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 pro-

duction, 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

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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

type (corn, sorghum, or both; temperate grass; tropical grass; sugarcane; alfalfa; other legume; and other forages), LAB species (*L. plantarum*, *P. pentosaceus*, *E. faecium*, *L. rhamnosus*, or mixed LAB species), LAB application rate ($\leq 10^4$, 10^5 , 10^6 , or $\geq 10^7$ cfu/g as fed), silo type (laboratory or farm-scale), ensiling duration, and whether or not the inoculant contained enzymes. The number of replicates, means, and variances (i.e., SE, SD) were extracted for the following response variables from both control and inoculant treatments: pH, DM recovery, concentrations of DM, NDF, lignin, CP, ADIN, WSC, ethanol, lactate, acetate, propionate and butyrate, in vitro DM digestibility (IVDMD-48 h), counts of LAB, yeasts, molds and clostridia (cfu/g as fed), and aerobic stability (h). The complete data set is available in an Excel (Microsoft Corp., Redmond,

WA) file in Supplemental File S1 and references are in Supplemental File S3 (<https://doi.org/10.3168/jds.2016-11815>).

Dairy Cow Performance. Based on the inclusion criteria, 31 peer-reviewed papers were selected and classified by first author, publication reference, forage type (temperate grass, alfalfa, or grass and legume mixture), LAB species (*L. plantarum* or LAB species combinations), LAB application rate ($< 10^4$, 10^5 , 10^6 , or $> 10^7$ cfu/g as fed), diet type [TMR or forage and concentrate separately offered (non-TMR)], the level of milk yield of the control cows (a median milk yield of < 22.8 kg/d or ≥ 22.8 kg/d). The number of replicates, means, and variances (i.e., SE, SD) were extracted for the following response variables for control and inoculant treatments: DMI, diet total-tract DM digestibility,

Figure 1. Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of the effects of homofermentative and facultative heterofermentative lactic acid bacteria inoculation on silage quality.

unadjusted milk yield, feed efficiency, and milk protein and fat concentrations and yields. The complete data set is available in an Excel file in Supplemental File S2; references are in Supplemental File S3; and performance data (DMI, feed efficiency, milk fat, and milk protein) are in Supplemental Figures S1, S2, S3, and S4 (<https://doi.org/10.3168/jds.2016-11815>).

Statistical Analysis

Meta-analysis was conducted using the *metafor* package of R Software (Viechtbauer, 2010) version 3.2.3. Forest plots were created using Stata software version 14.1 (StataCorp LP, College Station, TX). The effects of LAB inoculation on silage quality and the performance of dairy cows were evaluated by examining the raw mean differences (**RMD**) between uninoculated and inoculated treatment means (effect size), which were weighted by the inverse of the variance in the respective studies using the method proposed by DerSimonian and Laird (1986) for a random effect model.

Between-study variability (i.e., heterogeneity of effect size) was evaluated using the chi-squared (Q) test and the I^2 statistic, which measures the percentage of variation due to heterogeneity (Higgins et al., 2003). Negative values of I^2 were assigned a value of zero and values of <25, 25 to 50, and >50% were considered

indicative of low, moderate, and high heterogeneity, respectively (Higgins et al., 2003).

Using RMD as the dependent variable, meta-regression analysis was used to identify effects of the categorical covariates (previously described) for silage quality and dairy cow performance. A mixed meta-regression model was fitted to the data. The multiparameter Wald test was used to test the null hypothesis that the coefficients of the covariate are zero (Viechtbauer, 2010). The adjusted R^2 was calculated by comparing the estimated between-study variance when covariates were fitted (σ^2), with corresponding values when no covariates were fitted (σ_o^2); adjusted R^2 (%) = $(\sigma_o^2 - \sigma^2)/\sigma_o^2$. The adjusted R^2 represents the proportion of between-study variance (heterogeneity) explained by the covariates (Viechtbauer, 2010). When effects of a categorical covariate were significant ($P \leq 0.05$), the RMD was further analyzed by subgrouping.

Publication bias was assessed using funnel plots (Light and Pillemer, 1984) and was tested for funnel plot asymmetry (indicative of publication bias) by Egger's regression method between RMD and SE (Egger et al., 1997). Comparisons between inoculant and control treatments with standardized residuals >2.5 or < -2.5, and with Cook's distances (Cook, 1977) >5/ n were removed. The list of outliers removed is provided in Supplemental Files S1 and S2 (<https://doi.org/10.3168/jds.2016-11815>).

Figure 2. Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of the effects of homofermentative and facultative heterofermentative lactic acid bacteria inoculation of silages on dairy cattle performance.

Table 1. Effect of homofermentative and facultative heterofermentative lactic acid bacterial inoculants on chemical composition (DM basis; % of DM unless otherwise noted) and fermentation characteristics of ensiled forages

Item ¹	Control ² mean (SD)	N ³	RMD ⁴ (95% CI)		Heterogeneity ⁵	
			Random effect	P-value	P-value	I ² (%)
pH	4.10 (0.47)	236	−0.11 (−0.13, −0.10)	<0.01	<0.01	97.4
DM recovery (%)	92.02 (5.54)	51	0.78 (0.18, 1.374)	0.01	<0.01	77.5
DM (%)	29.93 (12.40)	209	0.32 (0.18, 0.46)	<0.01	<0.01	88.8
NDF	49.43 (12.90)	125	−0.25 (−0.56, 0.06)	0.14	<0.01	98.2
Lignin	5.23 (2.32)	25	−0.25 (−0.34, −0.15)	<0.01	<0.01	78.9
CP	12.55 (6.20)	120	0.05 (−0.07, 0.17)	0.91	<0.01	97.5
ADIN (% of N)	7.38 (3.91)	9	−0.13 (−0.39, 0.13)	0.32	0.10	41.6
NH ₃ -N (% of N)	7.82 (3.60)	134	−1.31 (−1.39, −1.24)	<0.01	<0.01	99.6
WSC	3.05 (2.59)	157	−0.09 (−0.15, −0.02)	<0.01	<0.01	98.6
IVDMD-48 h (%)	66.43 (9.17)	36	0.21 (−0.14, 0.55)	0.24	<0.01	66.3
Ethanol	0.22 (0.19)	98	−0.04 (−0.10, 0.01)	0.12	<0.01	95.8
Lactate	4.67 (2.99)	258	0.92 (0.79, 1.05)	<0.01	<0.01	99.8
Acetate	1.43 (1.26)	225	−0.20 (−0.23, −0.17)	<0.01	<0.01	99.1
Propionate	0.31 (0.53)	83	−0.005 (−0.01, 0.00)	0.18	<0.01	78.6
Butyrate	0.27 (0.46)	60	−0.05 (−0.06, −0.03)	<0.01	<0.01	96.4
LAB (log cfu/g)	7.04 (1.42)	83	−0.07 (−0.24, 0.11)	0.46	<0.01	98.7
Yeast (log cfu/g)	4.02 (1.66)	86	0.28 (0.09, 0.48)	<0.01	<0.01	99.5
Mold (log cfu/g)	3.00 (1.21)	32	−0.58 (−0.84, −0.32)	<0.01	<0.01	99.0
Clostridia (log/g)	3.54 (1.45)	8	−1.94 (−2.97, −0.91)	<0.01	<0.01	93.4
Aerobic stability (h)	90.93 (78.28)	40	−1.66 (−4.58, 1.26)	0.27	<0.01	81.0

