Meta-analysis of effects of inoculation with *Lactobacillus buchneri*, with or without other bacteria, on silage fermentation, aerobic stability, and performance of dairy cows

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ABSTRACT

A meta-analysis of 158 peer-reviewed articles was conducted to examine effects of inoculation with *Lactobacillus buchneri* (LB)-based inoculants (LBB) that did or did not include homolactic or obligate heterolactic bacteria on silage fermentation and aerobic stability. A complementary meta-analysis of 12 articles examined LBB inoculation effects on dairy cow performance. Raw mean differences between inoculant and control treatment means weighted by inverse variance were compared with a hierarchical effects model that included robust variance estimation. Meta-regression and subgrouping analysis were used to identify effects of covariates including forage type, application rate ($\leq 10^4$, 10^5 , 10^6 , or $\geq 10^7$ cfu/g as fed), bacteria type (LB vs. LB plus other bacteria), enzyme inclusion, ensiling duration, and silo type (laboratory or farm scale). Inoculation with LBB increased acetate (62%), 1, 2 propanediol (364%) and propionate (30%) concentration and aerobic stability (73.8%) and reduced lactate concentration (7.2%), yeast counts (7-fold) and mold counts (3-fold). Feeding inoculated silage did not affect milk yield, dry matter intake, and feed efficiency in lactating dairy cows. However, forage type, inoculant composition, and dose effects on silage quality measures were evident. Inoculation with LBB increased aerobic stability of all silages except tropical grasses. Adding

obligate homolactic or facultative heterolactic bacteria to LB prevented the small increase in DM losses caused by LB alone. The 10^5 and 10^6 cfu/g rates were most effective at minimizing DM losses while aerobic stability was only increased with 10^5 , 10^6 , and $\geq 10^7$ cfu/g rates. Inoculation with LBB increased acetate concentration, reduced yeast counts and improved aerobic stability but did not improve dairy cow performance.

Key words: corn silage, heterolactic bacteria, *Lactobacillus buchneri*, *Lactobacillus hilgardii*

INTRODUCTION

Silage is an integral component of most dairy cow diets in the United States and several other countries throughout the world, and previous research has been focused on developing strategies to improve silage quality and minimize nutrient losses during ensiling (Wilkins, 2003; Wilkinson et al., 2003). Silage inoculants have been the most commonly used additive for improving silage quality (Kung, 1998). Our recent meta-analysis showed that inoculation with homolactic and facultative heterolactic lactic acid bacteria (LAB) improved the fermentation of grass and legume silages and the performance of dairy cows but did not affect the fermentation of whole-plant corn, whole-plant sorghum, and sugarcane silages or aerobic stability of any silage (Oliveira et al., 2017). This meta-analysis (Oliveira et al., 2017) was only focused on inoculants for improving silage fermentation; hence, it intentionally excluded obligate heterolactic LAB that are added to improve aerobic stability. Among such obligate bacteria, only a few have been evaluated for their effects on silage fermentation and aerobic stability and these are primarily from the *Lactobacillus buchneri* (LB) group of the *Lactobacillus* genus; they include primarily *L. buchneri* and much less commonly *Lactobacillus brevis*, *Lactobacillus diolivorans*, *Lactobacillus hilgardii*, *Lactobacillus kefir*,

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and *Lactobacillus parafarraginis* (Muck et al., 2018). *Lactobacillus buchneri* improved the aerobic stability of silages in various laboratory studies (Muck, 1996; Weinberg et al., 1999; Arriola et al., 2011a) and field studies (Mari et al., 2009; Kristensen et al., 2010) in a strain- (Tabacco et al., 2011) and dose- (Driehuis et al., 1999; Ranjit and Kung, 2000) dependent manner. This effect of LB was confirmed by the meta-analysis of Kleinschmit and Kung (2006) in which aerobic stability of untreated corn silage (25 h) was increased to 35 h when inoculated with LB at or below 1×10^5 cfu/g and to 503 h at more than 1×10^5 cfu/g. This aerobic stability improvement by LB has been attributed to its ability to convert lactate into acetate and 1,2 propanediol under anaerobic conditions (Oude Elferink et al., 2001), and the corresponding reduction in yeast and mold counts due to the antifungal attribute of acetate (Driehuis et al., 2001). Similarly to *L. diolivorans* (Krooneman et al., 2002) and *Lactobacillus reuteri* (Sriramulu et al., 2008), a novel strain of *L. buchneri* A KKP 2047p, was recently reported to convert 1,2 propanediol into propionate (Zielińska et al., 2017) in the presence of cobalamine, potentially conferring greater antifungal effects. However, it is uncertain if naturally-occurring LB strains have this property (Muck et al., 2018), which may enhance aerobic stability even further due to the combined antifungal effects of propionate and acetate.

Earlier studies suggested that forage inoculation with heterolactic bacteria such as LB may increase DM losses during ensiling (Ranjit and Kung, 2000), and this was confirmed by the 1 to 1.8% increase reported when LB was applied to corn, grass, and small grain forages at high ($>1 \times 10^5$ cfu/g) doses in the meta-analysis of Kleinschmit and Kung (2006), though no effect was detected at lower doses ($\leq 1 \times 10^5$ cfu/g). The small increases in DM losses can be readily accepted if accompanied by substantial increases in aerobic stability (Kleinschmit and Kung, 2006). Nevertheless, to avoid or reduce DM losses and to enhance fermentation simultaneously, several inoculants now contain a mixture of homolactic or facultative heterolactic bacteria with obligate heterolactic bacteria. Studies have demonstrated that combining homolactic bacteria with LB improved aerobic stability without affecting DM losses (Driehuis et al., 2001; Jatkauskas and Vrotniakienė, 2011; Arriola et al., 2015).

Based on studies indicating associations between ingestion of acetate and reductions in DMI, (Buchanan-Smith, 1990; Gherardi and Black, 1991), concerns have been expressed that inoculating forages with LB and the attendant increases in acetate concentration may reduce feed intake in dairy cows (Kleinschmit and Kung, 2006). However, this has not been consistently

supported in dairy cow studies. Several studies reported that LB did not affect intake (Taylor et al., 2002; Kung et al., 2003; Arriola et al., 2011b) or milk yield (Taylor et al., 2002; da Silva et al., 2017), whereas others reported an increase in milk yield (Kung et al., 2003; Ben-Meir et al., 2018).

The objective of the present study was to examine effects of inoculation with LB-based bacteria (**LBB**), including LB alone or LB with homolactic or obligate heterolactic LAB, on silage fermentation, aerobic stability, and animal performance. We hypothesized that LBB inoculants would improve silage quality, aerobic stability, and milk yield but no effects would be observed on DMI.

