CORRELATION BETWEEN CRUDE NUTRIENTS IN FRESH GRASS AND PROPYLENE GLYCOL CONCENTRATIONS IN GRASS SILAGE TREATED WITH A MIXTURE OF HOMO- AND HETEROFERMENTATIVE LACTIC ACID BACTERIA

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ABSTRACT

Various strains of homo- and heterofermentative lactic acid bacteria (LAB) are used as silage inoculants. Homofermentative LAB convert water-soluble carbohydrates (WSC) into lactic acid, while heterofermentative LAB convert WSC into lactic and acetic acid. Some LAB strains (e.g. *Lentilactobacillus buchneri*) also produce alcohols such as propylene glycol (PG). In dairy cattle, PG is frequently used as glucogenic feed additive, to prevent ketosis at the onset of lactation. Even though high concentrations of PG in silages have been reported (up to 40 g/kg DM; Driehuis *et al.*, 2001; Nishino *et al.*, 2003), relatively little is known about compositional traits of grass, contributing to high PG yields. Therefore, this study was designed to identify compositional traits of grass that were associated with particularly high yields of PG in silages, inoculated with a mixture of homo- (*Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*) and heterofermentative (*Lentilactobacillus buchneri*) LAB.

Keywords: bacterial inoculant, grass silage, *Lentilactobacillus buchneri*, propylene glycol

INTRODUCTION

Based on their metabolic pathways and fermentation products, lactic acid bacteria (LAB) can be categorised as obligate homofermentative, facultative heterofermentative, or obligate heterofermentative. *Lentilactobacillus buchneri* is an obligate heterofermentative bacterium and frequently part of the native epiphytic microbiome (Daeschel *et al.*, 1987). Inoculation with *L. buchneri* can result in increased levels of acetic acid and 1,2-propanediol (= propylene glycol [PG]) in silages (Driehuis *et al.*, 2001), since L. buchneri can convert one mole of lactic acid into approximately 0.5 mol acetic acid, 0.5 mol PG and trace amounts of ethanol, under strictly anaerobic conditions (Oude-Elferink *et al.*, 2001). The main reason for using *L. buchneri* as a silage inoculant is its ability to improve aerobic stability by suppressing the growth of moulds and yeasts, primarily through the production of acetic acid (Muck, 1996; Driehuis *et al.*, 1999; Ranjit *et al.*, 2002). However, relatively little attention has been given to the fact that *L. buchneri* also produces propionic acid (PG), although PG is frequently used as a glucogenic feed supplement to prevent ketosis in postpartum dairy cows. Therefore, silage containing sufficiently high concentrations of PG could potentially mitigate the effects of negative energy balance at the onset of lactation. The present study was designed due to the lack of information concerning the maximum achievable PG concentrations. The aim was to examine the extent to which a silage inoculant containing a specially-selected *L. buchneri* strain would lead to the accumulation of PG in grass silage.

MATERIAL AND METHODS

This study consisted of 18 laboratory-scale ensiling experiments. Seven first, two second, six third, and three fourth cuts were ensiled over the course of two harvest seasons. The grass was inoculated with a mixture of homofermentative (*Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus*) and heterofermentative LAB (*Lentilactobacillus buchneri*) at a concentration of 3.0×10^5 CFU/g of fresh grass. Quadruplicate mini silos (1.5 L) were ensiled for each harvest time point (18 time points × 4 mini silos = 72 mini silos in total). Silos were stored for 90 d at 20 °C. The freshly cut grass was analyzed for ash, dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (aNDFom), acid detergent fiber (ADFom), fat, sugar, and enzyme-soluble organic matter (ESOM). To determine ensiling quality, pH and fermentation end products, including PG concentrations were measured. To explore any associations between the chemical composition of the grass samples and the concentration of PG in the silages, a correlation analysis (PROC CORR) between the DM, OM, CP, aNDFom, ADFom, fat, sugar and ESOM content of the grass before ensiling and the PG concentration (mean of quadruplicate samples per harvest date) in the silages was conducted (SAS, version 9.4). As measure of correlation between the respective parameters, the Pearson correlation coefficient (r) is reported.

RESULTS AND DISCUSSION

The DM and OM content of the grass before ensiling, as well as the aNDFom, ADFom, fat, sugar and ESOM, were all correlated with PG ($P < 0.05$; Table below). Increases in OM ($r = 0.553$), fat ($r = 0.789$), sugar ($r = 0.648$) and ESOM ($r = 0.612$) content of the

Tab. 1: Correlations between the dry matter, organic matter, crude protein, aNDFom, ADFom, fat, sugar, and enzyme-soluble organic matter (ESOM) content of grass before ensiling (d 0) and the propylene glycol (PG) content of grass silage (d 90), after inoculation with a mixture of homo-and heterofermentative lactic acid bacteria (*L. plantarum*, *L. rhamnosus*, *L. buchneri* at 3×10^5 CFU/g fresh matter; n = 18).

Category	r	P-value
Dry matter	−0.705	0.001
Organic matter	0.553	0.017
Crude protein	0.632	0.005
aNDFom[1]	−0.545	0.019
ADFom[2]	−0.571	0.013
Fat	0.789	<0.001
Sugar	0.648	0.004
Enzyme-soluble organic matter	0.612	0.007

[1]Neutral detergent fiber assayed with a heat stable amylase and expressed without residual ash

[2]Acid detergent fiber expressed without residual ash

https://doi.org/10.11118/978-80-7701-093-1-0164

grass before ensiling were all associated with an increase in PG in the mature silages. As expected, the WSC content was closely associated with high PG yields. Sugars are the primary energy source of LAB (McDonald *et al.*, 1991). The positive correlation between CP and high PG yields can be explained by the higher buffer capacity of grass with a higher CP content. As a result, the critical pH at which LAB growth is negatively affected is reached later in the fermentation process. This allows *L. buchneri* to convert more lactic acid into acetic acid and PG over a longer period of time. Increases in DM (r= –0.705), aNDFom (r= –0.545) and ADFom (r= –0.571) of the grass before ensiling were associated with a reduction in PG in the silages. Increases in DM, aNDFom and ADFom are characteristics of older, more mature grass lacking fermentable carbohydrates for LAB.

CONCLUSION

High concentrations of PG in grass silage treated with a mixture of *L. plantarum*, *L. rhamnosus* and *L. buchneri* are closely associated with low DM and high sugar contents of the grass at the time of ensiling. High concentrations of PG in grass silage (19.9 g/kg DM) can be achieved, if the right bacterial inoculants are used and the composition of the forage is suitable. Feeding experiments involving dairy cows experiencing a negative energy balance are needed to determine whether the intake of PG through specifically inoculated grass silage can be sufficiently high, to effectively reduce the risk of ketosis.

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