¹WSC = water-soluble carbohydrate; IVDMD = in vitro DM digestibility; LAB = lactic acid bacteria.

²Uninoculated treatment.

³N = number of comparisons of inoculated and uninoculated treatments (complete data set is available in Supplemental File S1; <https://doi.org/10.3168/jds.2016-11815>).

⁴RMD = raw mean differences between inoculated and uninoculated treatments.

⁵P-value for χ^2 (Q) test of heterogeneity; I² = proportion of total variation of size effect estimates that is due to heterogeneity.

[org/10.3168/jds.2016-11815](https://doi.org/10.3168/jds.2016-11815)). Significance was declared at $P \leq 0.05$ and tendencies at $0.05 > P > 0.10$.

RESULTS

Silage Quality

Among the 130 peer-reviewed studies selected to investigate the effects of LAB inoculation on silage quality, corn/sorghum, temperate and tropical grasses, sugarcane, alfalfa, other legumes (*Leucaena leucocephala*, *Lupinus albus*, *Pisum sativum*, *Trifolium pratense*, *Vicia faba*, and *Vicia* spp.), and other forages (safflower, cabbage, mixture of grass and legume, mixture of alfalfa and corn, TMR, *Hedychium gardnerianum*, and *Raphanus sativus*) accounted for 23.2, 30.2, 7.3, 9.5, 5.7, 12.1, and 12.1% of the silages, respectively. Approximately 67.0 and 27.3% of the studies used *L. plantarum* or a combination of LAB species, respectively, whereas *P. pentosaceus*, *E. faecium*, and *L. rhamnosus* accounted for only 3.8, 0.3, and 1.6% of the species used, respectively. The most commonly used application rates for LAB inoculation were 10^6 cfu/g as-fed basis (60.1%) and 10^5 cfu/g as-fed basis (34.1%), and LAB inoculation with $\leq 10^4$ or $\geq 10^7$ cfu/g represented only 3.5 and 2.5% of the selected studies. Laboratory-scale silos were

more commonly used (92.7% of the studies), whereas farm-scale silos were used in 7.3% of the selected studies. Only 2.4% of the studies reported use of inoculants containing enzymes.

The effects of LAB inoculation on silage quality across studies are shown in Table 1. Inoculation with LAB reduced ($P < 0.01$) silage pH and WSC concentration but increased ($P \leq 0.01$) DM concentration and recovery. In addition, LAB inoculation reduced lignin concentration ($P < 0.01$) but did not affect concentrations of NDF ($P = 0.14$), CP ($P = 0.91$), and ADIN ($P = 0.32$) or IVDMD-48 h ($P = 0.24$).

Lactate concentration was increased ($P < 0.01$), whereas acetate, butyrate, and NH₃-N concentrations were reduced ($P < 0.01$) with LAB inoculation. No effects were observed on ethanol ($P = 0.12$) or propionate ($P = 0.18$) concentrations. Furthermore, LAB inoculation reduced ($P < 0.01$) the counts of mold and clostridia and increased ($P < 0.01$) yeast counts but did not affect aerobic stability ($P = 0.27$).

High heterogeneity (I² statistic > 50%) was observed for all silage quality response variables, except for ADIN (I² = 41.6%). However, except for the response variables associated with lignin, NH₃-N, and acetate concentrations ($P < 0.01$), which were primarily attributable to the presence of outliers, no publication bias

was evident ($P > 0.05$) for the response variables, as is evident from funnel plots asymmetry test (Table 2).

Based on the results from the meta-regression analysis, no interactions were observed ($P > 0.10$) between the covariates (forage type, LAB species, application rate, and the silo type; data not shown). Forage type was the most consistent factor influencing the silage quality response because it accounted for some of the variability in pH, DM concentration and recovery, concentrations of NDF, ethanol, acetate, and LAB and mold counts ($P < 0.05$). Inoculation of corn and sorghum silages had no effects on silage pH ($P = 0.39$), DM concentration ($P = 0.87$), and DM recovery ($P = 0.34$); however, LAB inoculation of temperate and tropical grasses and alfalfa silages reduced silage pH and increased ($P < 0.05$) DM concentration and DM recovery (Figure 3). Inoculation of sugarcane silage reduced ($P < 0.05$) silage pH and DM recovery (Figure 3). Acetate concentrations were reduced by LAB inoculation in all forages except legume silages ($P < 0.05$). Mold counts were reduced by LAB inoculation ($P < 0.05$) in grass and alfalfa silages ($P = 0.55$) but not in corn and sorghum silages (Figure 3).

Species of LAB affected ($P < 0.01$) the RMD of $\text{NH}_3\text{-N}$, acetate, and LAB and mold counts. Inoculation with all LAB species reduced ($P < 0.05$) $\text{NH}_3\text{-N}$ concentrations, except for *P. pentosaceus*, which did not affect $\text{NH}_3\text{-N}$ ($P = 0.63$; Figure 4). Inoculation with *L. plantarum* reduced ($P < 0.01$) acetate (RMD = -0.26% DM) to a greater extent than inoculation with mixed LAB species (RMD = -0.09% DM). In addition, mold counts were reduced by *L. plantarum* inoculation ($P = 0.01$), but the mixed LAB species only tended ($P = 0.07$) to have the same effect. No effects were observed on acetate concentration when silage was inoculated with *E. faecium* or *P. pentosaceus* ($P > 0.05$), which was infrequent (<10 of the selected studies; Figure 4).