MATERIALS AND METHODS

Literature Search

A literature search was conducted to evaluate the effects of LB alone or with obligate heterolactic or homolactic LAB on silage fermentation, aerobic stability, and milk production. Peer-reviewed articles published from 1997 to 2020 were searched using the terms “silage” and “*Lactobacillus buchneri*,” using the Web of Science, Google Scholar, and Commonwealth Agricultural Bureaux International Abstracts databases (<https://apps.webofknowledge.com>, <https://scholar.google.com>, <https://www.cabi.org/publishing-products/cab-abstracts/>). In addition to these terms, “dairy cows” was included in the search for studies on effects of LB inoculants on dairy cow performance. Additional requests were made to individual authors of articles to identify data that might have been collected but not reported in the published paper.

Inclusion Criteria

Suitability for inclusion was determined initially by reading the abstract to ensure the experiment involved using LB with or without other bacteria to improve silage preservation. The materials and methods portion of the article was then read to exclude experiments that did not meet the inclusion criteria.

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, when the first article (Muck, 1996) on using LB for silage preservation was published, (3) have concurrently examined uninoculated and inoculated treatment groups, (4) have treatments of LB alone without or with other LAB, (5) have used at least 30 d of ensiling to ensure the silage was properly preserved, (6) have reported the inoculant application rate, (7) have used

temperature change to measure aerobic stability, which is the most common practice, and (8) have reported either standard error of the mean (**SEM**) or standard deviation (**SD**). The inclusion criteria for selecting studies that analyzed the effect of LB with or without other LAB on the performance of dairy cows were as follows: (1) was published in English-language peer-reviewed journals, (2) concurrently examined uninoculated and inoculated treatment groups, (3) included treatments comprising LB alone without or with other LAB, (4) use randomized design experiments with individual feeding of inoculated and inoculated silage-based diets to cows (5) reported either SEM or SD for the estimation of variance.

Data Extraction

Silage Quality. Figure 1 Shows a PRISMA diagram (Moher et al., 2009) depicting the data collection process for the meta-analysis. After initial screening, 295 full-text articles were assessed to determine their eligibility to be included in the meta-analysis and 137 articles were excluded for the following reasons: (1) no proper control used in the study (11 experiments); (2) no application rate of inoculants reported (15 experiments); (3) no SEM or SD reported (35 experiments); (4) LB was not one of the treatments (58 experiments); (5) chemical additives were used with LB (2 experiments); and (6) forages were ensiled for less than 30 d (16 experiments). Based on the inclusion criteria, 158 peer-reviewed papers (up to 542 comparisons) were selected to analyze LB inoculation effects on silage quality, and these were classified by first author, publication, and reference. Additional classifications included forage type [whole-plant corn, whole-plant sorghum, temperate grass, tropical grass, sugarcane, alfalfa, other legumes, grain, high moisture corn (**HMC**), and other forages]; LAB group {LB alone or with homolactic LAB [*Lactobacillus plantarum* (**LB+LP**), *Pediococcus pentosaceus* (**LB+PP**), *Lactobacillus plantarum* and *Pediococcus pentosaceus* (**LB+LP+PP**), *Lactobacillus plantarum* and *Enterococcus faecium* (**LB+LP+EF**)], LB plus other species such as *Lactococcus lactis*, *Lactobacillus casei*, *Pediococcus acidilactici* (**LB+others**), or obligate heterolactic [LAB *Lactobacillus hilgardii* (**LB+LH**)]]; LAB application rate ($\leq 10^4$, 10^5 , 10^6 or $\geq 10^7$ cfu/g fresh forage); silo type (laboratory or farm scale), and whether or not the inoculant contained enzymes.

The number of replicates, means, and SEM were extracted for the following response variables from both control and inoculant treatments: pH, DM recovery, concentrations of DM, NDF, CP, water-soluble carbohydrate (**WSC**), ethanol, 1,2 propanediol, lactate, ac-

etate, propionate, and butyrate, counts of LAB, yeast, and mold (log cfu/g fresh forage), and aerobic stability (h). The data set to evaluate silage quality measurements and corresponding references are presented in Supplemental Table S1 (<https://doi.org/10.7910/DVN/JQNWW6>) and Supplemental File S1 (<https://doi.org/10.7910/DVN/BVNRA1>), respectively.

Dairy Cow Performance. The database search for dairy cow performance in response to feeding LBB-inoculated silage retrieved 48 studies. A total of 32 studies were excluded because animals were not fed inoculated and uninoculated silage-based diets, 2 studies were excluded due to lack of a proper control treatment, 1 study was excluded because no SEM or SD was reported, and 1 study did not use LB as a treatment. Based on the inclusion criteria, 13 comparisons from 12 peer-reviewed studies were selected as shown in the PRISMA diagram for studies selected to examine the milk production response to inoculation (Figure 2). Studies were classified by first author, publication reference, forage type (whole-plant corn and other forages such as wheat, sugarcane, alfalfa, barley), LAB species (LB alone or with other LAB), LAB application rate (10^5 , 10^6 , and 10^7 cfu/g as fed), and level of milk yield of the control cows (a median milk yield of <31.7 kg/d or ≥ 31.7 kg/d). The number of replicates, means, and SEM were extracted from response variables for control and inoculated treatments: unadjusted milk yield, DMI, feed efficiency, milk fat and protein concentrations, and total-tract DM digestibility. The data set to evaluate the dairy cow performance response and corresponding references are presented in Supplemental Table S2 (<https://doi.org/10.7910/DVN/WYUORO>) and Supplemental File S1 (<https://doi.org/10.7910/DVN/BVNRA1>), respectively.

Statistical Analysis

Effect Size. The effects of inoculating LBB on silage quality and performance of dairy cows were evaluated using weighted raw mean differences (**WMD**) between uninoculated and inoculated silage means (estimated effect size). Weighting was performed by the inverse of the variance in a hierarchical effects model that included robust variance estimation, as proposed by Tipton (2015).

Heterogeneity. Variations among treatment level WMD were assessed using the I^2 statistic (Higgins et al., 2003), which measures the effect of heterogeneity on a meta-analysis [i.e., the proportion of true variance effects of the treatment (indicated by the τ^2 statistic) divided by the total variance observed in a treatment (Borenstein et al., 2017; Lean et al., 2018)]. The τ^2 statistic has also been described as the between-cluster

variance component, whereas the Ω^2 statistic represents the between-studies-within-cluster variance component (Hedges et al., 2010; Fisher et al., 2017). All 3 statistics are provided to allow readers to evaluate them.

Meta-Regression and Subgrouping. Meta-regression analysis was used to identify effects of the covariates (forage type, application rate, enzyme use, days of ensiling, LAB group, and silo type) on the LBB inoculation response for silage quality, using WMD as the dependent variable. The meta-regression analysis was performed using the robust variance estimation method with a hierarchical effects model (Tipton, 2015). In addition, subgrouping of the WMD was analyzed to evaluate the effects of the covariates on LBB inoculation response (WMD), as shown earlier (Oliveira et al., 2017).

