Effects of delayed sealing and additives on fermentation characteristics and dry-matter losses of red clover-grass silage contaminated with clostridia

L.M. Nazato[1], A. Jonsson[2], F. Dahlström[3], K. Weiss[4], J. Jakobsson[5], L. G. Nussio[1] and E. Nadeau[3]
[1]University of São Paulo, Piracicaba, São Paulo 13418-900, Brazil, Email: larissa.nazato@usp.br, [2]Research Institute of Sweden, RISE, Box 63, 532 21 Skara, Sweden, [3]Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Box 234, 532 23 Skara, Sweden, [4]Humboldt Universität zu Berlin, Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, D-10115 Berlin, Germany, [5]Konsil Scandinavia, Tvååker, Sweden

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Introduction The ensiling of low dry-matter (DM) grass-clover forage is highly susceptible to clostridial fermentation because of its low sugar content and high buffering capacity (Knicky and Spörndly, 2011). In addition, prolonged exposure to air before sealing the silo can affect the silage fermentation process negatively (Borreani et al., 2018). The aim of this project was to evaluate the effects of delayed sealing, additives and their interaction on clostridia counts, fermentation characteristics, DM losses, and aerobic stability of direct-cut red clover-grass silage, when contaminated with clostridia.

Materials and Methods A third-cut sward of red clover-grass (14% of DM) was harvested in south-west Sweden. The chopped forage was untreated control (UC) without *Clostridium* contamination or a control treatment (CC) and additive treatments with contamination of 10^3 cfu of *Clostridium* spores/g of forage. After addition of *Clostridium,* the following additives were applied: a bacterial inoculant containing both homofermentative (*Lactobacillus plantarum* and *Lactobacillus paracasei*) and heterofermentative (*Lactobacillus brevis*) lactic acid bacteria at a dosage of 210.000 cfu/g forage; a commercial blend of sodium nitrite, hexamine and sodium benzoate (Salt A) or of sodium nitrite, hexamine and potassium sorbate (Salt B), at 3 L/tonne; a commercial blend of formic acid, propionic acid and sodium formate at 5 L/tonne. A total of 36 laboratory silos of 1.7 L was used. Half of the silos were exposed to air for 24 h before being sealed, whereas the other half was sealed immediately after filling. The DM losses during storage were calculated according to Weissbach (2005). Aerobic stability was measured as described by Honig (1990). Silage samples were analysed for fermentation pattern at the Central Laboratory of Humboldt University, Berlin and for clostridia spore count by the plate count method (Jonsson, 1990) at SLU, Skara. The experiment had a completely randomized design with silo as the experimental unit in a 2 x 6 factorial arrangement of treatments with two levels of sealing treatment and six levels of additive treatment. Data were analysed by using the PROC MIXED procedure of SAS ver. 9.4 (Cary, NC, USA). When the global *F*-test showed significance at $P < 0.05$, the Tukey`s test was used for pairwise comparisons between least-square means (LSmeans).

Results and Discussion Water-soluble carbohydrates and lactic acid decreased whereas spore counts (log 4.9 vs. log 3.0 cfu/g), butyric acid, NH_3-N and DM losses increased with delayed sealing (Figure 1). The acid and the Salt A treatments had the lowest NH_3-N in direct sealing and the acid also had the lowest NH_3-N in the delayed sealing treatment, which shows inhibition of proteolysis. Both the acid and Salt A had more WSC than all other treatments and less DM losses than the control and the inoculant in direct sealing (Figure 1). The acid treatment promoted a good

fermentation even in the silos with delayed sealing as shown by higher WSC and lower butyric acid concentration and lower DM losses.

