J. Dairy Sci. 100:1–17
https://doi.org/10.3168/jds.2016-11815

Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows

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ABSTRACT

Forages are usually inoculated with homofermentative and facultative heterofermentative lactic acid bacteria (LAB) to enhance lactic acid fermentation of forages, but effects of such inoculants on silage quality and the performance of dairy cows are unclear. Therefore, we conducted a meta-analysis to examine the effects of LAB inoculation on silage quality and preservation and the performance of dairy cows. A second objective was to examine the factors affecting the response to silage inoculation with LAB. The studies that met the selection criteria included 130 articles that examined the effects of LAB inoculation on silage quality and 31 articles that investigated dairy cow performance responses. The magnitude of the effect (effect size) was evaluated using raw mean differences (RMD) between inoculated and uninoculated treatments. Heterogeneity was explored by meta-regression and subgroup analysis using forage type, LAB species, LAB application rate, and silo scale (laboratory or farm-scale) as covariates for the silage quality response and forage type, LAB species, diet type [total mixed ration (TMR) or non-TMR], and the level of milk yield of the control cows as covariates for the performance responses. Inoculation with LAB ($\geq 10^5$ cfu/g as fed) markedly increased silage fermentation and dry matter recovery in temperate and tropical grasses, alfalfa, and other legumes. However, inoculation did not improve the fermentation of corn, sorghum, or sugarcane silages. Inoculation with LAB reduced clostridia and mold growth, butyric acid production, and ammonia-nitrogen in all silages, but it had no effect on aerobic stability. Silage inoculation ($\geq 10^5$ cfu/g as fed) increased milk yield and the response had low heterogeneity. However, inoculation had no effect on diet digestibility and feed efficiency. Inoculation with LAB improved the fermentation of grass and legume silages and the performance of dairy cows but did not affect the fermentation of corn, sorghum, and sugar cane silages or the aerobic stability of any silage. Further research is needed to elucidate how silage inoculated with homofermentative and facultative heterofermentative LAB improves the performance of dairy cows.

Key words: ensiling, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Pediococcus pentosaceus*

INTRODUCTION

Ensiling is the most common forage preservation method in ruminant feeding systems (Weinberg and Muck, 1996). It is based on fermentation of water-soluble carbohydrates (**WSC**) in forages to organic acids (mainly lactic acid) by epiphytic bacteria under anaerobic environments (Weinberg and Muck, 1996). The low pH achieved as a result of accumulation of organic acids inhibits spoilage and pathogenic microbes, thereby preserving the nutritional value of the ensiled forage (Weinberg and Muck, 1996; Ogunade et al., 2016). During ensiling, processes such as plant respiration, plant microbial proteolytic activity, clostridial fermentation, microbial deamination, and decarboxylation of amino acids may negatively affect conservation efficiency, increase energy and nutrient losses, and cause accumulation of antinutritional compounds in silage (MacPherson and Violante, 1966; Muck, 1988). These processes

Received August 1, 2016.
Accepted December 14, 2016.
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contribute to silage DM losses and reduce the nutritive value, and they can adversely affect animal health and performance and food safety, thereby reducing the profitability of the dairy production system and increasing environmental pollution. Thus, silage management must aim to prevent or minimize these detrimental effects by optimizing lactic acid fermentation.

Homofermentative and facultative heterofermentative lactic acid bacteria (**LAB**) inoculation has been commonly used to improve lactic acid fermentation, inhibit deleterious epiphytic microbes, and preserve the nutritional quality of ensiled forages (Arriola et al., 2015; Ogunade et al., 2016; Silva et al., 2016). However, the results have been inconsistent. Some studies have reported positive (Filya et al., 2000) or no effects (Kleinschmit et al., 2005; Ogunade et al., 2016), but others have observed that LAB inoculation increased aerobic spoilage (Weinberg et al., 1993; Danner et al., 2003). This spoilage is because LAB inoculation typically reduces the concentration of acetate, which is strongly antifungal, and increases concentration of lactate, which is a growth substrate for spoilage yeasts (Weinberg et al., 1993).

Classical reviews have shown promising effects of homofermentative or facultative heterofermentative LAB inoculation on silage fermentation and animal performance; however, responses to silage inoculants could be influenced by several factors including type of forage, application rate of LAB inoculant, LAB species, and other ensilage management practices (Weinberg and Muck, 1996; Kung and Muck, 1997; Muck and Kung, 1997).

Although some reviews (Weinberg and Muck, 1996; Kung and Muck, 1997; Muck and Kung, 1997) suggested that homofermentative or facultative heterofermentative LAB inoculation improves both silage fermentation and animal performance, information on the magnitude of factors affecting the response are lacking. Furthermore, such reviews were not based on meta-analytic approaches. Meta-analysis is a statistical approach of summarizing multiple studies, which improves the power or ability to detect treatment effects and increases the capacity to explore sources of variation in responses (Glass, 1976; Higgins, 2008).

Our objective was to conduct a meta-analysis to evaluate the magnitude of effects of homofermentative or facultative heterofermentative LAB inoculation on silage quality and preservation and the performance of dairy cows. We also explored between-study sources of heterogeneity. We hypothesized that homofermentative or facultative heterofermentative LAB inoculation would improve silage quality and the performance of dairy cows but would not increase silage aerobic stability.

MATERIALS AND METHODS

Literature Search

A literature search was conducted using the Web of Science database on October 19, 2015, and an update was done on March 8, 2016. To evaluate effects of homofermentative or facultative heterofermentative LAB inoculation on silage quality, a total 1,747 peer-reviewed papers were retrieved using the terms "silage" and "*Lactobacillus plantarum*," "silage" and "*Pediococcus pentosaceus*," "silage" and "*Enterococcus faecium*," and "silage" and "*Lactobacillus rhamnosus*." To evaluate effects of feeding LAB-inoculated silage on the performance of dairy cows, a total of 206 published studies were retrieved using the terms "dairy cows," "silage," and "inoculant." In subsequent parts of this manuscript, the LAB acronym will refer to both homofermentative and facultative heterofermentative LAB but not to obligate heterofermentative LAB such as *Lactobacillus buchneri*.

Inclusion Criteria

A flowchart explaining the process of study identification and selection for analyzing the effects of LAB inoculant on silage quality is shown in Figure 1. The inclusion criteria for selecting studies were as follows. Studies had to (1) be published in English language peer-reviewed journals; (2) be published after 1996 because studies published earlier were included in earlier reviews (Weinberg and Muck, 1996; Kung and Muck, 1997; Muck and Kung, 1997); (3) concurrently examine uninoculated and inoculated treatment groups; (4) have treatments comprising only LAB; (5) use at least 30 d of ensiling to ensure the silage was properly preserved; (6) report the inoculant application rate; and (7) report the variance [i.e., standard error (**SE**) of the mean or standard deviation (**SD**)].

A flowchart detailing the process of study identification and selection for analyzing the effects of LAB inoculation of silage on the performance of dairy cows is shown in Figure 2. Inclusion criteria for the studies were as follows. Studies had to (1) be published in English language peer-reviewed journals; (2) concurrently examine uninoculated and inoculated treatment groups; (3) include treatments comprising only LAB; and (4) report the variance (i.e., SE, SD).

Data Extraction

Silage Quality. Based on the aforementioned inclusion criteria, 130 peer-reviewed papers were selected and classified by first author, publication reference, forage