Weighting. Each variance comparison between inoculant and control treatments was calculated as square of the pooled SD (Vesterinen et al., 2014). The SD for the inoculant and control for each comparison was calculated from SEM reported, where $SD = SEM \times \sqrt{n}$, where n = number of experimental units. When the standard deviation of the difference (**SED**) was reported in studies, SEM was calculated as: $SEM = SED / \sqrt{2}$. To prevent overweighting of studies with extremely low SEM, we truncated (i.e., trimmed) the SEM as shown earlier (Roman-Garcia et al., 2016). For the fermentation data set, $SEM < (0.25 \times \text{mean SEM})$ was trimmed to one-fourth of the mean SEM, such that the following percentages of parameter estimates were trimmed: pH (23%), aerobic stability (19%),

Figure 1. The PRISMA flow diagram from initial search and screening to final selection of studies included for the meta-analysis on the effect of *Lactobacillus buchneri* with or without homolactic or obligate heterolactic bacteria on silage fermentation and aerobic stability.

Figure 2. The PRISMA flow diagram from initial search and screening to final selection of studies included for the meta-analysis on the effect of *Lactobacillus buchneri* with or without homolactic or obligate heterolactic bacteria on dairy production.

DM recovery (24%), DM (18%), CP (19%), NDF (17%), lactate (15%), acetate (19%), propionate (22%), butyrate (37%), ethanol (40%), 1,2 propanediol (23%), $\text{NH}_3\text{-N}$ (29%), WSC (36%), yeast (31%), mold (38%), and LAB account (50%). For the dairy cow performance data set, the $\text{SEM} < (0.50 \times \text{mean SEM})$ was trimmed at half of the mean SEM: DMI (11% of observations), feed efficiency (25%), milk yield (20%), milk fat content (30%), milk protein content (33%), milk lactose content (29%), milk fat yield (0%), milk protein yield (0%), milk lactose yield (0%), and DM-total-tract digestibility (0%). This trimming process was done separately for mixed and fixed effects models because mixed models tend to have higher SEM (Littell et al., 1998).

Publication Bias and Outlier Analysis. Publication bias was examined using funnel plot (Light

and Pillemer, 1984) plot asymmetry and by Egger's regression method (Egger et al., 1997). Comparisons between uninoculated and inoculated treatments with standardized residuals >2.5 or <-2.5 , and with Cook's distances (Cook, 1977) $>5/n$ were removed (Oliveira et al., 2017).

Statistical Packages. The robumeta package (Fisher et al., 2017) of RStudio (version 1.3.1093; <https://cran.r-project.org/web/packages/robumeta/robumeta.pdf>) was used for overall WMD, forest plot, and meta-regression analysis. The metafor package (Viechtbauer, 2010) of R Software (version 1.3.1093; <https://cran.r-project.org/web/packages/metafor>) was used for subgrouping, publication bias, and outlier analysis. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Table 1. Effect of *Lactobacillus buchneri* (LB) with or without homolactic or obligate heterolactic bacteria on chemical composition, fermentation characteristics, and microbial count of ensiled forages

Item ¹	n ²	Control mean ³ (SD)	WMD ⁴ (95% CI)		Variance component ⁵		I^2 (%) ⁶	Funnel test ⁷ (<i>P</i> -value)
			Effect size	<i>P</i> -value	τ^2	Ω^2		
pH	490	4.13 (0.61)	0.059 (0.03, 0.09)	<0.01	0.01	0.02	97.6	0.47
DM, %	474	34.7 (14.1)	−0.14 (−0.31, 0.03)	0.11	0.13	0.61	90.4	0.16
DM recovery, %	142	93.8 (5.52)	−0.36 (−1.04, 0.32)	0.23	0.00	4.77	0.00	0.58
NDF, % of DM	225	46.9 (14.4)	0.42 (0.05, 0.79)	0.03	0.59	0.53	81.5	0.86
CP, % of DM	243	10.3 (4.54)	0.014 (−0.07, 0.10)	0.74	0.00	0.10	76.7	0.003
NH ₃ -N, % of DM	226	6.65 (5.97)	−0.087 (−0.26, 0.09)	0.31	0.19	0.00	81.7	0.002
WSC, % of DM	282	2.10 (2.07)	−0.27 (−0.40, −0.15)	<0.01	0.13	0.04	96.5	0.21
Lactate, % of DM	483	4.30 (2.51)	−0.31 (−0.51, −0.11)	0.003	0.55	0.51	97.8	0.004
Acetate, % of DM	494	1.70 (1.34)	1.05 (0.83, 1.27)	<0.01	0.68	0.80	99.3	0.38
Propionate, % of DM	233	0.27 (0.40)	0.083 (0.04, 0.13)	<0.01	0.00	0.13	99.7	0.46
Butyrate, % of DM	164	0.28 (0.59)	−0.027 (−0.05, −0.002)	0.03	0.004	0.002	89.4	0.63
Ethanol, % of DM	296	1.45 (2.80)	0.048 (−0.03, 0.13)	0.23	0.05	0.00	93.5	0.02
1,2 Propanediol, % of DM	100	0.22 (0.34)	0.80 (0.39, 1.22)	0.001	0.49	0.28	99.7	0.35
LAB, log cfu/g	236	7.18 (1.35)	0.60 (0.4, 0.80)	<0.01	0.00	3.20	93.4	0.49
Yeast, log cfu/g	286	4.19 (1.67)	−0.84 (−1.12, −0.57)	<0.01	0.00	1.11	92.5	0.85
Mold, log cfu/g	186	2.89 (1.52)	−0.40 (−0.66, −0.15)	0.003	0.00	0.86	84.1	0.97
Aerobic stability, h	241	111.2 (102.7)	82.1 (53.2, 111)	<0.01	7,247	2,294	99.3	0.98

¹WSC = water-soluble carbohydrate; LAB = lactic acid bacteria.²n = number of comparisons of inoculated and uninoculated treatments.³Uninoculated treatment.⁴WMD = weighted raw mean differences between LB-inoculated and uninoculated treatments, calculated using a robust regression hierarchical model to account for nesting of treatments within study (Tipton, 2015).⁵ τ^2 = between-cluster variance component; Ω^2 = between-studies-within-cluster variance component (Hedges et al., 2010; Fisher et al., 2017).⁶ I^2 = proportion of total variation of size effect estimates that is due to heterogeneity.⁷Egger's regression asymmetry test (Egger et al., 1997).