Silage pH was reduced by inoculation rates of $\leq 10^4$, 10^5 , or 10^6 cfu/g as fed ($P < 0.05$; Figure 4). However, no effect on silage pH was evident at the $\geq 10^7$ cfu/g rate ($P = 0.23$). This outcome was probably because the fewer comparisons ($n = 3$) available at this high rate limited the ability to detect significant differences. Dry matter recovery was increased ($P < 0.01$) by inoculation rates of 10^5 or 10^6 cfu/g, but it was reduced by the $\leq 10^4$ cfu/g rate ($P < 0.01$). Inoculation rates of 10^5 or 10^6 cfu/g reduced ($P < 0.01$) $\text{NH}_3\text{-N}$ and acetate concentration; however, inoculation at $\leq 10^4$ cfu/g increased ($P < 0.01$) $\text{NH}_3\text{-N}$ concentration and reduced ($P < 0.01$) acetate concentration to a lower extent ($P < 0.05$) than did the 10^5 or 10^6 cfu/g rates. Effects of the 10^5 and 10^6 cfu/g rates on pH, DM recovery, and $\text{NH}_3\text{-N}$ concentration did not differ, but the 10^6 cfu/g rate was

more effective at reducing acetate concentration than the 10^5 cfu/g rate.

Low variability was observed for LAB application rate, LAB species, and silo or type scale (Table 2). Silo type affected ($P < 0.05$) the RMD of DM, WSC, and $\text{NH}_3\text{-N}$ concentrations, and LAB and mold counts. Dry matter concentration was increased ($P < 0.05$), while $\text{NH}_3\text{-N}$ concentration, WSC concentration, and mold counts were reduced ($P < 0.05$) when LAB was inoculated in laboratory-scale silos. In contrast, with farm-scale silos, no effects were observed on DM concentration ($P = 0.49$), $\text{NH}_3\text{-N}$ concentration was reduced ($P < 0.01$), and WSC concentration was increased ($P = 0.03$; Figure 5).

Most of the covariates explained less than 50% of the heterogeneity (adjusted R^2) for almost all silage quality response variables (Table 2), indicating that unknown factors not identified in this meta-analysis could have affected the LAB inoculation response. Furthermore, it should be noted that the variability of certain covariates (LAB species, LAB application rate, and silo type) was low. However, for mold counts, the covariates explained 77% of the variability, indicating that forage type, LAB species, LAB application rate, and silo type together collectively explained most variation in the mold count response to LAB inoculation. This outcome may have been because the LAB type and inoculation rate determine the concentration of antifungal organic acids that inhibit mold growth, whereas forage and silo type may determine the epiphytic mold population (Kung, 1998).

Performance of Dairy Cows

Among the 31 peer-reviewed studies selected to investigate the effects of silage inoculation with LAB on performance of dairy cows, corn/sorghum, temperate grasses, alfalfa, and grass and legume mixtures accounted for 20.9, 44.2, 27.9, and 7.0% of the silages, respectively. *Lactobacillus plantarum* was used in 46.5% of studies, and combinations of various LAB species were used in the others (57.5%); no studies used *P. pentosaceus*, *E. faecium*, or *L. rhamnosus* as the sole inoculant. The LAB application rate of 10^5 cfu/g was used in 41.9% of the studies, while the 10^6 cfu/g rate was used in 27.9% of the studies. No information on application rate was provided in 27.9% of the studies, and one study used the $\leq 10^4$ cfu/g rate. Total mixed rations were used in 44.2% of the studies, while rest of the studies fed non-TMR (forage and concentrate separately offered) diets to the dairy cows.

Silage LAB inoculation increased milk yield (RMD = 0.37 kg/d; $P < 0.01$; Figure 6) and tended to increase

Table 2. Meta-regression of the effect of forage type, homofermentative and facultative heterofermentative lactic acid bacteria (LAB) species, LAB application rate, and silo type on raw mean differences (RMD) between inoculated and uninoculated treatments for silage quality parameters (% of DM unless otherwise noted)

Dependent variable ¹ (Y, RMD)	Meta-regression parameters (<i>P</i> -value) ²						Adjusted R ² (%) ³	N ⁴	Funnel test ⁵ (<i>P</i> -value)
	Intercept	Forage	LAB species	LAB rate	Silo type				
pH	0.09 (0.05)	-0.03 (<0.01)	-0.01 (0.56)	-0.02 (0.02)	-0.03 (0.36)		0.0	236	0.65
DM recovery (%)	-3.40 (0.04)	0.01 (0.03)	0.20 (0.18)	0.85 (<0.01)	—		32.2	51	0.29
DM (%)	0.54 (0.26)	0.10 (0.04)	0.07 (0.11)	-0.01 (0.94)	-0.74 (0.03)		1.9	209	0.45
NDF	-0.46 (0.47)	-0.11 (0.02)	-0.14 (0.09)	0.22 (0.46)	0.17 (0.65)		54.2	125	0.71
Lignin	-0.69 (<0.01)	0.02 (0.60)	0.01 (0.98)	0.11 (0.24)	—		0.0	25	<0.01
CP	0.02 (0.96)	0.03 (0.21)	0.02 (0.48)	0.01 (0.87)	-0.19 (0.48)		0.0	120	0.85
NH ₃ -N (% of N)	-1.24 (<0.01)	-0.01 (0.31)	-0.08 (<0.01)	0.12 (<0.01)	-0.27 (0.03)		35.1	134	<0.01
WSC	-0.25 (0.20)	0.02 (0.33)	0.03 (0.12)	-0.07 (0.11)	0.27 (0.04)		0.0	157	0.75
IVDMD-48 h (%)	0.17 (0.89)	-0.02 (0.83)	0.16 (0.11)	-0.21 (0.33)	0.45 (0.50)		18.9	36	0.45
Ethanol	0.18 (0.17)	-0.03 (0.05)	0.01 (0.98)	-0.04 (0.19)	0.03 (0.78)		0.0	98	0.15
Lactate	0.19 (0.60)	-0.01 (0.75)	0.06 (0.11)	0.07 (0.32)	0.35 (0.24)		6.8	258	0.88
Acetate	-0.44 (<0.01)	-0.01 (0.05)	0.02 (<0.01)	0.04 (<0.01)	0.07 (0.28)		40.3	225	0.02
Propionate	-0.015 (0.68)	-0.01 (0.78)	-0.01 (0.62)	0.01 (0.80)	0.01 (0.64)		0.0	83	0.10
Butyrate	0.15 (0.03)	-0.01 (0.69)	0.03 (0.38)	0.03 (0.38)	0.02 (0.68)		0.0	58	0.63
LAB (log cfu/g as fed)	1.04 (<0.01)	-0.21 (<0.01)	0.08 (0.02)	0.09 (0.11)	-0.76 (<0.01)		62.9	83	0.06
Yeast (log cfu/g as fed)	1.25 (0.22)	-0.05 (0.33)	-0.03 (0.68)	-0.15 (0.21)	-0.23 (0.61)		0.0	86	0.91
Mold (log cfu/g as fed)	1.63 (0.01)	-0.49 (<0.01)	-0.30 (<0.01)	-0.26 (0.49)	0.59 (0.04)		76.7	32	0.66
Aerobic stability (h)	3.2 (0.75)	-0.36 (0.62)	0.36 (0.70)	-1.32 (0.55)	—		0.0	40	0.35

