Bacterial Inoculants for Optimizing Silage Quality and Preservation and Enhancing Animal Performance

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Introduction

Silage accounts for up to 60% of dairy cow diets in the US and approximately 133 million tons of corn silage alone were produced in 2019 (Adesogan et al., 2020). However, significant wastage of silage worth over $2 billion occurs annually in the US due to spoilage and other losses that range from 14 to 24% on average on farms (Rotz and Muck, 1994). One of the effective ways of curtailing such losses is applying bacterial inoculants to forages at the point of ensiling. Several excellent reviews have been published on silage additives including inoculants. These include those of McDonald et al. (1991), Muck and Kung (1997), Kung et al (2003) and Muck et al. (2018). Addah et al. (2014) also discussed the cost effectiveness of silage inoculants. Rather than another review, the intention in this paper is to briefly describe the types and modes of actions of various bacterial inoculants, to summarize the findings of different meta analytical studies (Table 1) on their use, and to describe critical factors for ensuring inoculant effectiveness.

Inoculant Effects on Silage Preservation and Quality

Homofermentative LAB

The use of bacterial inoculants for silage preservation has increased substantially over the last few decades with increasing reliance of silage in diets of dairy and beef cattle. Traditional bacterial inoculants were selected to improve silage preservation by fermenting plant water-soluble carbohydrates (WSC) into organic acids to inhibit the growth of deleterious bacteria and fungi, minimize dry matter (DM) losses and preserve important nutrients. Since these effects are predicated on rapidly acidifying the silage, the focus has been to select bacteria that efficiently dominate the epiphytic bacteria and ferment sugars into organic acids that rapidly decrease the pH. Homolactic fermentation involves conversion of one molecule of hexose into a single acid, lactic acid. In the silage context, obligate and certain facultative heterofermentative lactic acid bacteria (HoLAB) ferment one molecule of glucose into two molecules of lactate via pyruvate. This is the most efficient fermentation pathway as it results in minimal energy losses and no losses of CO_2, and hence no dry matter losses. Facultative heterofermentative bacteria can also ferment pentoses into lactic and acetic acid via the phosphoketolase pathway. Most bacterial inoculants used for silage making are HoLAB including those belonging to the *Lactobacillus, Enterococcus*, and *Pediococcus* groups such as *Lactobacillus acidophilus, L. casei, L. curvatus, L. paracasei, L. plantarum, L. salivarius, Lactococcus acidilactici, Enterococcus faecium, Pediococcus acidilactici and P. pentosaceus.*

Table 1. Meta analyses on effects of bacterial inoculation on silage quality parameters and animal performance

Studies	**Years of data collected**	**# of articles**	**Forage**	**LAB species**	**Application rate (cfu/g)**
Kleinschmit and Kung, 2006	1996 - 2005	23	corn, grass and small grain (barley, sorghum, wheat, ryegrass, and pea:wheat mixture)	*L. buchneri*	$\leq 10^5$ $> 10^5$
Oliveira et al., 2017	1997 - 2016	130	corn, sorghum, temperate grass, tropical grass, sugarcane, alfalfa, other legume; and other forages	*L. plantarum, P. pentosaceus, E. faecium, L. rhamnosus*, or mixed LAB species	$\leq 10^4$, 10^5, 10^6, $\geq 10^7$
Blajman et al., 2018	1980 - 2017	104	corn	*L. buchneri*, *L. plantarum, P. acidilactici*	10^4 - 10^7
Rabelo et al., 2018	2006 - 2016	42	sugarcane	*L. plantarum L. buchneri*	0 - 1.8 x10^6 for Lp 0 – 2.5 10^{10} for Lb
Zhang et al., 2018	2003 - 2013	24	corn	*Lactobacillus plantarum, L. buchneri, L. brevis, P. pentosaceous, E. Faecium, L. rhamnosus, L. lactis, L. acidilactici, L. acidophilus*	$< 10^5$; $\geq 10^5$
Bernardi et al., 2019	1980 - 2017	140	corn	*L. brevis, L. buchneri, L. acidophilus, L. curvatus, L. paracasei, L. plantarum, L. salivarius, E. faecium, P. acidilactici, P. pentosaceus*	4.3×10^2 - 6.7×10^{10} 1×10^3 - 7×10^8 3.4×10^4 - 1×10^8
Blajman et al., 2020	1980- April 2018	48	alfalfa	*L. buchneri, L. plantarum, P. acidilactici, E. faecium*	10^4 - 10^7
Arriola et al., 2020	1997- 2018	120	whole-plant corn, whole-plant sorghum, temperate grass, tropical grass, sugarcane, alfalfa, other legumes, grain, high moisture corn, and other forages	*L. buchneri*, *L. plantarum, P. pentosaceus, E. faecium*, *L.hilgardii, L. casei, P. acidilactici, Lactococcus acidilactici*	$\leq 10^4$, 10^5, 10^6, $\geq 10^7$

