Therefore, probably the high acetic acid concentration observed in 25% DM silages explains the higher aerobic stability thereof. Meanwhile, high levels of acetic acid could be explained by the presence of clostridia, fungi and enterobacteria, which during the initial phase of the fermentation process generates large amounts of acetic acid (Wilkinson and Davies, 2012). The silages with 25% DM in addition to the high concentration of acetic acid, had higher water content, which acts by way of barrier preventing the flow of air into the silage mass. As a result, it would lower the aerobic spoilage due to the lower oxygen concentration in the silage (Wilkinson and Davies, 2012). Filya (2003) found significant differences between the Control and Inoculated treatment with *Lactobacillus buchneri*, but unlike the present study, the concentration of acetic acid was 1.1 and 2.1%, respectively. The concentration of acetic acid in Control treatment of Filya (2003) was lower with respect to this work and the Inoculated treatment was similar to the Control treatment of this study. Similarly, Nkosi *et al*. (2011) observed low concentration of acetic acid in the Control treatment (0.79%).

The propionic acid was different between the maturity stages before the rupture of the aerobic stability, whereas no significant differences were observed after the rupture of aerobic stability (0.06 ± 0.05%). In the maturity stages 25 and 35% DM the propionic acid levels were 85% higher compared to the stage with 45% DM.

The butyric acid before the rupture of the aerobic stability was not detectable and after the rupture of the aerobic stability it was detectable but did not have significant differences between the three states of maturity (0.05 ± 0.02%). Probably the initial absence of butyric acid is due to the low pH reached silage. However, once the loss occurred aerobic stability, the pH rises above 4.2, which allows the growth of microbes such as *Clostridium* fermenting the WSC to butyric acid (Muck, 2010).

The lactic acid concentration was different between the maturity stages before the rupture of the aerobic stability. Silages with 25% DM had the highest concentration of lactic acid, and this difference is 29% compared to 35% DM. In turn, the latter showed higher concentration compared to 45% DM silages, the difference being 18%. No effect on the maturity stage in the concentration of lactic acid after the rupture of the aerobic stability was observed. However, for the maturity stage 45% DM, a concentration of 5.3% was observed, while for the other two stages it was not detectable. Silages which have a good fermentation are characterized by predominance of lactic acid

Item	Treatments (T)		Maturity stages (MS)			T	MS	T * MS
	Control	Inoculated	25%	35%	45%			
N-NH_3 before, %	0.05	0.06	0.06	0.06	0.05	*p*=0.20	*p*=0.52	*p*=0.32
N-NH_3 after, %	0.07	0.04	0.05	0.05	0.07	*p*=0.08	*p*<0.07	*p*=0.10
WSC before, %	0.63	0.69	0.99 a	0.54 b	0.46 b	*p*=0.31	*p*<0.01	*p*=0.14
WSC after, %	0.68	0.65	1.11 a	0.44 b	0.44 b	*p*=0.51	*p*<0.01	*p*=0.92
C2 before, %	1.79	1.83	2.63 a	1.66 b	1.15 c	*p*=0.79	*p*<0.01	*p*=0.23
C2 after, %	0.08	0.06	0.09	0.08	0.05	*p*=0.20	*p*=0.10	*p*=0.25
C3 before, %	0.33	0.30	0.37 a	0.37 a	0.20 b	*p*=0.38	*p*<0.01	*p*=0.27
C3 after, %	0.06	0.07	0.09	0.06	0.04	*p*=0.70	*p*=0.17	*p*=0.34
C4 before, %	ND	ND	ND	ND	ND	--	--	--
C4 after, %	0.05	0.05	0.04	0.05	0.07	*p*=0.58	*p*=0.18	*p*=0.50
Lactic acid before, %	8.42	7.86	10.03 a	7.80 ab	6.59 b	*p*=0.09	*p*<0.01	*p*=0.47
Lactic acid after, %	5.54	4.85	ND	ND	5.31	*p*=0.84	--	--

Table 2. Effect the treatments and three maturity stages on ammonia nitrogen (N-NH3), water-soluble carbohydrates (WSC), acetic acid (C2), propionic acid (C3), butyric acid (C4), lactic acid measured before and after the rupture of aerobic stability.

Different letters between columns indicate significant differences (P<0.05). ND = not detected

because it reduces the pH of the ensiled mass more rapidly than other acids (McDonald *et al*., 2002).