¹WCS = water-soluble carbohydrate; IVDMD = in vitro DM digestibility.

²Forage = corn/sorghum, temperate and tropical grasses, sugarcane, alfalfa, other legumes, or other forages; LAB species = *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Enterococcus faecium*, *L. rhamnosus*, or mixture of LAB species; LAB application rate = $\leq 10^4$, 10^5 , 10^6 , or $\geq 10^7$ cfu/g as fed; Silo type = laboratory or farm-scale.

³Adjusted R² = proportion of the between-study variance (heterogeneity) explained by the forage, LAB, rate, and silo covariates.

⁴N = number of comparisons between inoculated and uninoculated treatments (complete data set is available in Supplemental File S1; <https://doi.org/10.3168/jds.2016-11815>).

⁵Egger's regression asymmetry test.

Figure 3. Subgroup analysis (subgroup = forage type) of the effects of silage inoculation with homofermentative and facultative heterofermentative lactic acid bacteria (LAB) on silage quality. RMD = raw mean differences between inoculated and uninoculated treatments.

Figure 4. Subgroup analysis [subgroup = homofermentative and facultative heterofermentative lactic acid bacteria (LAB) species and application rate] of the effects of silage inoculation with LAB on silage quality. RMD = raw mean differences between inoculated and uninoculated treatments. *E. faecium* = *Enterococcus faecium*; *P. pentosaceus* = *Pediococcus pentosaceus*; *L. plantarum* = *Lactobacillus plantarum*.

Figure 5. Subgroup analysis (subgroup = silo type) of the effects of silage inoculation with homofermentative and facultative heterofermentative lactic acid bacteria (LAB) on silage quality. RMD = raw mean differences between inoculated and uninoculated treatments.

DMI (RMD = 0.26 kg/d; $P = 0.08$; Supplemental Figure S1, <https://doi.org/10.3168/jds.2016-11815>), but it did not affect feed efficiency ($P = 0.18$; Supplemental Figure S2) and total-tract DM digestibility ($P = 0.31$) (Table 3). Inoculation tended to increase milk fat (RMD = 0.04%; $P = 0.08$; Supplemental Figure S3) and milk protein concentration (RMD = 0.02%; $P = 0.06$; Supplemental Figure S4; Table 3). For all performance variables, LAB inoculation effects were independent ($P > 0.10$) of the forage type, LAB species, diet type, level of milk yield of the control cows (19.5 ± 2.7 vs. 30.0 ± 6.1 kg/d), or LAB application rate (10^5 vs. 10^6 cfu/g as

fed; Table 4). No interactions between covariates were detected ($P > 0.10$; data not shown). The covariates collectively explained less than 45% of the heterogeneity in the performance variables (Table 4).

Heterogeneity was low for milk yield (I^2 statistic = 28.3%; Figure 6) and feed efficiency ($I^2 = 0\%$), moderate for milk protein concentration ($I^2 = 42.3\%$), and high for DMI ($I^2 = 71.5\%$) and milk fat concentration ($I^2 = 69.2\%$; Table 3). However, funnel plot asymmetry was not observed ($P > 0.10$) for any performance variable, indicating that publication bias was not evident (Figure 7).

Table 3. Effect of silage inoculation with homofermentative and facultative heterofermentative lactic acid bacterial inoculants on the performance of dairy cows

Item	Control ¹ mean (SD)	N ²	RMD ³ (95% CI)		Heterogeneity ⁴	
			Random effect	P-value	P-value	I ² (%)
DMI (kg/d)	17.80 (3.72)	32	0.26 (−0.03, 0.54)	0.08	<0.01	71.5
DM digestibility (%)	69.9 (4.42)	6	−0.42 (−1.22, 0.39)	0.31	0.47	0.0
Milk yield (kg/d)	25.04 (7.13)	38	0.37 (0.09, 0.65)	<0.01	0.06	28.3
Feed efficiency	1.49 (0.26)	10	0.03 (−0.01, 0.06)	0.18	0.68	0.0
Milk fat (%)	3.77 (0.43)	40	0.04 (0.00, 0.08)	0.08	<0.01	69.2
Milk protein (%)	3.06 (0.23)	37	0.02 (0.00, 0.03)	0.06	<0.01	42.3

¹Uninoculated treatment.²N = number of comparisons between inoculated and uninoculated treatments (complete data set is available in Supplemental File S2; <https://doi.org/10.3168/jds.2016-11815>).³RMD = raw mean difference between inoculated and uninoculated treatment means.⁴P-value to χ^2 (Q) test of heterogeneity; I² = proportion of total variation of size effect estimates that is due to heterogeneity. **Figure 6.** Forest plot showing the effects of silage inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on milk yield (kg/d) of dairy cows. The x-axis shows the raw mean difference (RMD); diamonds to the left of the solid line represent a reduction in the measure, whereas diamonds to the right of the line indicate an increase. Each diamond represents the mean size effect for that study, and the size of the diamond reflects the relative weighting of the study to the overall size effect estimate, with larger diamonds representing greater weight. The lines connected to the diamond represents the upper and lower 95% confidence interval for the size effect. The dotted vertical line represents the overall size effect estimate. The diamond at the bottom represents the mean response across the studies, and the solid vertical line represents a mean difference of zero or no effect.

