# An *in vitro* Study on the Influence of Residual Sugars on Aerobic Changes in Grass Silages

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**Introduction** How do residual sugars in high dry matter grass silages influence microbial metabolism? To answer this question a simple laboratory method was developed using pH as main indicator for aerobic changes.

**Materials and methods** Three grass silages made from *Lolium perenne* (35-40% dry matter (DM)) prone to aerobic deterioration were investigated. The silages were extracted with sterile water in the ratio 1:4 (g:ml) for 5 min in a Stomacher. The extracts served as a complex medium in an agitated batch culture system. Fungi and bacteria were provided by the indigenous microflora in the silage extracts. Fructose was added (0, 3 or 6% in fresh matter (FM)) to investigate the effect of different levels of residual sugar. Three replicate samples consisting of 40 ml aliquots of silage extract in each Erlenmeyer flask (100 ml) covered with aluminium foil were agitated on an orbital shaker at 175 rpm at 25°C for 2 d. pH was measured at the beginning (0 h) and after 21, 34, 45 and 51 h. Volatile fatty acids were analysed by HPLC after 0, 21 and 45 hours. Numbers of yeasts and lactic acid bacteria (LAB) were counted on modified malt extract and Rogosa agar.

**Results** The 3 silages used for the extracts contained: yeasts 6-7 log cfu/g FM, LAB 5-6 log cfu/g FM, lactate 8.8-11.7 g/kg FM, WSC 47-69 g/kg FM (fructose, glucose, sucrose and not including fructans) and had a pH in the range 4.6-4.8. During the first 21 h there was a pH decline with all sugar levels. Lactic acid was produced within that time in 6 out of 7 cases. Acetic acid contents rose in all media. Further changes in pH were directly affected by WSC content. The lower the WSC content the faster and steeper was the pH rise. After 45 h the five WSC groups differed significantly from each other by at least 0.09 pH units (Tukey test) (descending): 47, 59-69, 77, 89-99, 119 (g/kg FM initial WSC content). At the same time acetic acid contents rose further in general contrast to values for lactic acid content.

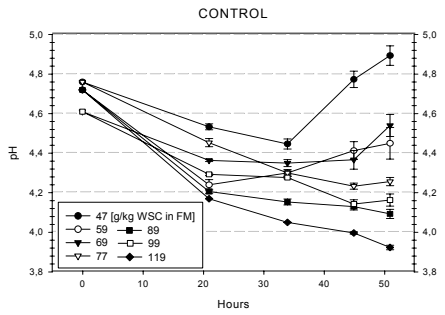


**Figure 1** Changes of pH in silage extracts with different WSC contents (error bars = s.d.)

**Table 1** Lactic and acetic acid contents (mg/ml) of media with different WSC levels after 0, 21 and 45 h

| WSC | Lactic acid | | | Acetic acid | | |
|---|---|---|---|---|---|---|
| (g/kg FM) | 0 h | 21 h | 45 h | 0 h | 21 h | 45 h |
| 47 | 2.9 | 3.1 | 1.6 | 0.9 | 1.3 | 3.3 |
| 59 | 2.2 | 3.0 | 1.9 | 0.8 | 1.3 | 2.1 |
| 69 | 2.4 | 2.6 | 2.4 | 0.9 | 1.0 | 1.6 |
| 77 | 2.9 | 2.7 | 2.6 | 0.9 | 1.3 | 5.5 |
| 89 | 2.2 | 2.9 | 2.4 | 0.8 | 1.5 | 3.3 |
| 99 | 2.4 | 2.5 | 2.6 | 0.9 | 1.2 | 2.4 |
| 119 | 2.2 | 2.8 | 2.5 | 0.8 | 2.2 | 3.9 |

The regression analysis for the whole measurement period showed a significant correlation between pH and WSC x time (t): $pH_t = 5.19-0.024*t$ [h]$+0.0003*t^2-0.06*$WSC [% in FM], ($r^2 = 0.88$).

**Conclusions** In this defined *in vitro* system it was shown that the higher the residual WSC content of grass silage the higher was the tendency to increase the $H^+$ ion concentration in the initial phase of exposure to air. This was caused partly by lactate production from LAB metabolism within the first 21 h and partly by acetate production from LAB and yeasts under aerobic conditions during the 2 d of measurement (Condon, 1987; Flikweert, 1999). The method described can easily be applied and adapted to study microbial processes and their manipulation in many types of silages that have undergone a natural fermentation.

**References**

Flikweert, M.T. (1999). *Physiological roles of pyruvate decarboxylase in Saccharomyces cerevisiae*. PhD thesis, Delft University of Technology, Delft.
Condon, S. (1987). Responses of lactic acid bacteria to oxygen. *FEMS Microbiology Reviews*, 46, 269-280.