RESULTS

Data from 158 peer-reviewed studies were collected to investigate the effects of inoculation of LBB on silage fermentation and aerobic stability of whole-plant corn (38.2% of the studies), whole-plant sorghum (6.1%), temperate grass (17.2%), tropical grass (3.3%), sugarcane (4.2%), alfalfa (6.6%), other legumes (1.1%), grain (1.3%), HMC (7.9%), and other forages (14%). The most common application rates for LBB inoculation were 10⁵ and 10⁶ cfu/g fresh forage, and these represented 60.3 and 29.5% of the selected studies, respectively. A total of 57.7% of the studies evaluated the effect of inoculation of LB alone, and combinations of LB+PP, LB+other, or LB+LP represented 8.3, 11.8, and 8.9% of studies, respectively. Most of the studies used laboratory silos (96.3%), and only a small portion used farm-scale silos (3.7%). Enzymes were included with inoculants for only 19.6% of the studies.

Inoculation Effects on Silage Quality

Overall, LBB inoculation increased silage pH (+1.43%; *P* < 0.01) and NDF concentration (+0.90%; *P* = 0.03), whereas no effects were observed on DM recovery (*P* = 0.23; Table 1). Water-soluble carbohydrate

(−12.9%; *P* < 0.01), butyrate (−9.64%; *P* = 0.03), and lactate concentrations (−7.21%; *P* < 0.01) were reduced, whereas acetate (+61.8%; *P* < 0.01), propionate (+30.7%; *P* < 0.01), and 1,2 propanediol (+364%; *P* < 0.01) concentrations were increased with LBB inoculation. The LAB counts were increased 4-fold (*P* < 0.01), yeast counts decreased 7-fold (*P* < 0.01), and mold counts decreased 3-fold (*P* < 0.01) with LBB inoculation resulting in markedly greater aerobic stability (+73.8%; *P* < 0.01).

Based on the results from the meta-regression analysis (Table 2), forage type, application rate, enzyme inclusion, days of ensiling, and silo type contributed to the variability of some variables. Forage type reduced mold counts (*P* = 0.05) and acetate concentration (tendency, *P* = 0.09). Application rate tended to reduce NH₃-N (*P* = 0.05), butyrate (*P* = 0.05), and ethanol (*P* = 0.06) concentrations. Enzyme inclusion in the inoculant tended to reduce CP concentration (*P* = 0.09), whereas days of ensiling increased LAB (*P* < 0.01) and tended to reduce CP (*P* = 0.07) concentration and yeast counts (*P* = 0.06). Species of LBB increased WSC (*P* = 0.03), lactate concentration (*P* = 0.04) and reduced acetate concentration (*P* < 0.01), whereas silo type increased DM % (*P* = 0.04), reduced DM recovery (*P* < 0.01) and mold counts (*P* = 0.02),

Table 2. Meta-regression of the effect of forage type, *Lactobacillus buchneri*-based (LBB) inoculant species, inoculation rate, enzyme inclusion, ensiling duration, and silo type on weighted raw mean difference (WMD) between inoculated and uninoculated treatments for silage quality parameter estimates

Dependent variable (Y, WMD) ¹	Meta-regression variables (<i>P</i> -value) ²						Variance component ³		
	Intercept	Forage	Rate	Enzyme	Days of ensiling	LBB species	Silo type	τ^2	Ω^2
pH	0.35 (0.003)	-0.0026 (0.22)	-0.042 (0.16)	0.00068 (0.98)	0.00017 (0.56)	-0.0067 (0.20)	-0.18 (0.03)	0.02	0.02
DM, %	-1.48 (0.02)	0.0049 (0.83)	0.24 (0.13)	0.075 (0.67)	-0.0018 (0.29)	0.046 (0.14)	0.68 (0.04)	0.13	0.62
DM recovery, %	2.44 (0.24)	0.020 (0.76)	-0.099 (0.89)	0.49 (0.51)	-0.0025 (0.45)	0.051 (0.57)	-3.19 (0.002)	0.00	3.57
NDF, % of DM	1.09 (0.60)	-0.0079 (0.89)	0.034 (0.91)	-0.28 (0.55)	-0.0029 (0.26)	0.067 (0.48)	-0.23 (0.88)	0.73	0.57
CP, % of DM	0.05 (0.84)	-0.012 (0.42)	0.080 (0.10)	-0.20 (0.09)	-0.00093 (0.07)	0.011 (0.50)	0.12 (0.48)	0.00	0.08
NH ₃ -N, % of DM	1.43 (0.02)	-0.023 (0.34)	-0.30 (0.05)	0.20 (0.40)	-0.0017 (0.43)	-0.055 (0.12)	-0.59 (0.09)	0.23	0.00
WSC, % of DM	-0.77 (0.10)	0.025 (0.23)	-0.042 (0.71)	0.11 (0.26)	0.00044 (0.54)	0.047 (0.03)	0.18 (0.43)	0.14	0.07
Lactate, % of DM	-1.74 (0.03)	0.036 (0.23)	0.19 (0.23)	0.11 (0.58)	-0.0018 (0.35)	0.12 (0.04)	0.51 (0.37)	0.60	0.55
Acetate, % of DM	1.82 (0.009)	-0.057 (0.09)	0.033 (0.80)	0.014 (0.97)	0.0022 (0.23)	-0.085 (0.005)	-0.57 (0.12)	0.80	0.58
Propionate, % of DM	0.03 (0.83)	0.0024 (0.69)	0.031 (0.47)	0.057 (0.46)	0.000094 (0.91)	-0.0024 (0.75)	-0.097 (0.28)	0.001	0.13
Butyrate, % of DM	0.19 (0.04)	-0.0028 (0.53)	-0.031 (0.08)	0.025 (0.58)	0.000029 (0.79)	-0.0064 (0.16)	-0.13 (0.05)	0.003	0.002
Ethanol, % of DM	0.47 (0.07)	-0.011 (0.28)	-0.12 (0.06)	0.11 (0.21)	0.00013 (0.75)	-0.011 (0.40)	-0.22 (0.11)	0.08	0.00
1,2 Propanediol, % of DM	1.67 (0.35)	-0.031 (0.53)	0.79 (0.28)	-0.49 (0.16)	0.0014 (0.35)	-0.088 (0.14)	-1.68 (0.10)	0.58	0.25
LAB, log cfu/g	0.86 (0.19)	-0.012 (0.64)	-0.065 (0.65)	-0.16 (0.33)	0.0049 (0.005)	0.020 (0.40)	-0.34 (0.37)	0.00	3.40
Yeast, log cfu/g	-0.16 (0.85)	-0.043 (0.35)	-0.099 (0.52)	0.21 (0.64)	-0.0032 (0.06)	-0.027 (0.52)	-0.16 (0.70)	0.00	1.07
Mold, log cfu/g	1.11 (0.13)	-0.067 (0.05)	-0.28 (0.14)	0.085 (0.84)	-0.00052 (0.66)	-0.0066 (0.83)	-0.64 (0.02)	0.00	0.76
Aerobic stability, h	-2.96 (0.97)	2.47 (0.56)	4.77 (0.71)	60.1 (0.23)	0.29 (0.29)	-1.74 (0.66)	-30.1 (0.53)	8,803	1,724
									237

¹Y = dependent variable. WMD = weighted raw mean differences between *Lactobacillus buchneri*-inoculated and uninoculated treatments, calculated using robust regression method-hierarchical model with small-sample corrections, to account for nesting of treatments within study (Tipton, 2015). WSC = water-soluble carbohydrate; LAB = lactic acid bacteria.