Table 4. Intercepts and slopes of the meta-regression of effects of forage type, homofermentative and facultative heterofermentative lactic acid bacteria (LAB) species, diet type, and milk yield level on raw mean differences (RMD) between inoculated and uninoculated treatments for dairy cow performance parameters

Dependent variable (Y, RMD)	Meta-regression parameters (<i>P</i> -value) ¹						Adjusted R ² (%) ²	N ³
	Intercept	Forage type	LAB species	Diet type	Milk group	LAB rate		
DMI (kg/d)	0.67 (0.64)	0.01 (0.96)	-0.02 (0.85)	0.14 (0.79)	-0.11 (0.81)	-0.12 (0.64)	0.0	32
DM digestibility (%)	7.04 (0.24)	0.61 (0.78)	-0.31 (0.19)	2.34 (0.55)	—	2.63 (0.47)	0.0	6
Milk yield (kg/d)	-1.44 (0.41)	0.09 (0.36)	0.04 (0.59)	-0.50 (0.36)	0.65 (0.17)	0.28 (0.29)	5.0	38
Feed efficiency	0.47 (0.46)	0.03 (0.11)	-0.01 (0.60)	-0.14 (0.12)	-0.13 (0.56)	-0.02 (0.79)	0.0	10
Milk fat (%)	0.33 (0.02)	-0.02 (0.19)	-0.01 (0.96)	0.04 (0.94)	-0.04 (0.45)	-0.04 (0.11)	44.8	40
Milk protein (%)	0.08 (0.22)	0.01 (0.37)	-0.01 (0.43)	-0.02 (0.29)	-0.01 (0.47)	-0.01 (0.69)	0.0	37

¹Forage = corn, temperate grass, alfalfa, or grass and legume mixture; LAB species = *Lactobacillus plantarum* or mixture of LAB species; diet type = TMR or non-TMR; milk group = milk yield control group <22.8 or ≥22.8 kg/d, median = 22.8 kg/d; LAB application rate = 10⁵ or 10⁶ cfu/g as fed.

²Adjusted R² = proportion of the between-study variance (heterogeneity) explained by the covariates forage, LAB, diet type, milk group, and LAB application rate.

³N = number of comparisons between inoculated and uninoculated treatments (complete data set is available in Supplemental File S2; <https://doi.org/10.3168/jds.2016-11815>).

DISCUSSION

Silage Quality

Our meta-analysis provides strong evidence that in temperate and tropical grasses and in alfalfa and other legumes, but not in corn, sorghum, and sugarcane, LAB inoculation with 10⁵ or 10⁶ cfu/g as fed increased silage fermentation by increasing lactate accumulation and reducing pH and growth of deleterious epiphytic microbes such as clostridia and molds. Homofermentative or facultative heterofermentative LAB inoculation improved silage DM recovery in temperate and tropical grasses but not in corn and sorghum, probably by reducing detrimental biological processes such as clostridial fermentation, proteolysis, and amino acid deamination and decarboxylation (Muck, 1988). The increased yeast counts observed in response to LAB inoculation could be due to increased substrate availability (greater lactate accumulation) for their growth and reduced antifungal components in silage (reduced acetate concentrations). Though greater yeast counts often increase aerobic deterioration of silage (Weinberg et al., 1993), the increase in yeast population by LAB inoculation was insufficient to affect aerobic stability.

The effects of LAB inoculation on silage quality showed considerable heterogeneity, which represents any kind of variability between studies in a systematic review (Higgins and Green, 2011). Silage fermentation is a dynamic and complex multistage biological process. The extent and rate of fermentation during ensiling is determined by various factors including anaerobic conditions in the silo, WSC concentration, epiphytic bacteria, DM concentration, and buffering capacity of the pre-ensiled mass (Muck, 1988). We used meta-regression to identify potential sources of heterogeneity followed by subgroup analysis to examine their influences on the response (Higgins et al., 2003). Forage type was the most consistent factor affecting the silage quality response to the LAB inoculation. Low variability among the LAB species, LAB application rate and silo type may have limited our ability to examine the effects of these factors.

The lack of effects of LAB inoculation on the fermentation and DM recovery of corn and sorghum silages is notable because these forages are perhaps the most widely ensiled for dairy production in the United States. The lack of response to inoculation is probably because these forages contained sufficient WSC concentrations for the fermentation as well as high epiphytic bacterial populations and low buffering capacities (Weinberg and Muck, 1996; Carvalho et al., 2014). The efficacy of silage inoculant bacteria is based on their ability to compete effectively with the epiphytic flora in forages.

Figure 7. Funnel plots showing the effects of silage inoculation homofermentative and facultative heterofermentative lactic acid bacteria on the performance of dairy cows. The horizontal line indicates the raw mean difference (RMD) estimate and the vertical line indicates their corresponding standard errors (SE). *P*-value refers to the test for funnel plot asymmetry by Egger's regression method between RMD and SE. Funnel plot asymmetry is indicative of publication bias.

A 2-fold increase in numbers of inoculated bacteria over epiphytic flora is required for the inoculant to improve the fermentation (Pahlow, 1991). Unlike in corn and sorghum silages, LAB inoculation improved the fermentation of legume and grass silages probably because of

their low epiphytic flora, low WSC concentrations, and high buffering capacities (particularly for the legumes) (Arriola et al., 2015; Ogunade et al., 2016; Silva et al., 2016). The causes of the reduction in DM recovery by LAB inoculation of sugarcane silages are not clear, but

they are probably associated with excessive fermentation due to the high WSC concentration of sugarcane.

Most (92.7%) of the studies that examined LAB inoculation effects on silage quality involved mini-silos rather than farm-scale silos. Typical differences between both silo types can influence the fermentation and aerobic stability of silage because of differences in the extent to which oxygen is excluded, the degree of compaction and heat transfer, and the unloading rates (Weinberg and Muck, 1996). The trends for farm-scale silos to have greater WSC concentration and fewer LAB may be because the harvested forage for the former is left in the field for longer periods than that for mini-silos. The longer drying durations may have decreased epiphytic bacterial viability, increased moisture loss, and decreased plant respiration, and collectively, these factors likely increased the residual WSC in forage (Weinberg et al., 2010).

