Effects of a chemical additive on the fungal community and aerobic stability of whole-plant corn silage challenged with air stress during storage

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Introduction Chemical additives can be used in silage production to overcome the risks brought by unwanted crop characteristics, challenging environmental conditions, or bad management practices, such as air-stress during storage. Specifically, some additives can inhibit microorganisms that are stimulated by oxygen infiltration and cause undesirable fermentations or spoilage (Henderson, 1993). The objectives of this experiment were to evaluate the effects of an additive containing sodium benzoate, potassium sorbate, and sodium nitrite on the composition of the fungal community of whole plant corn silage; and to determine its effectiveness on overcoming an air-stress challenge applied during the last half of the storage period compared to silage that was air-stressed over the entire duration of the storage period.

Materials and Methods Whole corn plants were harvested at 40% DM, chopped, and untreated (CTRL), or treated with Safesil (Salinity, Göteborg, Sweden; active ingredients: 20% sodium benzoate, 10% potassium sorbate, and 5% sodium nitrite) at 2 L/t of fresh forage weight (SF). Fifteen individually replicated silos (7.5 L) for each treatment were packed at a density of 224 kg of DM/m^3. Silos from each CTRL or SF treatments were not air-stressed (NS), air-stressed during the entire ensiling period (3 h/week for 8 weeks) (WK1-8), or air-stressed only during the second half of the ensiling period (3 h/week from week 4 to 8) (WK4-8). Silos used for air stress challenge had three holes of 1.60 cm diameter that were plugged with rubber stoppers and sealed with silicon glue. Silages were analyzed after 8 weeks of ensiling for pH, numbers of yeasts on malt extract agar plates, DM recovery, aerobic stability (defined as the number of h that the silage temperature took to raise its temperature 2ºC above the baseline), and fungal community composition through the sequencing of the ITS1 region using Illumina MiSeq platform. Data were analyzed as 2 × 3 factorial arrangement of treatments with the main effects of additive treatment, air stress, and their interaction.

Results and Discussion We found that silages challenged with air stress during the entire storage period (WK1-8) had higher ($P < 0.01$) pH than NS and WK4-8 (Table 1). Silages WK4-8 had higher numbers of yeasts ($P < 0.01$) than NS and WK1-8. For silages treated only with SF, those without air stress had higher ($P < 0.05$) DM recovery than WK1-8, whereas WK4-8 had intermediate DM recovery. There was an interaction between additive treatment and air stress for aerobic stability ($P < 0.01$). For CTRL silages, air-stress reduced the aerobic stability. Specifically, CTRL/NS had the highest, CTRL/WK4-8 intermediate, and CTRL/WK1-8 the lowest aerobic stability. Although CTRL and SF when not air-stressed had equally high aerobic stability, when silages were subjected to air stress, SF/WK1-8 silages had numerically higher aerobic stability and SF/WK4-8 statistically higher aerobic stability ($P < 0.05$) than CTRL/WK1-8 and CTRL/WK4-8,

respectively. Silages subjected to air stress during the last half of the storage period had higher ($P < 0.05$) relative abundance of the fungi *Monascus* and lower abundance of the yeast *Candida* than NS and WK1-8. The genus *Candida* comprises yeasts strains that can assimilate lactate and increase silage pH, initiating the deterioration process. Therefore, the lower ($P < 0.05$) relative abundance of *Candida* in SF compared to CTRL could possibly explain why silages treated with the additive had higher aerobic stability even though the numbers of agar culturable yeasts were the same on CTRL and SF.

Table 1. The pH, numbers of yeasts (log cfu/g of fresh forage), DM recovery (%), aerobic stability (h), and relative abundance of fungal genera (%) of corn silage ensiled for 8 weeks untreated (CTRL) or treated with an chemical additive at 2L/t of fresh forage weight, not air-stressed (NS), air-stressed during the entire ensiling period (3 h/week for 8 weeks) (WK1-8), or air-stressed only during the second half of the ensiling period (3 h/week from week 4 to 8) (WK4-8).

		pH	Yeasts	DM recovery	AS[1]	Relative abundance		
						Monascus	*Aspergillus*	*Candida*
Additive means	CTRL	3.74	2.68	95.29	143	30.82	0.00	44.35^{a}
	SF[2]	3.74	2.47	94.41	194	16.33	15.04	35.38^{b}
Air stress means	NS	3.73^{b}	2.15^{b}	95.88	254	0.00^{b}	0.00	55.15^{a}
	WK1-8	3.77^{a}	2.28^{b}	94.08	71	0.00^{b}	0.07	61.90^{a}
	WK4-8	3.73^{b}	3.30^{a}	94.58	180	70.73^{a}	22.49	2.55^{b}
Additive × Air stress	CTRL/NS	3.73	1.96	95.11^{ab}	248^{a}	0.00	0.00	59.40
	CTRL/WK1-8	3.77	2.30	95.11^{ab}	55^{c}	0.00	0.00	69.17
	CTRL/WK4-8	3.73	3.77	95.65^{ab}	127^{b}	92.46	0.00	4.48
	SF/NS	3.72	2.33	96.66^{a}	260^{a}	0.00	0.00	50.90
	SF/WK1-8	3.77	2.25	93.05^{b}	88^{bc}	0.00	0.14	54.62
	SF/WK4-8	3.73	2.84	93.51^{ab}	234^{a}	49	44.98	0.63
	SEM	0.01	0.29	0.74	9	11.87	10.60	5.19
P-value[3]	A	0.68	0.39	0.15	<0.01	0.15	0.10	0.05
	AS	<0.01	<0.01	0.06	<0.01	<0.01	0.08	<0.01
	A × AS	0.51	0.09	0.03	<0.01	0.14	0.08	0.60

[1]AS, aerobic stability (number of hours that the silage took to increase its temperature 2ºC above the baseline).
[2]SF = additive containing sodium benzoate, potassium sorbate, and sodium nitrite.
[3]A= effect of additive, AS= effect of air stress, A × AS= effect of the interaction between additive and air stress.

Conclusions Exposing silage to air during each week of the ensiling period resulted in silage with a higher pH and lower aerobic stability. Although the additive did not reduce the total numbers of yeasts on agar plate it reduced the relative abundance of *Candida*. This yeast genus contains strains able to assimilate lactate and initiate aerobic spoilage of corn silage. The additive improved the aerobic stability of silages that were air stressed during only the last half of storage, but when the air stress was every week during storage, the additive, at the dose tested in this study, was not able to overcome this challenge. Results from our study confirms that the additive used can improve aerobic stability of air stressed silage but that it should not be expected to overcome extremely stressful conditions.

References Henderson, N. 1993. Silage additives. Anim. Feed Sci. Technol. 45:35-56.