The highest pH values the silages were observed with 45% DM, being a 5 to 10% higher with respect to the silages with 25 and 35% DM, respectively (table 3). The silages with 25% DM were 5% higher compared to 35% DM. The pH remained nearly constant in both treatments and at different maturity stages before the rupture of the aerobic stability and consequent increase in temperature. In this experiment, the value remained below 4.2, which according to Kung and Shaver (2001) is beneficial for the proper conservation of the corn silage.

The pH of silage before the rupture of the aerobic stability was higher for the maturity stage 45% DM with respect to the other two stages (8% higher). However, after the rupture of the aerobic stability, silage with lower percentages DM had higher pH values with respect to the stage with 45% DM (38% higher). The pH is an indicator of aerobic deterioration of silage, since the lactic acid is consumed by the yeast and fungi during the exposure to oxygen, thereby permitting the increase thereof (Basso *et al*., 2012). The higher pH observed in the silage with 25 and 35% DM is probably explained by the greater concentration of WSC in them, which would allow the multiplication of yeasts and fungi with consequent reduced levels of lactic acid.

The average ambient temperature during the experiment was 22.2 ± 2.0 °C (table 4), thus achieving the recommended temperature to assess the aerobic stability of the silage after opening.

The aerobic stability of this experiment was between the values informed by Owen (2002). The silage with 25% DM were more stable with respect to silages with 35 and 45% DM, this difference being 115%. Silages with 25% DM also needed more time to reach the maximum temperature, 110% higher compared the silages with 35 and 45% DM. The maximum temperature reached with 35% DM was 19% (5.5ºC) higher compared to the silages with 25 and 45% DM. Silages with 25% DM took longer hours to break the aerobic stability and achieve the maximum temperature. The same was observed by McDonald *et al*. (1991), with lower DM the longer time to break the aerobic stability and achieve the maximum temperature of silage, associated with higher water content thereof. The temperature increase is larger in treatments with higher DM because less heat is required to raise the temperature of the dry material (Wilkinson and Davies, 2012). In addition, when increasing crop maturity there is more concentration of the aspergillus at pre-harvest period (Oldenburg, 1999) and this has a high negative correlation with aerobic stability (Jonsson *et al.,* 1990; Borreani *et al.,* 2018).

Dry matter losses were higher in silages with 45% DM compared with 25 and 35% DM. The greatest loss of dry matter would be associated with undesirable nutrient intake during the storage period prior to the opening of the same silage. The same was observed by Griswold *et al.* (2009), that DM loss increased markedly at higher silage DM concentrations and similar to the average of silages treated with *L. Buchneri* and homofermentative bacteria observed by Wilkinson and Davies (2012).

Item	Treatments (T)		Maturity stages (MS)			T	MS	T * MS
	Control	Inoculated	25%	35%	45%			
pH silage	3.84	3.86	3.85 b	3.68 c	4.03 a	*p*=0.45	*p*<0.01	*p*=0.23
pH before	3.94	4.02	3.87 b	3.90 b	4.20 a	*p*=0.31	*p*<0.01	*p*=0.57
pH after	6.41	6.32	6.63 a	7.38 a	5.08 b	*p*=0.75	*p*<0.01	*p*=0.20

Table 3. Effect of the treatments and the three maturity stages on pH before and after the rupture of aerobic stability.
Different letters between columns indicate significant differences (P<0.05).

Item	Treatments (T)		Maturity stages (MS)			T	MS	T * MS
	Control	Inoculated	25%	35%	45%			
AS, hs	79.3	70.0	116.0 a	59.0 b	49.0 b	*p*=0.48	*p*<0.01	*p*=0.37
Máx. T., hs	88.7	75.3	126.0 a	67.0 b	53.0 b	*p*=0.33	*p*<0.01	*p*=0.48
Máx. T., ºC	30.4	29.8	28.6 b	33.7 a	27.9 b	*p*=0.47	*p*<0.01	*p*=0.70
Average T., ºC			22.0	21.5	23.3			
DM losses, %	14.5	14.3	13.6b	13.2b	16.5a	*p*=0.59	*p*<0.01	*p*=0.99

Table 4. Effect of treatments and maturity state on the aerobic stability (AS), time to reach maximum temperature (Max. T.), maximum temperature, average temperature (Average T.) and loss of dry matter (DM losses).
Different letters between columns indicate significant differences (P<0.05).

CONCLUSION

The fermentation parameters and aerobic stability of corn silage were not affected by bacterial inoculation, if there was an effect of maturity stage. At a higher maturity stage there were higher pH levels and loss of dry matter, with less aerobic stability and hours to reach the maximum temperature. So, when the harvest is delayed the aerobic stability of silage is lower.

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