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Effects of storage temperature and combined microbial inoculants on fermentation end products and microbial populations of Italian ryegrass (*Lolium multiflorum* Lam.) silage

Short Title: Combined lactic acid bacteria and their effects

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Abstract

Aims: To examine five lactic acid bacteria (LAB) strains (LCG9, LTG7, I5, TG1 and LI3) isolated from the Tibetan Plateau, and evaluate their combined effects on the silage quality of Italian ryegrass (*Lolium multiflorum* Lam.) at three temperatures (10°C, 15°C and 25°C).

Methods and Results: The isolated strains were evaluated by morphological, physiological and biochemical tests. Six combined inoculants LCG9+LI3, LCG9+I5, LCG9+TG1, LTG7+LI3, LTG7+I5 and LTG7+TG1 were added to Italian ryegrass for ensiling 30 days in laboratory silos (1L) at various temperatures, respectively. All the isolates could grow normally at 5-20°C, pH 3.5-7.0 and NaCl (3.0%, 6.5%). Compared to three corresponding controls, all the inoculants improved the silage quality of Italian ryegrass at different temperatures, indicated by markedly ($P<0.05$) higher lactic acid (LA) contents and ratios of lactic acid/acetic acid (LA/AA), and lower pH and ammonia nitrogen (NH₃-N) contents and undesirable microorganism counts. At 10°C, LTG7+LI3, I5 or TG1 inoculants performed superior than LCG9+LI3, I5 or TG1 inoculants, evidenced by distinctly ($P<0.05$) higher LA contents and ratios of LA/AA, and lower pH and NH₃-N contents. LTG7+LI3 or TG1 silages had obviously ($P<0.05$) higher LAB counts than LTG7+I5 silage at 10°C.

Conclusions: Therefore, the combined inoculants LTG7+LI3 or TG1 are recommended as starter cultures for Italian ryegrass silage at low temperatures.

Significance and Impact of the Study: Temperature is a key factor affecting ensilage. In cold regions, low temperature could be an adverse environmental condition during ensiling. However, few studies have focused on improving silage quality at low temperatures. Moreover, analysis of effects of combined LAB strains with their physiological and

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