²Forage = whole-plant corn, whole-plant sorghum, temperate grasses, tropical grasses, sugarcane, alfalfa, other legumes, grain, high moisture corn, other forages; LBB application rate = $\leq 10^4$, 10^5 , 10^6 , $\geq 10^7$ cfu/g as fed; with or without enzyme; LBB species = *Lactobacillus buchneri*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Pediococcus pentosaceus*, *Lactococcus lactis*, *Pediococcus acidilactici*, *Enterococcus faecium*; Silo type = laboratory or farm scale.

³ τ^2 = between-cluster variance component; Ω^2 = between-studies-within-cluster variance component (Hedges et al., 2010; Fisher et al., 2017).

⁴n = number of comparisons of inoculated and uninoculated treatments.

and tended to reduce butyrate ($P = 0.05$) and $\text{NH}_3\text{-N}$ ($P = 0.09$) concentration.

The ensuing sections discuss the most important sources of variation from the meta-regression and subgrouping analysis for understanding the LBB inoculation responses. The effects of covariates including forage type (Figure 3), type of LBB species (Figure 4), and inoculation rate (Figure 5) on DM recovery, acetate and 1,2 propanediol concentrations, mold counts, yeast counts, and aerobic stability are presented in the main

body of the article, whereas effects of the respective covariates on pH, lactate, propionate, and ethanol are presented in the supplemental section (Supplemental Figures S1, S2, S3, <https://doi.org/10.7910/DVN/ANYKJ5>). The effects of ensiling duration on aerobic stability and yeast counts (Figure 6) and that of silo type on mold counts (Figure 7) are presented but their other effects are not shown as they represented only a few studies or were considered less important for understanding the LBB response.

Figure 3. Forage type (subgroup A to F) effect on silage fermentation and aerobic stability responses to inoculation with *Lactobacillus buchneri* (LB)-based inoculants (LBB) with or without homolactic or obligate heterolactic bacteria. WMD = weighted raw mean differences between LB-inoculated and uninoculated silage. HMC = high moisture corn; other forages = pea-wheat, rice, triticale, clover-ryegrass, alfalfa-ryegrass, oat, potato-wheat, sweet potato, potato hash. Error bars represent the confidence interval (95%).

Effect of Forage Type on the Inoculation Response

Inoculation with LBB increased the pH of all silages ($P < 0.01$) except ($P > 0.10$), temperate grasses, sugarcane, alfalfa, and grains (Supplemental Figure S1a) but reduced the pH of tropical grasses ($P = 0.02$). Inoculation with LBB reduced DM recovery of whole-plant corn silage (0.78 percentage points; $P < 0.01$;

Figure 3A) but increased those of tropical grasses (6.87 percentage points; $P = 0.01$) and alfalfa ($P = 0.07$, tendency) without affecting others ($P > 0.10$).

Inoculation with LBB reduced lactate concentration ($P < 0.05$) of most forage types except temperate grasses ($P = 0.11$), tropical grasses ($P = 0.22$), alfalfa ($P = 0.35$), and other forages ($P = 0.13$; Supplemental Figure S1b). Acetate concentration was greater with

Figure 4. Lactic acid bacteria type (subgroups A to F) effects on silage fermentation and aerobic stability responses to inoculation with *Lactobacillus buchneri* (LB)-based inoculants (LBB) with or without homolactic or obligate heterolactic bacteria. WMD = weighted raw mean differences between LB-inoculated and uninoculated silage; LB = *Lactobacillus buchneri* alone; LB+LP = *L. buchneri* with *Lactobacillus plantarum*, LB+PP = *L. buchneri* with *Pediococcus pentosaceus*; LB+LP+EF = *L. buchneri* with *L. plantarum* and *Enterococcus faecium*; LB+LP+PP = *L. buchneri* with *L. plantarum* and *P. pentosaceus*; LB+LH = *L. buchneri* with *Lactobacillus hilgardii*; LB+other = *L. buchneri* with other species such as *Lactococcus lactis*, *Lactobacillus casei*, *Pediococcus acidilactici*. Error bars represent the confidence interval (95%).

LBB inoculation ($P < 0.05$) in all forage types; however, effects were less pronounced for HMC (Figure 3B). *Lactobacillus buchneri*-based bacteria inoculation increased propionate concentration ($P < 0.01$) for whole-plant corn silage, temperate grasses, and other forages silage, whereas no effect was observed on HMC, grain, alfalfa, sugarcane, tropical grasses, and whole-plant sorghum silage ($P > 0.10$; Supplemental Figure S1c). Ethanol concentration reduced with LBB inoculation of tropical grasses and sugarcane silages ($P \leq 0.01$; Supplemental Figure S1d) and increased with temperate grasses ($P < 0.01$) and whole-plant

corn silage ($P = 0.07$, tendency). Inoculation increased 1,2 propanediol concentration ($P < 0.05$) in all forage types except in tropical grasses ($P = 0.84$), and the magnitude of the increase was greatest for sugarcane silage (Figure 3C).

Inoculation with LBB reduced yeast counts ($P < 0.05$) in all silage types; however, the response was lower with tropical grasses (-0.26 cfu/g) and other legumes (-0.39 cfu/g) than in other forages (-1.17 cfu/g) and temperate grasses (-1.19 cfu/g), which had the greatest responses (Figure 3D). Inoculation with LBB also reduced mold counts ($P < 0.05$) in all silage

Figure 5. Inoculant application rate (subgroups A to F) effect on silage fermentation and aerobic stability responses to inoculation with *Lactobacillus buchneri* (LB) with or without homolactic or obligate heterolactic bacteria. WMD = weighted raw mean differences between LB-inoculated and uninoculated silage. Error bars represent the confidence interval (95%).

Figure 6. Effects of ensiling duration (days of ensiling) on aerobic stability and yeast responses to inoculation with *Lactobacillus buchneri* with or without homolactic or obligate heterolactic bacteria. WMD = weighted raw mean differences between inoculated and uninoculated silage. Error bars represent the confidence interval (95%).

types, except in whole-plant sorghum ($P = 0.60$) and other legumes ($P = 0.54$; Figure 3E).