The greater variability in concentrations of $\text{NH}_3\text{-N}$, DM, WSC, and counts of yeasts and molds in the farm-scale silos is probably because prevailing conditions during the fermentation are more variable when silage is made on farms than in laboratories. This variability is due to climatic and management factors that can influence the fermentation but are more difficult to control on the farm than in the laboratory such as DM at harvest, porosity, humidity, wind speed, temperature, packing density, sealing time, and so forth. As reported earlier (Xiccato et al., 1994; Weinberg and Muck, 1996) and based on results from the current study, the effects of bacterial inoculation on silage quality depend on the silo type; hence, caution is needed when extrapolating responses obtained with mini-silos to those from farm-scale silos. Differences between other silage quality measures in farm-scale and mini-silo studies may have been detected if more studies on the former were available. Therefore, more studies are needed on effects of bacterial inoculation on silage preserved in farm silos as well as on how silo type affects the silage quality response to LAB inoculation.

Lactobacillus plantarum has been reported to be the most commonly used silage inoculant (Kung, 2001; Muck, 2010), and this fact was confirmed by our meta-analysis. However, some LAB species also have been selected as silage inoculants because of their faster growth at high pH values (>5) than *L. plantarum* (Kung, 2001). Consequently, some inoculant preparations contain synergistic mixtures of bacteria that target different phases of the fermentation. For instance, combination inoculants may contain *E. faecium*, which unlike *L. plantarum*, grows rapidly at high pH (>5) and thus rapidly dominates epiphytic flora at the initial stages of the fermentation, as well as *L. plantarum*, which rapidly acidifies the forage at later stages once

the pH drops below 5 because it grows best at low pH conditions that inhibit the growth of *E. faecium* (Kung et al., 2011). The rapid growth of *E. faecium* at high pH (Kung et al., 2011) probably explains its greater lactate concentration relative to those of the other individual bacteria. This higher acidification potential may have rapidly inhibited plant and microbial proteolytic activity (Muck, 1988), thereby resulting in a lower $\text{NH}_3\text{-N}$ concentration relative to those of the other individual bacteria.

Synergistic effects among bacteria applied together may explain why inoculation with combinations of bacteria resulted in lower acetate concentration and greater LAB counts than those inoculated with single bacteria. Despite reducing acetate concentration by a lesser amount, combination inoculants may have been more effective at reducing mold counts than *L. plantarum* because of production of other antifungal compounds.

Pediococcus pentosaceus inoculation did not affect $\text{NH}_3\text{-N}$ concentration in silage for unknown reasons, which may be related to its slower growth rate than the other bacteria (Kung, 2011). Only 5.7% of the examined studies used *P. pentosaceus*, *E. faecium*, or *L. rhamnosus* as individual silage inoculants, and this infrequent use may have limited our ability to detect LAB-species related effects on measures of silage quality. Consequently, more research is needed on effects of these and other infrequently used LAB on silage fermentation.

Our meta-analysis shows that an inoculation rate of at least 10^5 cfu/g as fed is required to effectively improve silage quality because the $\leq 10^4$ cfu/g rate only reduced pH and acetate concentration (to a lesser extent than the 10^5 or 10^6 cfu/g rates) but did not increase DM recovery or reduce $\text{NH}_3\text{-N}$ concentration. The recommended application rate for silage inoculants varies by region, with 10^5 cfu/g being common in the United States, 10^6 cfu/g being common in Europe, and $\leq 10^4$ cfu/g being common in some Asian and South American countries. This study demonstrates that the most effective rates for improving the fermentation are 10^5 and 10^6 cfu/g, and the latter rate is more effective at reducing acetate concentration. More research is needed on the efficacy of the $\geq 10^7$ cfu/g rate on silage quality parameters due to the paucity of data on this rate in the literature, which may be because it is not an economically viable rate in most cases.

Reductions in butyrate and $\text{NH}_3\text{-N}$ accumulation in response to LAB inoculation of all silages were the most consistent and among the most important benefits observed in the present study. Fermentation of sugars and lactate to butyrate is associated with high DM and energy losses during ensiling and low DM intake in ruminants (Muck, 1988), whereas $\text{NH}_3\text{-N}$ is indica-

tive of proteolytic activity and amino acid deamination and decarboxylation (Ohshima and McDonald, 1978; Scherer et al., 2015), which typically reduce the nutritive value of silages (Buchanan-Smith and Phillip, 1986; Phuntsok et al., 1998; Broderick, 1995). The decreases in butyrate and $\text{NH}_3\text{-N}$ concentrations in the present study are probably because LAB inoculation reduced the growth of clostridia (Muck, 1988).

Performance of Dairy Cows

Silage inoculation with LAB at rates of 10^5 or 10^6 cfu/g as fed was associated with increased milk yield. The response had low heterogeneity, and it was independent of forage type, LAB species (*L. plantarum* or mixture of LAB), diet type, level of milk yield of control cows, and LAB application rate (10^5 vs. 10^6 cfu/g as fed). However, inoculation did not affect feed efficiency probably because it increased DMI without affecting diet digestibility. The LAB species may have not affected milk yield because it had few and mostly small effects on silage quality and no effects on in vitro or total-tract in vivo DM digestibility and DM intake.

We observed variation by region in the LAB application rate used in studies. In studies from Europe, 10^6 cfu/g was more common, while in those from the Americas, 10^5 cfu/g was more common. However, we did not find any evidence of differences in performance of dairy cows due to applying 10^5 cfu/g instead of 10^6 cfu/g of LAB.

The improved milk yield in response to LAB inoculation at an application rate of 10^5 or 10^6 cfu/g as fed is probably attributable to increased DMI, which may have been mediated by reduced accumulation of hypophagic compounds such as butyrate, ammonia, and biogenic amines in inoculated silages. Hypophagic effects of butyrate are mediated by increased ruminal osmolality, leading to stimulation of osmoreceptors and reducing meal size (Allen, 2000). Increased NH_3 concentration in silages might affect palatability, resulting in reduced DMI (Kertz et al., 1982). Furthermore, it might lead to energetic imbalance in the brain tissue as a result of the increased glutamate use for ammonia clearance, the inhibition of decarboxylation of α -ketoglutarate in the tricarboxylic acid cycle, or both (Wilson et al., 1975; Kertz et al., 1982; Hertz and Kala, 2007). Biogenic amines are products of amino acid decarboxylation, and they have been negatively associated with intake by reducing ruminal motility (Phuntsok et al., 1998; Scherer et al., 2015). Furthermore, the reduced lignin concentration observed in response to LAB inoculation could have potentially contributed to the increased DMI (Oliveira et al., 2011).

