Comparison of propylene glycol production in grass silages induced by different silage inoculants

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Introduction: Numerous different strains of homofermentative and heterofermentative lactic acid bacteria (LAB) are contained in silage additives. Thereby, homofermentative LAB utilize water soluble carbohydrates (WSC) to produce lactic acid and heterofermentative LAB transform WSCs to lactic and acetic acid. Particular LAB strains (e.g. *Lb. buchneri*) produce, next to above mentioned fermentation acids, alcohol and propylene glycol (PG; Driehuis et al., 2001). With regard to the specialized fermentation pathway of heterofermentative LAB, dry matter (DM) and energy losses are in general higher compared over homofermentative LAB (Driehuis et al., 2001). In postpartum dairy cows, synthetic PG is often supplemented as a glucogenic precursor in order to support energy metabolism at the beginning of lactation (Nielsen and Ingavartsen, 2004). Due to those studies the consumption of silages containing increased PG levels might be advantageous in the realm of performance parameters as well as in reducing the risk of ketosis (Nishino et al., 2002). The following study intends to evaluate the production of PG in grass silages containing various DM, WSC and neutral detergent fiber ($aNDF_{om}$) levels.

Material and Methods: In this study several (n=19) batch silage experiments were conducted. To evaluate the effects of silage inoculants on grasses of different maturity stages, first, second, third, and fourth crops of two different years (2016 and 2017) were used as substrates. Following the design of different treatments (quadruplicate): (1) control (*no inoculant*), (2) inoculant containing a mixture of homofermentative LAB at 3.0 x 10^5 CFU/g fresh grass (*Lb. plantarum, Lb. rhamnosus – hoLAB*), and (3) inoculant containing a mixture of homo- and heterofermentative LAB 3.0 x 10^5 CFU/g fresh matter (*Lb. rhamnosus*, *Lb. plantarum, Lb. buchneri – hoheLAB)*. The different treated samples varied in DM content (23.6% to 78.5%), level of WSCs (6.6 ± 1.4% to 23.2 ± 0.13%) and NDF content (42.2 ± 0.18 to 55.5 ± 0.12%; all on DM basis). The duration of the experiments was 90d and grasses were ensiled in mini silos (1500ml). The following parameters were evaluated: chemical composition of fresh and ensiled substrate (Methods according to VDLUFA, 2012) and determination of enzyme soluble organic matter (ELOS) in a Daisyll incubator (Stadler and Henkelmann, 2012). Occurring fermentation gas losses (FGL) were defined by taking the mass difference before and after ensiling into account which were than declared as percentage fresh matter (Weissbach, 1998). In 1995 Weissbach and Kuhla defined DM losses (DML) as the mass difference before and after ensiling in association with the level of DM and the proportion of dissolved CO_2 in the ensiled material. Silage DM contents were adjusted for losses of ethanol, PG, volatile fatty acids and lactic acid due to drying (Weissbach and Kuhla, 1995). High performance liquid chromatography (HPLC) was used to evaluate concentration of PG, ethanol and fermentation acids. For quantitative enumeration of LAB counts, samples were plated on MRS agar, and yeast and mold counts on YGC agar. ANOVA was used to test for differences in between the treatments (control, hoLAB, hoheLAB) and comparison of means was subsequently conducted using the Tukey Test. Significances were declared at $P < 0.05$ (SAS, 2013), whereby the model included the fixed effect of treatment as well as the mini silo as random effect.

Results and Discussion: DM losses in hoheLAB treated silages were observed to be higher (4.98%) compared over hoLAB treated silages (4.34%, $P < 0.05$). As expected, pH after 90d was lower in hoLAB (4.21) compared to hoheLAB (4.42) and the control respectively (4.36; P< 0.05). Significant higher concentrations of PG were measured in silages treated with hoheLAB (4.98% DM) compared to the control (0.10% DM) and hoLAB (0.04% DM; P < 0.001). With regard to the varying level of DM and WSCs of the grasses used in this study, an impact of both variables (DM, see Fig 1 and WSC, see Fig. 2) could infer for the formation of PG. Considerably higher levels of PG were observed when grasses containing <40% DM and ≥12% WSC (% of DM) were ensiled. Amounts of PG differed between 0.09% of DM (contents of fresh grass: 53.4% DM; $aNDF_{om}$ 42.4% of DM; WSC 20.1% of DM) and 4.1% of DM (contents of fresh grass: 24.2% DM; $aNDF_{om}$ 43.5% of DM; WSC 14.7% of DM). Taking the results of the fermentation acids and fermentation metabolites into consideration, a positive influence of lower DM contents (<50% DM) on LAB activity is confirmed. The results of this study justify the claim of elevated PG production in silages inoculated with hoheLAB. Results also identified increasing PG levels with decreasing levels of DM: (I) 20-30% DM on average 3.2 ± 0.94% of DM (n=4); (II) 30-40% DM on average 2.2 ± 0.94% of DM (n = 9); (III) >40% on average 0.60 ± 0.41% of DM (n=6) after 90 days of storage.

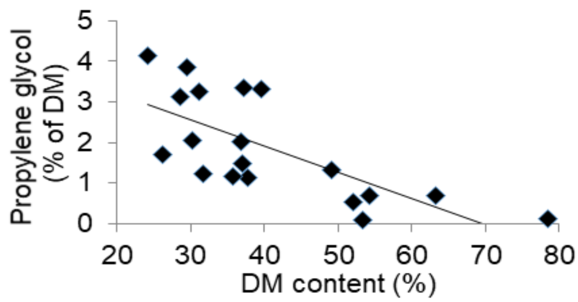

Figure 1. Propylene glycol content in ensiled grass (supplemented with hoheLAB) in relation to the DM content of fresh grass.

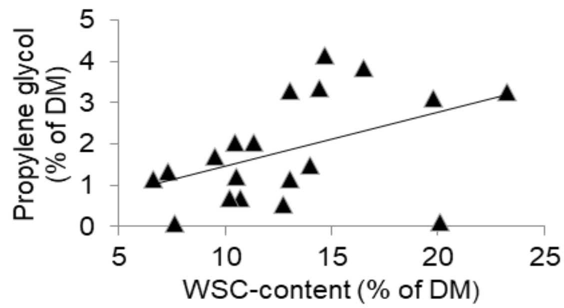

Figure 2. Propylene glycol content in ensiled grass (supplemented with hoheLAB) in relation to the content of WSC of fresh grass.

Conclusion: The results show, the level of PG in grass silages is primarily dependent on the choice of silage inoculant. Further influencing factors on PG formation in grass silages are the content of DM and WSCs of the fresh material.

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