Inoculation with LBB increased aerobic stability ($P < 0.05$) in all silage types, with the noteworthy exception of tropical grass silage ($P = 0.20$; Figure 3F). The aerobic stability increases ($P < 0.01$) were greatest for grain silages (199 h) and HMC (169 h); intermediate for alfalfa (89 h), temperate grasses (112 h), whole-plant corn (69 h), and whole-plant sorghum silage (53 h); and lower for sugarcane (19 h) and other legumes (17 h).

Effect of the LAB Species Combination on LB Inoculation Response

Application of LB ($P < 0.01$), and LB+LH ($P < 0.01$) increased silage pH while LB+other ($P = 0.07$), and LB+LP+EF ($P = 0.09$) tended to increase silage pH (Supplemental Figure S2a). Inoculation with LB+LP ($P = 0.10$) and LB+PP ($P = 0.06$) tended to decrease silage pH. Dry matter recovery was decreased by LB ($P < 0.01$) and increased by LB+PP ($P = 0.02$), but other inoculants had no effect ($P > 0.10$; Figure 4A).

Application of LB ($P < 0.01$) and LB+LH ($P < 0.01$) decreased lactate concentration, and only LB+LP+PP ($P < 0.01$) increased the response; LB+other had a similar tendency ($P = 0.06$; Supplemental Figure S2b). All inoculants increased acetate concentration ($P < 0.01$; Figure 4B); however, propionate concentrations were only increased by LB ($P < 0.01$), and LB+LP+EF ($P < 0.01$), and a tendency for improvement was observed with LB+other ($P = 0.06$; Supplemental Figure S2c). Ethanol concentration was increased by LB+LH ($P = 0.01$), LB+LP+EF ($P = 0.04$) and LB ($P = 0.02$) and decreased by LB+other ($P < 0.01$), and it tended to decrease by LB+LP ($P = 0.07$; Supplemental Figure S2d).

Inoculation with all inoculants increased ($P < 0.01$) 1,2 propanediol concentration except LB+LP+PP, which tended ($P = 0.10$; Figure 4C) to reduce the concentration but resulted in greater yeast count reductions ($P < 0.01$; Figure 4D) than LB alone ($P > 0.05$). Application of all LB inoculants reduced mold counts ($P < 0.05$; except LB+LH, $P = 0.11$; Figure 4E). Inoculation with LB+LP+PP was associated with a greater aerobic stability response than LB+PP, LB+LP+EF or LB+other bacteria (Figure 4F).

Effect of the Application Rate on LB Inoculation Response

Inoculation rates of 10^5 or 10^6 cfu/g increased ($P < 0.01$) and $\leq 10^4$ tended to increase ($P = 0.10$) silage pH, whereas $\geq 10^7$ cfu/g reduced the response ($P < 0.01$; Supplemental Figure S3a). Dry matter recovery was reduced when inoculants were applied at $\leq 10^4$ ($P \leq 0.01$) and 10^6 cfu/g ($P = 0.02$) but not 10^5 cfu/g ($P = 0.37$; Figure 5A).

Figure 7. Effects of silo type (laboratory vs. farm scale) on mold count responses to inoculation with *Lactobacillus buchneri* with or without homolactic or obligate heterolactic bacteria. WMD = weighted raw mean differences between inoculated and uninoculated silage. Error bars represent the confidence interval (95%).

Lactate concentration was increased by applying $\geq 10^7$ cfu/g ($P < 0.01$), decreased ($P < 0.01$) by applying 10^5 or 10^6 cfu/g, and unaffected by applying $\leq 10^4$ cfu/g ($P = 0.52$; Supplemental Figure S3b). Acetate concentration was increased ($P < 0.05$) at all application rates (Figure 5B). Similarly, propionate concentration was increased by all application rates ($P \leq 0.02$) except the $\leq 10^4$ cfu/g rate ($P = 0.53$; Supplemental Figure S3c). Ethanol concentration was increased ($P < 0.01$) by applying 10^5 ($P < 0.01$) and $\leq 10^4$ cfu/g ($P < 0.01$) and reduced by applying $\geq 10^7$ cfu/g ($P < 0.01$; Supplemental Figure S3d). The 1,2 propanediol concentration was increased to a greater extent by the 10^6 cfu/g rate ($P < 0.01$) compared with 10^5 and $\leq 10^4$ cfu/g rates ($P < 0.01$), but unaffected ($P = 0.84$) by the $\geq 10^7$ cfu/g rate (Figure 5C).

Inoculation with 10^5 , 10^6 , and $\geq 10^7$ cfu/g reduced ($P < 0.05$) yeast counts (Figure 5D). Mold counts were reduced ($P < 0.05$) with 10^5 , 10^6 , and $\geq 10^7$ cfu/g (Figure 5E). No effects were observed on yeast and mold counts with the 10^4 cfu/g rate. All inoculation rates increased aerobic stability ($P < 0.01$); however, the magnitude of improvement was lower with $\leq 10^4$ cfu/g (Figure 5F).

Effects of Ensiling Duration on the Inoculation Response

The effects of ensiling duration on aerobic stability are presented in Figure 6. Ensiling for > 90 d resulted in greater ($P < 0.01$; Figure 6A) improvements in aerobic stability relative to ensiling for 7 to 30 or 31 to 60 d, which had a similar response. Similarly, greater reduction of yeast counts was observed after 90 d of ensiling ($P < 0.01$; Figure 6B) relative to ensiling for 7 to 30 d, and a similar trend was evident for the 61 to 90 d duration.

Effects of Silo Type on the Inoculation Response

Application of LBB reduced mold counts, regardless of the type of silo used (Figure 7), but the magnitude of reduction compared with uninoculated controls was greater (4.8-fold) for farm scale silos compared with laboratory silos (2.3-fold).

Effects of Feeding Inoculated Silage on Performance of Lactating Dairy Cows

Data from 12 peer-reviewed studies were analyzed to investigate the effects of inoculation of LBB on dairy cow performance. Six of the studies fed corn silage, whereas the rest fed other forages including wheat, sugarcane, alfalfa, and barley silage to lactating dairy

Table 3. Effect of forage silage inoculation with *Lactobacillus buchneri* (LB) with or without homolactic or obligate heterolactic bacteria on the performance of dairy cows

