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Effect of different inocula on aerobic stability of corn silage

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Introduction Aerobic deterioration of silages causes dry matter (DM) and nutritive value losses, and leads to risks for human and animal health (Borreani et al. 2018). In order to improve the aerobic stability of silages, heterolactic bacteria, mainly *Lactobacillus buchneri* inocula have been used because of their ability to produce a greater amount of acetic acid, which inhibits yeasts and increases aerobic stability. New strains of LAB inoculant have been evaluated in recent years (Assis et al., 2014) to meet the needs of dairy farmers to feed silages from early opening silos (after 30 to 40 d of ensiling). The aim of the work was to evaluate the effect of *L. hilgardii* (LH), alone or in combination with *L. buchneri,* on aerobic stability of corn silage after different ensiling durations.

Material and Methods Corn was harvested as a whole plant (42% DM), and not treated **(C)** or inoculated with *L. buchneri* NCIMB 40788 **(LB)** [(theoretical application rate of 300,000 cfu/g fresh matter (FM)], *L. hilgardii* CNCM I-4785 **(LH-)** (theoretical application rate of 100,000 cfu/g FM), *L. hilgardii* CNCM I-4785 **(LH+)** (theoretical application rate of 300,000 cfu/g FM) and with a combination of *L. hilgardii* and *L. buchneri* **(LB+LH)** (theoretical application rate of 150,000 cfu/g FM of each one). The fresh forage was ensiled in 20-L plastic silos and opened after 15, 30 and 100 d of ensiling. At opening, the silages were analysed for DM content, pH, fermentative profile and microbial counts. The DM content was determined at 60°C for 72 h and corrected in order to consider the losses of volatile compounds. The fermentative products were determined in the acid extract by HPLC. Yeast and mold counts were determined using the pour plate technique on Yeast Extract Glucose Chloramphenicol agar. The weight losses due to fermentation were calculated as the difference between the weight of the forage placed in each plastic silo at ensiling and the weight at the end of conservation, and were expressed on a DM basis. At each opening, the silages were subjected to an aerobic stability test by continuously measuring the temperature during exposure to air. Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 2°C above room temperature. The obtained data were analyzed for their statistical significance, via analysis of variance, using version 24 of SPSS for Windows (SPSS Inc., Chicago, IL). The data were analyzed utilizing the inocula as the fixed factor, with five replicates. When the calculated values of F were significant, the REGWF test ($P < 0.05$) was used to interpret any significant differences among the mean values.

Results and Discussion DM content, pH, fermentative profile, yeast count and aerobic stability are reported in Table 1. The pH decreased as the duration of the ensiling increased, with a significant effect among treatments on 30 and 100 d, Lactic acid increased in all the treatments during conservation, with higher values in C and LH- silages after 100 d of ensiling, even though the differences were not of practical relevance. No differences were found in the acetic acid content between treatments after 15 d of ensiling, whereas it was higher in the LH+ and LB+LH than in the C silages at 30 and 100 d. In line with Assis et al. (2014), the fermentative results indicate the presence of 1,2-propanediol in silages treated with *L. hilgardii*. The yeast count decreased as the ensiling duration increased, with the lowest value in LH treated silages. The aerobic stability increased as the ensiling duration increased, mainly due to a reduction in the yeast count (Figure 1), with a *P-value* <0.001 and an adjusted R^2 of 0.77. The highest value of aerobic stability was observed in LH+ silages at 100 d. Confirming results reported by several authors, the increase in aerobic stability was related to the large amount of acetic acid and lower yeast count (Kleinschmit & Kung 2006).

Conclusion The use of *L. hilgardii* improved the aerobic stability at 15 d of ensiling when used in combination with *L. buchneri*, and at 100 d of ensiling when used alone with comparable effect to *L. buchneri*. The reduction in the yeast count during ensiling have been confirmed to be the most relevant factor influencing the increase of aerobic stability.

Table 1. Dry matter (DM) content, fermentative profile, yeast count and aerobic stability of corn silage after 15, 30 and 100 d of ensiling.

Days	C	LB	LH-	LH+	LB+LH	*P-value*	SEM
			DM (%)				
15	42.1^{ab}	42.4^{a}	41.5^{ab}	41.9^{ab}	41.2^{b}	0.023	0.142
30	42.7	42.2	41.5	41.9	42.3	0.270	0.175
100	41.6	41.5	41.8	40.9	40.7	0.353	0.200
			pH				
15	3.80	3.81	3.80	3.80	3.81	0.697	0.00321
30	3.76^{bc}	3.77^{ab}	3.76^{c}	3.77^{ab}	3.77^{a}	0.002	0.00170
100	3.74^{c}	3.77^{a}	3.74^{c}	3.75^{b}	3.77^{a}	<0.001	0.00261
			Lactic acid (g/kg DM)				
15	31.4	30.0	31.6	28.5	29.5	0.175	0.464
30	32.9^{b}	35.6^{ab}	37.4^{a}	34.8^{ab}	35.6^{ab}	0.022	0.459
100	39.6^{a}	35.0^{b}	39.8^{a}	38.5^{ab}	36.6^{ab}	0.021	0.572
			Acetic acid (g/kg DM)				
15	6.1	6.2	6.1	5.9	6.1	0.840	0.0911
30	6.3^{c}	6.7^{bc}	7.7^{a}	7.8^{a}	7.3^{ab}	<0.001	0.141
100	8.5^{b}	10.0^{b}	9.7^{b}	12.4^{a}	12.0^{a}	<0.001	0.363
			1,2-propanediol (g/kg DM)				
15	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.17^{a}	0.014	0.0203
30	0.00^{d}	0.34^{c}	0.57^{b}	0.88^{a}	0.41^{c}	<0.001	0.0616
100	0.00^{c}	1.86^{ab}	1.31^{b}	3.32^{a}	2.78^{ab}	<0.001	0.280
			Yeast (log_{10} cfu/g)				
15	5.11^{abc}	5.38^{ab}	5.38^{a}	5.00^{bc}	4.89^{c}	0.006	0.0575
30	4.49^{ab}	4.71^{a}	4.40^{b}	4.23^{b}	4.39^{b}	0.003	0.0438
100	4.00^{a}	3.18^{ab}	2.88^{b}	2.80^{b}	2.98^{b}	0.010	0.132
			Aerobic stability (h)				
15	51^{b}	50^{b}	52^{b}	53^{b}	74^{a}	0.014	2.822
30	58^{ab}	55^{b}	57^{ab}	67^{ab}	67^{a}	0.037	1.644
100	96^{c}	160^{ab}	170^{ab}	191^{a}	137^{b}	<0.001	7.778
			Weight losses (% DM)				
15	1.81	1.66	1.73	1.70	1.82	0.556	0.0339
30	1.96	1.86	1.87	1.89	1.97	0.551	0.0236
100	2.20	2.23	2.18	2.16	2.34	0.180	0.0261

C = control; DM = dry matter; LB = *L. buchneri*; LH = *L. hilgardii*; SEM = standard error of the mean.

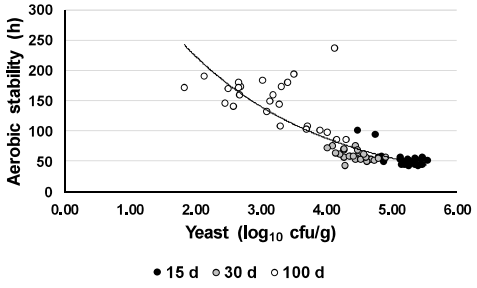

Figure 3. Relation of the aerobic stability and yeast count at silo opening, as affected by different ensiling period.

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