Control of pathogenic *Escherichia coli* O157:H7 in contaminated grass silage with dual-purpose silage inoculant

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Introduction Poor management of the silage-making process results in leftover air inside the forage mass. Residual oxygen, or air penetration into the bunker during the feed-out phase, promotes the development of yeast and mold as well as pathogenic bacteria such as *Escherichia coli* O157:H7 (Queiroz *et al.*, 2018). Spoiled and contaminated silages lead to less nutritive value forage, cause diseases in ruminants, and constitute a vehicle of transmission of pathogens on the farm. Silage inoculants are known for their positive effects improving fermentation and curtailing the growth of pathogens such as *E. coli* O157:H7 (Ogunade *et al.*, 2016; Pedroso *et al.*, 2010). The objectives of this study were to evaluate the effects of a dual strain silage inoculant on fermentation parameters, aerobic stability (AS) and its ability to control the growth of *Enterobacteriaceae* including *E. coli* O157:H7 in silage during the fermentation phase and after AS.

Materials and Methods Twelve mini-silos were filled with wilted grass (600±0.5 g, 28.0% DM). The following treatments were applied in triplicate with a manual sprayer: (i) Milli Q water (Control, C), (ii) SiloSolve® FC (FC), (iii) 10^6 cfu of *E. coli* O157:H7 DSM17076 per g of forage (EC) and (iv) EC + FC (EFC). 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. The mini-silos were then stored for 7 days at 25°C. After one week of fermentation, each mini-silo followed an AS test for 7 days. The AS test was performed by aerobically challenging the mini-silos and monitoring the temperature with a datalogger placed in the middle of the forage mass. AS is defined as the number of hours the silage temperature remains below the threshold + 3°C room temperature. The contents of the mini-silos were mixed thoroughly and subsampled for analysis of total lactic acid bacteria (LAB), yeast, mold, *Bacillus* spores and *Enterobacteriaceae* populations (including *E. coli* O157:H7) before and after AS. Fermentation parameters and chemical composition were also evaluated at opening and after AS test.
Data were analyzed in agreement to 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 EC (i.e., challenge) and their first order interaction. Significance was declared for a $P<0.05$.

Results and Discussion Fermentation and microbiological parameters are summarized in Table 1. Mini-silos treated with SiloSolve® FC had greater aerobic stability (increase > 120 hours, $P<0.05$) than C. The pH was lower in FC and EFC treated silages compared to C or EC respectively ($P<0.05$). This was due to the high concentrations of acetate ($P<0.05$) and lactate ($P<0.05$) in FC

and EFC. The use of SiloSolve® FC (FC and EFC) also reduced the yeast population significantly upon opening (P<0.05) and both yeast and mold populations after the AS challenge (P<0.05). After fermentation, *Bacillus* spores were numerically lower (P=0.05) in FC treated silages (average of 2.74 log cfu/g) compared to control silages (average of 3.18 log cfu/g), while after AS, differences were observed (P<0.05) between control silages (average of 7.74 log cfu/g) and treated silages (average of 2.87 log cfu/g). In addition, similarly to the study conducted by Ogunade *et al.* (2016), the lactose-fermenting *Enterobacteriaceae* population including *E. coli* remained detectable during ensiling in the absence of treatment with SiloSolve® FC (average of 6.41 log cfu/g). However, the addition of the inoculant inhibited the growth of lactose-fermenting *Enterobacteriaceae* after one week of fermentation and suppressed them after AS (P<0.05).

Table 1 *Effect of inoculant on fermentation end-products, microbial enumeration and aerobic stability in grass silage ensiled for seven days.*

Items	Treatment				s.e.	*P*-value inoculant	*P*-value challenge	*P*-value Inoculant*Challenge
	C	FC	EC	EFC				
AS (hours)	34.33	159.00	35.33	159.00	3.95	<0.0001	0.9025	0.9025
pH	4.63	4.05	4.63	4.11	0.05	<0.0001	0.5226	0.6073
pH post-AS	6.39	3.99	6.31	4.05	0.09	<0.0001	0.9044	0.4485
DM (%)	32.52[a]	32.18[a]	28.12[b]	31.95[a]	0.57	0.0158	0.0036	0.0065
Fermentation end product (%DM)								
Acetate	0.869	3.035	1.070	2.375	0.218	<0.0001	0.3239	0.0839
Lactate	1.424	3.272	1.873	3.529	0.370	0.0015	0.3688	0.8023
Microbial enumeration (log cfu/g)[1]								
LAB	9.59[a]	8.00[b]	8.61[b]	8.00[b]	8.86	0.0215	0.0420	0.0420
LAB post-AS	10.33	10.32	10.25	10.20	9.23	0.5286	0.0347	0.5946
Yeast	4.87	2.75	5.33	2.85	4.53	0.0028	0.0727	0.0732
Yeast post-AS	8.22	2.39	8.30	3.46	7.52	0.0006	0.6376	0.6376
Mold	4.64	<2.00	4.22	<2.00	4.22	0.1080	0.4421	0.4410
Mold post-AS	9.44	<2.00	9.28	3.77	8.35	<0.0001	0.0993	0.0993
Bacillus spores	3.17	2.73	3.19	2.75	2.63	0.0547	0.9183	0.9675
Bacillus spores post-AS	7.14[a]	2.82[a]	8.34[b]	2.93[a]	7.18	<0.0001	0.0001	0.0001
Enterobacteriaceae	5.27	<2.00	7.55	4.90	6.94	0.0757	0.0772	0.0783
Enterobacteriaceae post-AS	8.07[a]	2.00[a]	9.31[b]	<2.00[b]	8.41	0.0033	0.0061	0.0061

Mean of 3 replicates per treatment. [a-c] Means within a row with different superscript differ (P<0.05).[1] When microbiological counts were below detection limit (< 2 log cfu/g), the value of 2 was used to carry out statistical analysis.

Conclusion This study showed that the use of SiloSolve® FC in grass silages increased aerobic stability. Furthermore, the inoculant reduced the growth of yeast, mold, *Bacillus* spores and *Enterobacteriaceae* during ensiling and after AS even after a short period of fermentation. SiloSolve® FC could be used as a natural microbial solution in silage making process to control and reduce pathogenic *E. coli* O157:H7 during fermentation and at the feed-out stage.

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