Item	n ¹	Control mean ² (SD)	WMD ³ (95% CI)		Variance component ⁴			Funnel test ⁶ (P-value)
			Effect size	P-value	τ^2	Ω^2	I^2 (%) ⁵	
DMI, kg/d	12	21.8 (4.03)	-0.15 (-0.65, 0.35)	0.51	0.00	8.29	36.0	0.20
DM digestibility, %	5	70.2 (3.08)	-0.22 (-1.65, 1.22)	0.64	0.00	0.00	0.00	0.36
Milk yield, kg/d	13	32.2 (7.89)	-0.068 (-0.98, 0.84)	0.87	0.00	88.0	75.2	0.62
Feed efficiency, kg of milk/kg of DMI	5	1.32 (0.38)	0.0070 (-0.01, 0.03)	0.31	0.00	0.03	0.00	0.68
Milk fat, %	12	3.46 (0.39)	-0.023 (-0.09, 0.04)	0.43	0.00	0.47	0.00	0.77
Milk protein, %	12	3.13 (0.21)	-0.013 (-0.04, 0.02)	0.37	0.00	0.17	0.00	0.62
Milk lactose, %	10	4.76 (0.47)	0.016 (-0.005, 0.04)	0.11	0.00	0.00	0.00	0.79
Milk fat, kg/d	8	1.10 (0.22)	-0.016 (-0.08, 0.05)	0.54	0.00	0.06	0.00	0.16
Milk protein, kg/d	8	1.09 (0.22)	-0.029 (-0.06, 0.004)	0.08	0.00	0.06	0.00	0.48
Milk lactose, kg/d	3	1.29 (0.49)	0.021 (-0.04, 0.08)	0.17	0.00	0.00	0.00	0.84

¹n = number of comparisons of inoculated and uninoculated treatments. LB alone (n = 9), LB + *Pediococcus pentosaceus* (n = 1), LB + others (n = 3). Whole-plant corn silage (n = 7) and other forages (n = 6). Application rate: < 10⁴ (n = 1), 10⁵ (n = 9) and 10⁶ (n = 3) cfu/g fresh forage.

²Uninoculated treatment.

³WMD = weighted raw mean differences between LB-inoculated and uninoculated treatments, calculated using robust regression method-hierarchical model with small-weighted sample corrections, to account for nesting of treatments within study (Tipton, 2015).

⁴ τ^2 = between-cluster variance component; Ω^2 = between-studies-within-cluster variance component (Hedges et al., 2010; Fisher et al., 2017).

⁵ I^2 = proportion of total variation of size effect estimates that is due to heterogeneity.

⁶Egger's regression asymmetry test (indicative of publication bias).

cows. The most common application rates for LBB inoculation were 10⁵ and 10⁶ cfu/g, accounting for 66.7 and 25% of the studies, respectively. A total of 66.7% of the studies evaluated the effect of inoculation of LB alone, and the others used LB with other LAB (33.3%).

Inoculation with LBB did not affect milk yield ($P = 0.87$; Table 3; Figure 8), DMI ($P = 0.51$), feed efficiency ($P = 0.31$), DM digestibility ($P = 0.64$), milk fat ($P = 0.43$), and milk lactose ($P = 0.11$) concentration; however, it tended to reduce milk protein concentration by a small amount (WMD = -0.01%; $P = 0.08$). Funnel plot asymmetry was not observed ($P > 0.10$) for any performance variable, indicating that publication bias was not evident.

Heterogeneity was high for milk yield ($I^2 = 75.2$) and low for DMI ($I^2 = 36.0$; Table 3). Based on the subgroup analysis of milk yield responses, which should be cautiously interpreted due to the few studies involved, feeding LBB-inoculated whole-plant corn as the basal forage tended to reduce milk yield ($P = 0.09$) but no effects were observed with feeding other inoculated forages ($P = 0.21$; Supplemental Figure S4a, <https://doi.org/10.7910/DVN/ANYKJ5>). Similarly, no effects were observed on milk yield when cows were fed silage inoculated with LB alone ($P = 0.16$); however, feeding forages inoculated with LBB reduced milk yield ($P < 0.01$; Supplemental Figure S4b; n = 3). Application rate did not affect the milk yield response ($P > 0.10$; Supplemental Figure S4c). Basal forage type, inoculant LAB composition and inoculation rate did not affect DMI (data not shown).

DISCUSSION

Silage Quality and Stability

Across forage types, LBB inoculation decreased lactate concentration; increased pH, DM losses, and concentrations of acetate, 1,2 propanediol and propionate; decreased yeast and mold counts; and increased aerobic stability. Silages inoculated with LB typically contain less lactate and more acetate than untreated silages because the bacterium converts lactate to acetate and 1,2-propanediol during silage fermentation (Oude Elferink et al., 2001). The 1,2 propanediol can be converted into propionate by *L. diolivorans* (Krooneman et al., 2002), *L. reuteri* (Sriramulu et al., 2008) and by a novel strain of *L. buchneri* A KKP 2047p in the presence of cobalamine (Zielińska et al., 2017). Due to their antifungal nature, the greater acetate and propionate concentrations of LB-inoculated silages inhibit spoilage yeast and molds (Moon, 1983), resulting in improved aerobic stability (Oude Elferink et al., 2001; Pahlow et al., 2003). Additionally, LB may also produce other

Figure 8. Forest plot showing the effects of forage inoculation with *Lactobacillus buchneri* with or without homolactic or obligate heterolactic bacteria on milk yield (kg/d) by dairy cows. The x-axis shows the WMD; squares to the left of the line represent reduction, whereas squares to the right of the line indicate an increase in milk yield (kg/d). Each square represents the mean size effect for that study, and the size of the squares reflects the relative weighting of the study to the overall size effect estimate, with larger squares representing greater weight. The lines connected to the squares represent 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. WMD = weighted raw mean differences between LB-inoculated and uninoculated treatments. (Sutton et al., 2002; Bayatkouhsar et al., 2011; Mohammadzadeh et al., 2011, 2012; Andrade et al., 2016; Santos et al., 2017).

antimicrobial substances, such as buchnericin, a bacteriocin that may contribute to increased aerobic stability (Yildirim, 2001).

The meta-analysis of Kleinschmit and Kung (2006) reported greater aerobic stability in LB-treated corn silage ($>10^5$ cfu/g), grass, and small grain silages ($\leq 10^5$ and $>10^5$ cfu/g) than in untreated silages. The results of the current meta-analysis support the latter by

showing that inoculation with LBB improved aerobic stability in all forages examined except tropical grasses which were not specifically examined in the previous meta-analysis. The increased aerobic stability in most forages in the current study is largely because LB increased acetate concentration at the expense of lactate concentration, reduced yeast counts in all forages, and reduced mold counts in most forages.

The lack of an aerobic stability response for tropical grasses may be due to the relatively low number of observations ($n = 6$), wide variation in responses and in particular the insufficient levels of lactate substrate for LB to convert to acetate and propionate (via 1,2 propanediol), which are the main antifungal compounds in silage. The high variability in responses for tropical grasses partly reflects wide morphological and chemical differences in tropical forages (bermudagrass vs. elephant grass vs. guinea grass), which may have contributed to differences in porosity and density after packing.