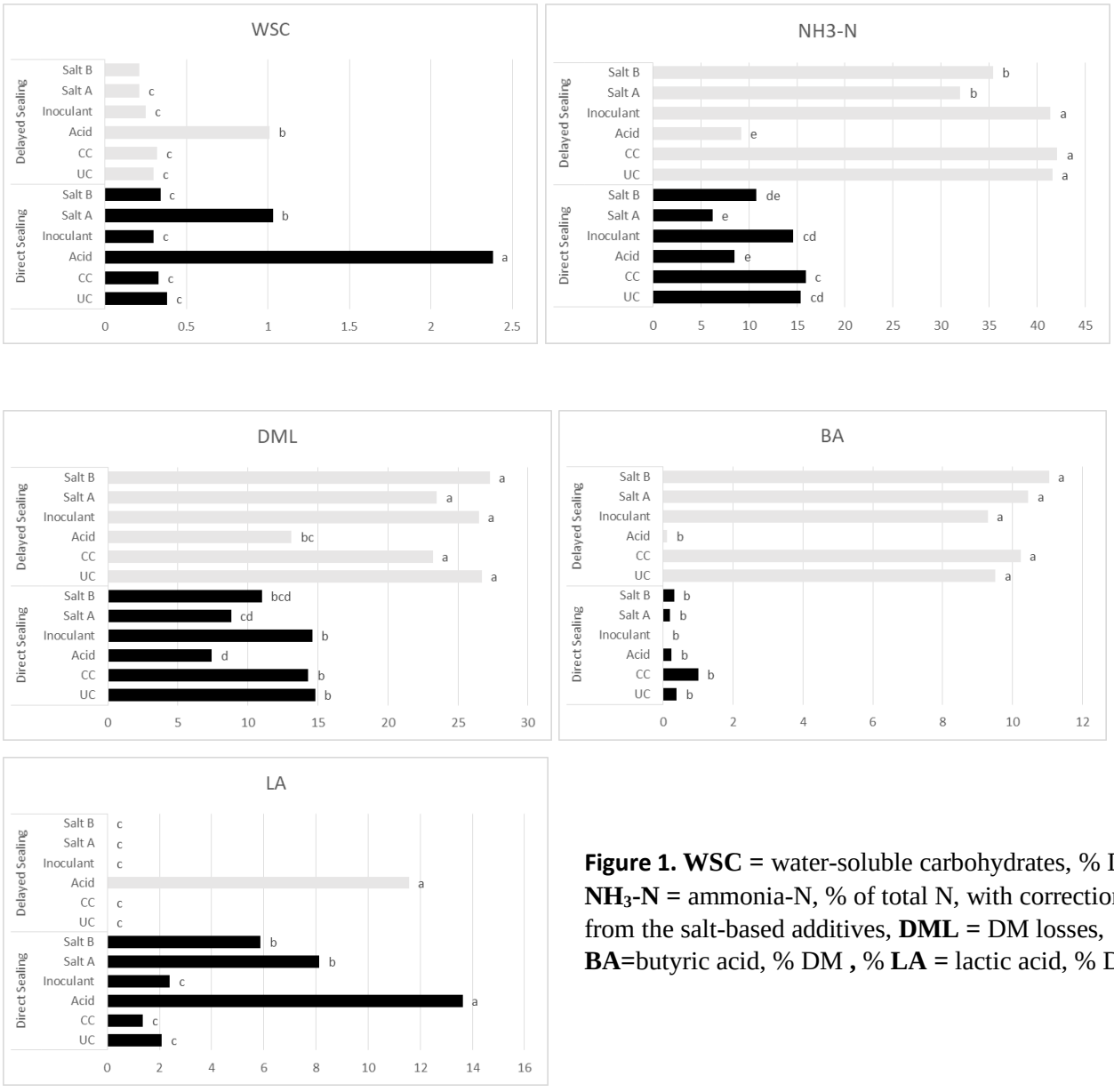

Figure 1. WSC = water-soluble carbohydrates, % DM, **NH_3-N** = ammonia-N, % of total N, with correction for N from the salt-based additives, **DML** = DM losses, **BA=**butyric acid, % DM **,** % **LA** = lactic acid, % DM,

Conclusion Delayed sealing caused clostridia fermentation in the silage. The acid-treated silage showed the most effective conservation in both direct-sealed and delayed-sealed silos, whereas the silage treated with a salt containing sodium nitrite, hexamine and sodium benzoate resulted in improved fermentation when the silos were sealed immediately. Direct sealing of the silo and the use of chemical additives are advised to achieve high-quality red clover-grass silage of extremely low DM content.