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Effects of a Chemical Additive on Aerobic Stability and Fungal Microbiome of Corn Silage

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Introduction

Exposure of silage to air allows for the growth of lactate-assimilating yeasts, causing an increase in silage pH which spoils silage and ultimately decreases the nutritive value of silage and increases dry matter loss (Merrill et al., 2012). Previous research has shown that antimicrobial compounds such as potassium sorbate and sodium benzoate are effective in inhibiting yeasts and improving the aerobic stability of silages (Knicky and Spörndly, 2011). However, specific changes in the microbial communities caused by these additives have not been well studied in silage. Furthermore, silages are often fed as part of TMR and thus it is of interest to know if the stability of a TMR can be improved when it contains treated silage.

Objectives

The objective of this experiment was to determine the effectiveness of Safesil (**SF**; 20% sodium benzoate, 10% potassium sorbate, 5% sodium nitrite) from Salinity, Sweden on improving the aerobic stability of corn silage alone and when that silage was combined into a TMR. We also determined the effects of the additive on the silage fungal community by analysis of the internal transcribed space 1 (ITS1).

Materials and Methods

Whole plant corn was harvested at 39% DM, chopped, and untreated (CTRL) or treated with SF (2 L/t). Four replicated silos (7.5 L) were packed at a density of 224 kg of DM/m³. After 85 d of ensiling, aerobic stability (h before a 2°C increase above baseline after exposure to air at 22°C) was determined on

1) CTRL silage
2) SF silage
3) TMR-CTRL, CTRL silage incorporated into a TMR comprised of 40% corn silage, 20 % alfalfa silage and 40% of a grain mix (DM basis)
4) TMR–SF, SF silage made into a TMR
5) TMR-RT-SF, CTRL silage incorporated into a TMR but treated with the equivalent amount of additive used in the SF corn silage.

Three replicates of each fresh forage, and CTRL and SF after 85 d of ensiling were analyzed for fungal microbiome composition. DNA extraction using MoBio PowerMag Soil kit, amplification of the ITS1 with the primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2aR (GCTGCGTTCTTCATCGATGC), and library preparation and sequencing by the Illumina MiSeq (San Diego, CA, USA) platform were performed by the Research and Testing Laboratory (Lubbock, TX, USA). Data were analyzed using JMP 12.0, and bioinformatics analysis was done on QIIME 1.9.1 and Rstudio 1.0.136.

Table 1. pH, yeast counts (log cfu/g of fresh material), and ethanol concentration (% of DM) of corn silage after 85 d of ensiling.

Treatment[1]	pH	Yeast	Ethanol
CTRL	3.74[a]	3.96[a]	2.39[a]
SF	3.65[b]	2.00[b]	1.27[b]
SEM	0.01	0.30	0.09
P-value	<0.01	<0.01	<0.01

[a-b] Means in columns within an experiment with unlike superscripts differ ($P < 0.05$).
[1]Untreated silage (CTRL) and silage treated with Safesil 2L/t (SF).

Results

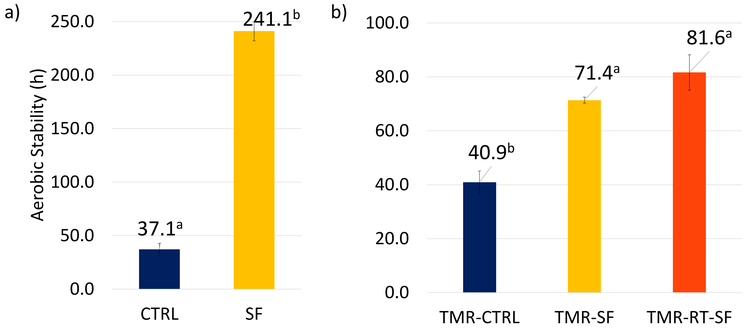

Figure 1. a) Aerobic stability of untreated silage (CTRL) and silage treated with Safesil 2 L/t (SF) after 85 days of ensiling, and b) aerobic stability of TMR with untreated silage (TMR-CTRL), TMR with silage treated with Safesil 2 L/t before ensiling (TMR-SF), and TMR made with silage treated with Safesil 2L/t after ensiling (TMR-RT-SF). [a-b] Means bars with unlike letters differ ($P < 0.05$).

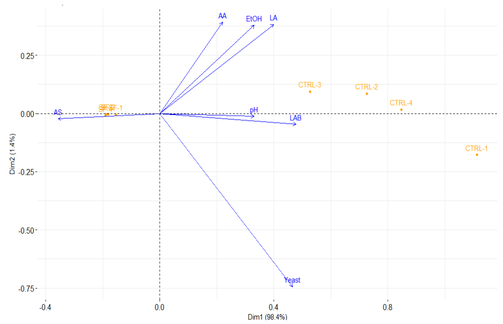

Figure 2. Canonical correspondence analysis (CCA) of fermentation variables on silage treatments. Arrows demonstrate the direction and magnitude of the fermentation variables. CTRL, untreated silage ensiled for 85 d; SF, silage treated with Safesil 2 L/t ensiled for 85 d; AS, aerobic stability; LAB, lactic acid bacteria; LA, lactic acid; AA, acetic acid.

