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

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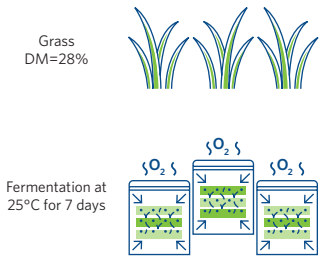
INTRODUCTION

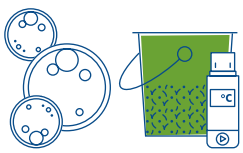
Poor management of the silage-making process results in leftover air inside the forage mass. Residual oxygen or air penetration into the bunker during the feed-out phase promote the development of yeast and mold as well as pathogenic microorganisms such as *Escherichia coli* O157:H7 (Queiroz *et al.* 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 grass silage during the fermentation phase and after AS.

MATERIAL AND METHODS



- **Control (C)**
- **SILOSOLVE® FC (FC)**
  Lactobacillus buchneri DSM22501 and *Lactococcus lactis* DSM11037 applied at 150,000 cfu/g of forage
- *E. coli* O157:H7 (EC) DSM17076 applied at 10^6 cfu/g of forage
- EC + FC



- Fermentation parameters analysis: pH, acetate and lactate quantification
- Microorganism enumeration
  Total count of lactic acid bacteria (LAB), yeast, mold, *Bacillus* spores and *Enterobacteriaceae*
- Aerobic stability test for 7 days at room temperature

Data were analyzed in agreement to a completely randomized design with a 2 x 2 factorial arrangement of treatments. The tested main effects were use 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

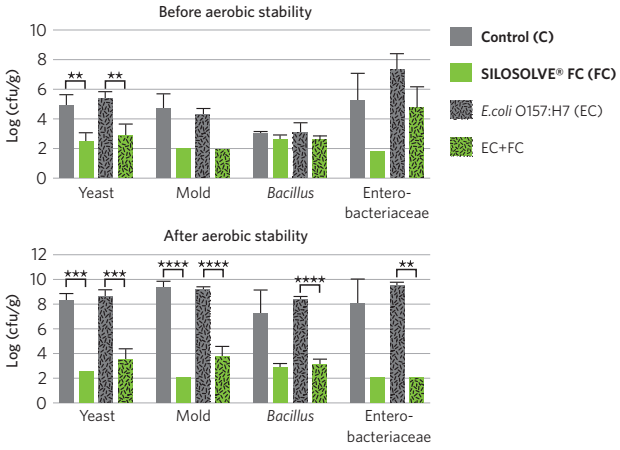
The use of **SILOSOLVE® FC** resulted, after one week of fermentation, in (i) a greater aerobic stability (AS) (increase > 120 hours), (ii) a lower pH before and after AS, and (iii) an increase in lactate and acetate levels (Table 1). **SILOSOLVE® FC** also significantly reduced the yeast population upon opening and both yeast and mold populations after AS (Figure 1). After fermentation, *Bacillus* were numerically lower in silages treated with **SILOSOLVE® FC** while after AS, significant differences were observed (Figure 1). Similarly to the study conducted by Ogunade *et al.* (2016), *Enterobacteriaceae* remained detectable in control mini-silos (C and EC) fermented for one week. However, the addition of **SILOSOLVE® FC** inhibited the growth of *Enterobacteriaceae* during ensiling and after AS (Figure 1).

Table 1. Effect of inoculant on DM, pH and fermentation end-products in grass silage ensiled for seven days.

Items	Treatments				s.e.	P-value Inoculant	P-value Challenge	P-value Inoculant* Challenge
	C	FC	EC	EC+FC				
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
Acetate (% DM)	0.87	3.04	1.07	2.38	0.22	<0.0001	0.3239	0.0839
Lactate (% DM)	1.42	3.27	1.87	3.53	0.37	0.0015	0.3688	0.8023

Mean of 3 replicates per treatment. [a,b] Means within a row with different superscript differ ($P<0.05$).

Figure 1. Microorganism enumeration in grass inoculated without additive or with **SILOSOLVE® FC** after one week of fermentation and after aerobic stability. $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$



CONCLUSION

> The present study showed the efficacy of **SILOSOLVE® FC** in grass silages to increase aerobic stability and control the growth of yeast, mold, *Bacillus* and *Enterobacteriaceae* during ensiling and after AS, following a short period of fermentation. Furthermore, **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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