Aerobic stability was increased by the different LBB inoculants in this study as in others (Blajman et al., 2018; Zhang et al., 2018; Bernardi et al., 2019). For unknown reasons, the magnitude of improvement in aerobic stability was greater when LB+LP+PP was applied instead of other bacterial combinations, except LB alone and LB+LP. Interestingly, the response of LB alone did not differ from those of combinations of LB and other LAB. This is because, for most of such combinations, LB was the main bacteria contributing to aerobic stability, as the other LAB were added to improve the fermentation and DM recovery. The exception could be LH, which similarly to LB is also an obligate heterolactic bacterium added to improve aerobic stability earlier than LB (Ferrero et al., 2019a; Arriola et al., 2021). The metabolic pathway for converting lactate into acetate and 1,2 propanediol is common for both LH and LB (Heinl et al., 2012); hence, previous studies have shown the efficacy of LH at improving aerobic stability in sugar cane (Avila et al., 2012, Carvalho et al., 2015), whole-plant corn silage (Reis et al., 2018, Ferrero et al., 2019b) and whole-plant sorghum silage (Ferrero et al., 2019a; Arriola et al., 2021).

Schmidt et al. (2009) suggested that LB inoculants need at least 45 d to improve aerobic stability. This is supported by this study because the magnitude of improvement in aerobic stability was greater after 90 d of ensiling, and a similar trend was evident after 61 to 90 d versus shorter ensiling durations. Nevertheless, it is noteworthy that, though less pronounced, aerobic stability increases were evident after 7 to 30 d of ensiling. This is one of the reasons why LH has been examined as an alternative to LB, but the results of early studies are inconclusive for forages (Ferrero et al., 2019a; Nair et al., 2020; Arriola et al., 2021) but promising for HMC (da Silva et al., 2020).

Early concerns that the heterolactic nature of LB would cause large losses of DM from silages were not substantiated by the meta-analyses of Kleinschmit and Kung (2006), who reported DM losses of 1 (corn silages) to 1.8% (grass and small grain silages) with LB inoculation. In the current meta-analysis, inoculation with

LBB only decreased DM recovery of whole-plant corn silage by a small amount (-0.78%) but increased or did not affect those of others. Applying LB alone reduced DM recovery (by 0.7%) but no reduction was evident when other bacteria were applied with LB or when it was applied at the recommended 10^5 cfu/g dose. The current meta-analysis therefore supports the notion that losses of DM due to heterofermentation by LBB inoculants are generally small and are forage, inoculant composition, and dose specific (Kleinschmit and Kung, 2006). In addition, adding complementary LAB such as LP, PP, and LP+PP, to LB prevented the increase in pH and or decrease in DM recovery that resulted from applying obligate heterolactic LB or LB+LH. Therefore, addition of homolactic or facultative heterolactic LAB to LB increased acidification in some cases and prevented the small losses in DM that occurred when LB alone was applied. The meta-analysis of Bernardi et al. (2019) also showed that applying inoculants that combine heterolactic bacteria with obligate homolactic or facultative heterolactic bacteria improved silage fermentation, reduced yeast and mold counts and therefore improved aerobic stability. However, unlike in the current study, Bernardi et al. (2019) showed that homolactic and obligate heterolactic bacteria increased DM losses. This difference may reflect the inclusion of older studies (1980 to 2017), as well as those published in Portuguese and Spanish in that study.

The recommended dose for LAB inoculants in parts of Asia and South America is $\leq 10^4$ cfu/g; however, it is typical to use 10^5 cfu/g in the United States and 10^6 cfu/g in Europe (Oliveira et al., 2017). All inoculation rates, except $\leq 10^4$ cfu/g, reduced yeast counts. Accordingly, aerobic stability was greater at higher doses than at the $\leq 10^4$ cfu/g dose. This was probably because the $\leq 10^4$ cfu/g rate had insufficient bacteria to dominate the epiphytic population.

The considerable increase in lactate concentration, relatively small increase in acetate, and lack of a 1,2 propanediol response at the $\geq 10^7$ cfu/g rate, suggests that the classical fermentation of lactate into 1,2 propanediol and acetate by LB was curtailed at this high dose. However, the result should be cautiously interpreted because only a few studies used this high rate ($n = 2$ for such studies that measured 1,2 propanediol). Why the high dose would curtail the typical LB response is unclear, but it may be related to pH effects on lactic acid degradation by LB. This high dose is typically too expensive for routine use on farms, and it did not improve DM recovery, acetate concentration, yeast and mold counts, or aerobic stability relative to the 10^5 or 10^6 cfu/g rates. Therefore, the 10^5 or 10^6 cfu/g were the best application rates for improving silage preservation in this study.

Performance of Dairy Cows

The effects of feeding silage treated with LB on performance of dairy cows in the literature have been inconsistent. Although some studies have observed a greater milk yield response (Kung et al., 2003), others did not (Taylor et al., 2002; Arriola et al., 2011b; da Silva et al., 2017). Kleinschmit and Kung (2006) questioned whether the high acetate concentrations of LB-inoculated forages would reduce DMI. However, studies conducted to date have found no adverse effect on DMI when LB-inoculated silage was fed to dairy cows (Taylor et al., 2002; Kung et al., 2003; Arriola et al., 2011b). In the present study, no effects of feeding LBB-inoculated silage on DMI, milk yield, DM digestibility, feed efficiency, and milk fat percent were detected. However, inoculation of silage was associated with a small (−0.6 percentage units) reduction in milk protein concentration, which is unclear because inoculation with LAB generally improves ruminal function by stimulating rumen microbes, increasing VFA production (Weinberg et al., 2003), increasing NDF degradability (Weinberg et al., 2007) or increasing microbial protein synthesis (Contreras-Govea et al., 2011).

Bernardi et al. (2019) showed that applying heterolactic LAB alone to corn silage did not affect milk yield by dairy cows but applying LBB reduced milk yield. This study differed in scope from the current study as they included studies that were older, that involved different bacteria and were published in Portuguese and Spanish. Reasons for trends in this study for LBB inoculation of corn but not for other forages or for LBB but not LB inoculation to reduce milk yield are not clear. The latter milk yield responses should be cautiously interpreted because few studies were involved; the tendencies observed suggest that statistical power may have been insufficient. More research is needed on the effects of LBB inoculants on the performance of dairy cows.

CONCLUSIONS

Silage inoculation with LBB increased aerobic stability due to greater acetate concentration and lower yeast counts for all inoculant combinations, rates of inoculation, and forage types except tropical forages. Aerobic stability was improved by LBB inoculation at all ensiling durations examined but was greater with >90 d of ensiling. The DM losses with LBB inoculation were generally small and were forage, inoculant composition, and dose specific. Adding other bacteria to LB prevented the small loss in DM caused by LB alone. The best application rates for preventing DM losses, increasing acetate concentrations, reducing yeast

counts and increasing aerobic stability were 10^5 and 10^6 cfu/g fresh forage. Feeding LBB-inoculated silage had no effects on DMI, DM digestibility, and feed efficiency.

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