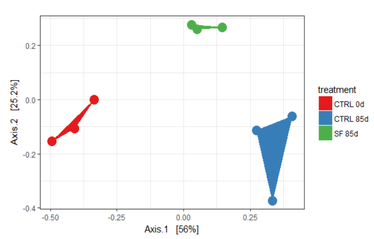

Figure 3. Principal coordinate analysis (PCoA) plot of the fungal microbiome of fresh forage (CTRL 0d), and silage untreated (CTRL 85d) or treated with Safesil 2 L/t (SF 85d) ensiled for 85 d.

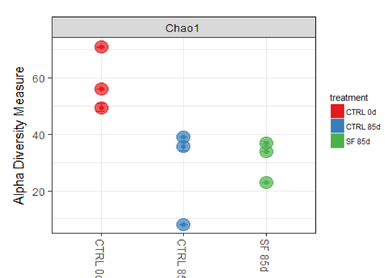

Figure 4. Alpha diversity measure, Chao1 index , for fresh forage (CTRL 0d), and silage untreated (CTRL 85d) or treated with Safesil 2 L/t (SF 85d) ensiled for 85 d.

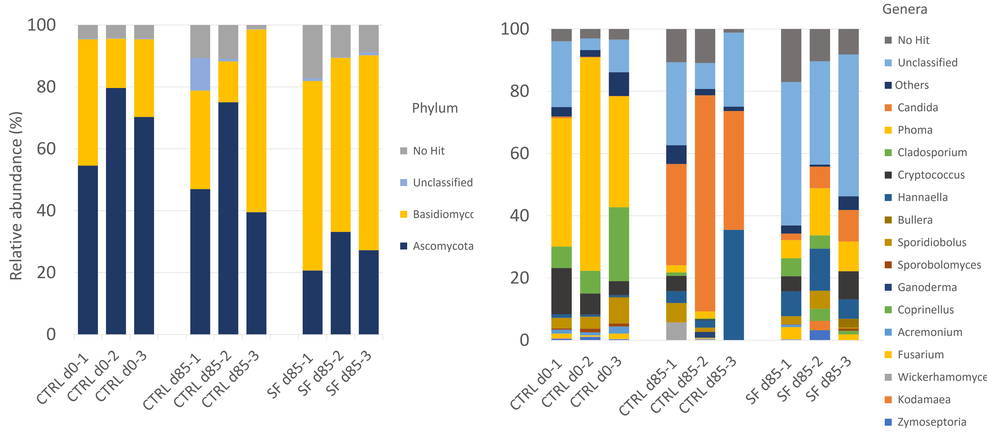

Figure 5. Relative abundance of fungi phylum and genera on fresh forage (CTRL d0), untreated silage (CTRL d85) or silage treated with Safesil 2 L/t (SF d85) ensiled for 85 d.

- Silage treated with SF improved the aerobic stability of silage alone. When SF-treated silage was used to make a TMR, its aerobic stability was better when compared to a TMR made with untreated silage. Adding SF to a TMR with untreated silage also improved the aerobic stability of resulting TMR.
- Chao1 index indicated a decrease ($P < 0.05$) in diversity after ensiling compared to fresh forage.
- SF had lower *Ascomycota* abundance ($P < 0.05$) compared to fresh forage, and numerically lower abundance compared to CTRL.
- Relative abundance of genus *Candida*, which includes strains of lactate-assimilating yeasts, was lower ($P < 0.01$) in SF (6.3%) than CTRL (46.2%) after 85 d of ensiling.
- Lower total yeast counts and lower abundance of lactate-assimilating yeasts can explain the increase in aerobic stability in silage treated with the chemical additive.

Conclusions

The use of SF greatly improved the aerobic stability of silage alone and a TMR made with treated silage. Additionally, SF has the potential to be used as a TMR stabilizer, if applied to silage after ensiling. The improvement on aerobic stability by the chemical additive might be due to its capacity to reduce total numbers of yeasts, and to decrease specific populations of yeasts associated with lactate assimilation, such as *Candida*.

References

Knicky, M., and R Spörndly. 2011. The ensiling capability of a mixture of sodium benzoate, potassium sorbate, and sodium nitrite. J. Dairy Sci. 94: 824-831.

Merrill, C. A., T. P. Roth, M. A. Santos, M. C. Der Bedrosian, and L. Kung Jr. 2012. Characterization of aerobic deterioration of corn silage treated with stabilizers. J. Dairy Sci. 95 (Suppl. 2): 461. (Abstr.)

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