Aerobic stability evaluation, after a short fermentation period, in artificially contaminated alfalfa silages inoculated with a dual-purpose microbial solution

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Introduction As extensively reviewed by Kung *et al.* (2018), an incorrect silo management could compromise ensiling phases, thus exposing silages to the risk of air penetration. Aerobic deterioration could cause nutrient and dry matter (DM) losses, heat damage of nutrients, proteolysis or proliferation of undesirable microorganisms, such as yeast and mold. The negative effects of aerobic activity could be more severe in specific areas of the silage, especially in the lateral and apical parts of the ensiled crop, which are generally packed and sealed with difficulty (Vissers *et al.*, 2007; Borreani and Tabacco, 2010). The objective of this study was to evaluate the effects of a dual strain silage inoculant on fermentation parameters, aerobic stability (AS) and its ability to control the growth of undesirable microorganisms in alfalfa silage (fermented for only 14 days) and artificially challenged with yeast and mold isolated from top layer spoilage.

Materials and Methods Twenty 20-L mini-silos were filled with wilted alfalfa (6.75±0.2 kg, 37.1% DM). The following treatments were applied with a manual sprayer: (i) physiological isotonic saline solution (Control or C), (ii) SiloSolve® FC (FC), (iii) yeast and mold spoiled silages (YMC) and (iv) YMC + FC (YMFC). SiloSolve® FC (50:50 of *Lactobacillus buchneri* DSM22501 and *Lactococcus lactis* O224 DSM11037) was applied at 1.5 x 10^5 cfu/g of forage. A 10^8 cfu/ml yeast and 10^8 cfu/ml mold solution was obtained by extracting microorganisms from aerobically unstable top layer alfalfa silage sampled in a commercial bunker (0.25 kg of silage in a 1 L 0.85% NaCl physiological isotonic saline solution), then added as 5 L in 500 kg fresh chopped forage before ensiling. An equal amount of physiological isotonic saline solution was added to C and FC. After two weeks of fermentation (at 25°C), each mini-silo followed an AS test for 14 days. The AS test was performed by aerobically challenging the mini-silos and monitoring the temperature with a datalogger. AS is defined as the number of hours the silage temperature remains below the threshold of +3°C above ambient temperature. At opening the contents of the mini-silos were subsampled for analysis of total lactic acid bacteria (LAB), yeast and mold. Data were analyzed in agreement with a completely randomized design with a 2 x 2 factorial arrangement of treatments. The tested main effects were used of SiloSolve® FC (i.e., inoculant), challenge with yeast and mold (i.e., challenge) and their first order interaction. Significance was declared for a $P < 0.05$.

Results and Discussion Fermentation and microbiological parameters are reported in Table 1. All the treated silages (FC and YMFC) were more stable (+106 hours on average, $P < 0.05$) than untreated silages (C and YMC) even after only 14 days of fermentation. The DM loss at opening of mini-silos was numerically higher in YMC with respect to other treatments (inoculum*challenge effect, $P = 0.083$). Acetate levels were higher in SiloSolve® FC treated mini-silos and increased when yeast and mold were added to the silages ($P = 0.051$). The increases in acetate levels due to challenge were higher in YMFC (i.e., + 1.6% DM compared to FC) than YMC (i.e., + 1.0% DM compared to C). Higher levels of lactate were observed in SiloSolve® FC treated mini-silos, but an opposite trend was observed when silages were challenged with yeast and mold (inoculum*challenge effect, $P = 0.107$). In particular, the decreases in lactate level due to yeast and mold challenge were lower in YMFC (i.e., - 0.3% DM compared to FC) than in YMC (i.e., - 1.6% DM compared to C). The use of SiloSolve® FC slightly increased the LAB population ($P < 0.05$). As expected after fermentation, the challenge model (addition of yeast and mold solution) increased only the yeast levels (inoculum*challenge effect, $P < 0.05$) while mold levels remained unchanged ($P = 0.759$). SiloSolve® FC treated silages reported numerically lower yeast ($P = 0.661$) and mold levels ($P = 0.145$) compared to untreated alfalfa silages.

Table 1 *Effect of inoculant on fermentation end-products, microbial counts and aerobic stability in alfalfa silage ensiled for 14 days.*

Items	Treatment				√MSE	*P*-value inoculant	*P*-value challenge	*P*-value Inoculant*Challenge
	C	FC	YMC	YMFC				
AS (hours)	157	256	109	222	51.0	<0.05	0.897	0.145
pH	4.57	4.53	4.72	4.69	0.263	0.788	0.207	0.994
DM corrected[1] (%)	41.5	40.3	39.8	38.3	1.34	0.446	0.381	0.341
DM loss (% DM)	2.6	3.0	7.5	2.0	3.53	0.133	0.232	0.083
Fermentation end-products (%DM)								
Acetate	2.3	3.2	3.3	4.8	1.40	0.074	0.051	0.612
Lactate	3.6	3.8	2.0	3.5	0.87	<0.05	<0.05	0.107
Microbial enumeration[2] (log_{10} cfu/g)								
LAB	8.5	9.1	8.9	8.9	0.29	<0.05	0.400	0.058
Yeast	2.3[a]	2.4[a]	3.6[b]	3.1[ab]	0.97	0.661	0.479	<0.05
Mold	2.2	<2	2.2	<2	0.29	0.145	0.759	0.759

[1] The DM concentration was corrected for the volatile losses that occurred during oven drying through equations adopted by NorFor (2011) formula.
[2] When microbiological counts were below the detection limit (log_{10} cfu/g < 2), the value of 2 was used to carry out statistical analysis.
[a-c] Means (n = 5) within a row with different superscript differ ($P < 0.05$).

Conclusion This study showed that the use of SiloSolve® FC in alfalfa silages increased aerobic stability even when bad season conditions where mimicked by artificially challenging the silage with a top layer yeast and mold suspension (and even challenged with a short fermentation period). The challenge model was a good method to artificially increase the yeast level and mimic challenge conditions that the farmers could face depending on the season. The different trends measured in challenged silages for acetate and lactate levels deserve further investigation to be completely understood.

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