The improvement in silage protein preservation (i.e., reduced $\text{NH}_3\text{-N}$) with LAB inoculation could have increased ruminal microbial protein synthesis independently of DMI and thus contributed to the increase in milk yield. Basso et al. (2014) reported that inoculation with *L. plantarum* alone or in combination with other LAB increased the performance of lambs by increasing ruminal microbial protein synthesis. However, the effects of silage LAB inoculation on ruminal protein synthesis are inconclusive. Although LAB inoculation increased in vitro ruminal microbial biomass production in some studies (Contreras-Govea et al., 2011, 2013), no effects were observed in others (Jalc et al., 2009a,b). No study that examined the influence of silage inoculation on milk yield in this meta-analysis also examined effects on ruminal microbial protein synthesis. Therefore, future studies should examine if the milk yield response to inoculation is mediated by increased ruminal microbial protein synthesis.

Silage inoculation with LAB tended to increase milk fat concentration independent of forage, LAB species, diet type, and the level of milk yield of the control cows, but the underlying mechanism is unclear. Previous studies have indicated that LAB can biohydrogenate and degrade linoleic and linolenic fatty acids during ensiling (Ogawa et al., 2005; Ding et al., 2013). We speculate that by reducing the concentration of unsaturated fatty acids, inoculation may have altered ruminal biohydrogenation pathways, thereby reducing the abundance of biohydrogenation intermediates that could potentially inhibit mammary lipogenesis such as C18:2 *trans*-10,*cis*-12 and subsequently milk fat (Harvatine et al., 2009). Clearly, the underlying mechanism by which LAB inoculation of silage increases the milk fat response needs further investigation.

Silage inoculation with LAB tended to increase milk protein concentration independently of forage type, LAB species, diet type, and the level of milk yield of the control cows; however, the response was modest (RMD = 0.02%). Although the mechanism by which LAB silage inoculation increases milk protein concentration is not clear and needs further investigation, we hypothesize that the inoculation increases the availability of metabolizable protein and energy for milk protein synthesis by reducing proteolysis and amino acid deamination and decarboxylation during ensiling and by increasing DMI.

CONCLUSIONS

Silage inoculation with homofermentative or facultative heterofermentative LAB (application rate $\geq 10^5$ cfu/g as fed) markedly enhanced silage fermentation

and increased DM recovery in temperate and tropical grasses and in alfalfa and other legumes, independently of LAB species. However, inoculation did not improve the fermentation of corn, sorghum, and sugarcane silages. Inoculation also increased yeast counts across forages but did not affect aerobic stability. In addition, inoculation with a rate of at least 10^5 cfu/g of LAB improved dairy cattle performance by increasing milk yield, irrespective of the type of ensiled forage, LAB species, diet type, and level of milk yield of cows in the control treatment. Inoculation also had a similar tendency on milk protein and fat concentrations. However, no effects of inoculation were detected on total-tract DM digestibility or feed efficiency. Future studies should examine how homofermentative or facultative heterofermentative LAB inoculants increase the performance of dairy cows.

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REFERENCES

- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624.
- Arriola, K. G., O. C. M. Queiroz, J. J. Romero, D. Casper, E. Muniz, J. Hamie, and A. T. Adesogan. 2015. Effect of microbial inoculants on the quality and aerobic stability of bermudagrass round-bale haylage. *J. Dairy Sci.* 98:478–485.
- Basso, F. C., A. T. Adesogan, E. C. Lara, C. H. S. Rabelo, T. T. Berchielli, I. A. M. A. Teixeira, G. R. Siqueira, and R. A. Reis. 2014. Effects of feeding corn silage inoculated with microbial additives on the ruminal fermentation, microbial protein yield and growth performance of lambs. *J. Anim. Sci.* 92:5640–5650.
- Broderick, G. A. 1995. Desirable characteristics of forage legume for improving protein utilization in ruminants. *J. Anim. Sci.* 73:2760–2773.
- Buchanan-Smith, J. G., and L. E. Phillip. 1986. Food intake in sheep following intraruminal infusion of extracts from lucerne silage with particular reference to organic acids and products of protein degradation. *J. Agric. Sci.* 106:611–617.
- Carvalho, B. F., C. L. S. Avila, J. C. Pinto, J. Neri, and R. F. Schwan. 2014. Microbiological and chemical profile of sugar cane silage fermentation inoculated with wild strains of lactic acid bacteria. *Anim. Feed Sci. Technol.* 195:1–13.
- Contreras-Govea, F., R. Muck, D. Mertens, and P. Weimer. 2011. Microbial inoculant effects on silage and in vitro ruminal fermentation, and microbial biomass estimation for alfalfa, BMR corn, and corn silages. *Anim. Feed Sci. Technol.* 163:2–10.
- Contreras-Govea, F. E., R. E. Muck, G. A. Broderick, and P. J. Weimer. 2013. *Lactobacillus plantarum* effects on silage fermentation and in vitro microbial yield. *Anim. Feed Sci. Technol.* 179:61–68.
- Cook, R. D. 1977. Detection of influential observation in linear regression. *Technometrics* 19:15–18.
- Danner, H., M. Holzer, E. Mayrhuber, and R. Braun. 2003. Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microbiol.* 69:562–567.
- DerSimonian, R., and N. Laird. 1986. Meta-analysis in clinical trials. *Control. Clin. Trials* 7:177–188.
- Ding, W. R., R. L. Long, X. L. Long, and X. S. Guo. 2013. Effects of plant enzyme inactivation or sterilization on lipolysis and proteolysis in alfalfa silage. *J. Dairy Sci.* 96:2536–2543.
- Egger, M., G. D. Smith, M. Schneider, and C. Minder. 1997. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315:629–634.
- Filya, I., G. Ashbell, Y. Hen, and Z. G. Weinberg. 2000. The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. *Anim. Feed Sci. Technol.* 88:39–46.
- Glass, G. V. 1976. Primary, secondary, and meta-analysis of research. *Educational Researcher* 5:3–8.
- Harvatine, K. J., Y. R. Boisclair, and D. E. Bauman. 2009. Recent advances in the regulation of milk fat synthesis. *Animal* 3:40–54.
- Hertz, L., and G. Kala. 2007. Energy metabolism in brain cells: Effects of elevated ammonia concentrations. *Metab. Brain Dis.* 22:199–218.
- Higgins, J. P. T. 2008. Heterogeneity in meta-analysis should be expected and appropriately quantified. *Int. J. Epidemiol.* 37:1158–1160.
- Higgins, J. P. T., and S. Green, ed. 2011. *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 [updated March 2011]. <http://www.cochrane-handbook.org>.
- Higgins, J. P. T., S. G. Thompson, J. J. Deeks, and D. G. Altman. 2003. Measuring inconsistency in meta-analysis. *BMJ* 327:557–560.
- Jalc, D., A. Laukova, Z. Varadyova, P. Homolka, and V. Koukolova. 2009a. Effect of inoculated grass silages on rumen fermentation and lipid metabolism in an artificial rumen (RUSITEC). *Anim. Feed Sci. Technol.* 151:55–64.
- Jalc, D., Z. Varadyova, A. Laukova, P. Homolka, and F. Jancik. 2009b. Effect of inoculated corn silage on rumen fermentation and lipid metabolism in an artificial rumen (RUSITEC). *Anim. Feed Sci. Technol.* 152:256–266.
- Kertz, A. F., M. K. Koepke, L. E. Davidson, N. L. Betz, J. R. Norris, L. V. Skoch, B. R. Cords, and D. T. Hopkins. 1982. Factors influencing intake of high urea-containing rations by lactating dairy cows. *J. Dairy Sci.* 65:587–604.
- Kleinschmit, D. H., R. J. Schmidt, and L. Kung. 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* 88:2130–2139.
- Kung, L. 1998. A review on silage additives and enzymes. Pages 121–135 in *Proceedings 59th Minneapolis Nutrition Conference*, Minneapolis, MN. Department of Animal Science, University of Minnesota, St. Paul.
- Kung, L., Jr. 2011. Silage fermentation & additives. Accessed Oct. 25, 2016. [http://www1.foragebeef.ca/\\$Foragebeef/frgebeef.nsf/all/frg69/\\$FILE/silagefermentationadditives.pdf](http://www1.foragebeef.ca/$Foragebeef/frgebeef.nsf/all/frg69/$FILE/silagefermentationadditives.pdf).
- Kung, L., Jr., and R. E. Muck. 1997. Animal Response to Silage Additives. Pages 200–210 in *Proc. Silage: Field to Feedbunk North American Conference*. NRAES-99. Northeast Regional Agricultural Engineering Service, Ithaca, NY.
- Light, R. J., and D. B. Pillemer. 1984. *Summing Up: The Science of Reviewing Research*. Harvard University Press, Cambridge, MA.
- MacPherson, H. T., and P. Violante. 1966. Ornithine, putrescine and cadaverine in farm silage. *J. Sci. Food Agric.* 17:124–127.
- Muck, R. E. 1988. Factors influencing silage quality and their implications for management. *J. Dairy Sci.* 71:2992–3002.

- Muck, R. E. 2010. Silage microbiology and its control through additives. *Rev. Bras. Zootec.* 39:182–191.
- Muck, R. E., and L. Kung Jr. 1997. Effects of silage additives on ensiling. Pages 187–199 in *Proc. Silage: Field to Feedbunk North American Conference*. NRAES-99. Northeast Regional Agricultural Engineering Service, Ithaca, NY.
- Ogawa, J., S. Kishino, A. Ando, S. Sugimoto, K. Mihara, and S. Shimizu. 2005. Production of conjugated fatty acids by lactic acid bacteria. *J. Biosci. Bioeng.* 100:355–364.
- Ogunade, I. M., D. H. Kim, Y. Jiang, Z. G. Weinberg, K. C. Jeong, and A. T. Adesogan. 2016. Control of *Escherichia coli* O157:H7 in contaminated alfalfa silage: Effects of silage additives. *J. Dairy Sci.* 99:4427–4436.
- Ohshima, M., and P. McDonald. 1978. A review of the changes in nitrogenous compounds of herbage during ensilage. *J. Sci. Food Agric.* 29:491–505.
- Oliveira, A. S., E. Detmann, J. M. S. Campos, D. S. Pina, S. M. Souza, and M. G. Costa. 2011. Meta-analysis of the impact of neutral detergent fiber on intake, digestibility and performance of lactating dairy cows. *Rev. Bras. Zootec.* 40:1587–1595.
- Pahlow, G. 1991. Role of microflora in forage conservation. Page 26–36 in *Forage Conservation Towards 2000*. G. Pahlow and H. Honig, ed. Inst. Grassl. Forage Res., Braunschweig, Germany.
- Phuntsok, T., M. A. Froetschel, H. E. Amos, M. Zheng, and Y. W. Huang. 1998. Biogenic amines in silage, apparent postruminal passage, and the relationship between biogenic amines and digestive function and intake by steers. *J. Dairy Sci.* 81:2193–2203.
- Scherer, R., K. Gerlach, and K. H. Sudekun. 2015. Biogenic amines and gamma-amino butyric acid in silage: Formation, occurrence and influence on dry matter intake and ruminant production. *Anim. Feed Sci. Technol.* 210:1–16.
- Silva, V. P., O. G. Pereira, E. Leandro, T. Da Silva, K. Ribeiro, H. Mantovani, and S. Santos. 2016. Effects of lactic acid bacteria with bacteriocinogenic potential on the fermentation profile and chemical composition of alfalfa silage in tropical conditions. *J. Dairy Sci.* 99:1895–1902.
- Viechtbauer, W. 2010. Conducting meta-analysis in R with the metafor package. *J. Stat. Softw.* 36:1–48.
- Weinberg, Z. G., G. Ashbell, Y. Hen, and A. Azrieli. 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. *J. Appl. Bacteriol.* 75:512–518.
- Weinberg, Z. G., P. Khanal, C. Yildiz, Y. Chen, and A. Arieli. 2010. Effects of stage of maturity at harvest, wilting and LAB inoculant on aerobic stability of wheat silages. *Anim. Feed Sci. Technol.* 158:29–35.
- Weinberg, Z. G., and R. E. Muck. 1996. New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiol. Rev.* 19:53–68.
- Wilson, G., F. A. Martz, J. R. Campbell, and B. A. Becket. 1975. Evaluation of factors responsible for reduced voluntary intake of urea diets for ruminants. *J. Anim. Sci.* 41:1431–1437.
- Xiccato, G., M. Cinetto, A. Carazzolo, and M. E. Cossu. 1994. The effect of silo type and dry matter content on the maize silage fermentation process and ensiling loss. *Anim. Feed Sci. Technol.* 49:313–323.