In addition to using HoLAB to improve the fermentation, reduce DM losses, and preserve nutrients. increasing aerobic stability and digestibility are other desirable outcomes. To our knowledge, only one meta analytical study examined the efficacy of HoLAB at achieving these goals over a wide range of forage types, application rates and bacterial species. Based on 130 studies, Oliveira et al. (2017) showed that inoculation with HoLAB reduced silage pH and proteolysis and increased DM recovery of legume, tropical and temperate grass silages (Figure 1). This was achieved by increasing lactate production and reducing ammonia-N, acetate, and butyrate production. However, DM digestibility was not affected. No other studies have examined HoLAB effects across different forages, but some have examined specific forages. For instance, the meta-analysis of Bernardi et al., (2019) revealed that inoculating corn silage with HoLAB increased lactic acid concentration but still increased DM losses. Similarly, Rabelo et al. (2018) also showed that inoculating sugarcane silage with HoLAB (*L. plantarum* alone) increased DM losses. Collectively these studies suggest that applying HoLAB alone to corn, sorghum and sugarcane silages may not reduce DM recovery. This is probably because the latter forages have low buffering capacities and sufficient nonstructural carbohydrates to allow a natural homofermentative pathway to prevail even without inoculation. However, application of HoLAB to forages like alfalfa or grasses, that have lower nonstructural carbohydrate concentrations or high buffering capacities, improves fermentation, and reduces DM losses.

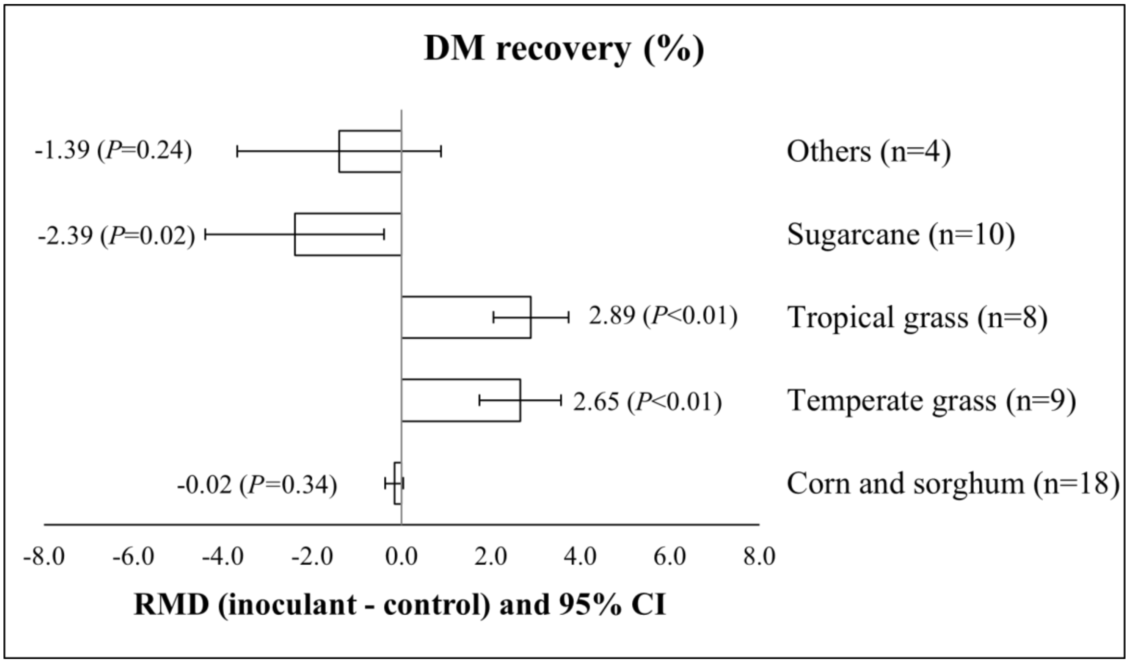

Figure 1. Effects of silage inoculation with homofermentative and facultative heterofermentative lactic acid bacteria (LAB) on silage DM recovery as affected by forage type. RMD = raw mean differences between inoculated and uninoculated treatments. Adapted from Oliveira et al. (2017).

The Oliveira et al. (2017) meta-analysis reported that HoLAB inoculants also reduced clostridia and mold growth but had no effect on aerobic stability. These results support the literature review of Muck and Kung (1997) and meta analyses on sugarcane (Rabelo et al., 2018) and corn (Bernardi et al., 2019) silages that had similar findings. The failure of HoLAB to reliably improve aerobic stability has led to development of combination inoculants that include heterofermentative bacteria (HeLAB), which increase aerobic stability, as well as HoLAB in inoculants.

Single Obligate Heterofermentative LAB

Obligate heterolactic bacteria ferment one molecule of hexose into lactic acid, carbon dioxide and ethanol. Following conversion of hexose into pyruvate, acetate and CO_2 via the pentose phosphate pathway, pyruvate and acetate are further reduced to lactate as well as ethanol and CO_2, respectively. The CO_2 and ethanol produced during heterofermentation contribute to DM losses, hence this pathway is less efficient than homofermentation. The main group of obligate heterolactic bacteria used in silage fermentation belong to the *Lactobacillus buchneri group,* among which *L. buchneri, L. brevis, L. diolivorans, L. hilgardii, L. kefiri, L. parafarraginis* have been tested on silage (Muck et al., 2018).

The most widely used obligate heterolactic bacterium is *Lactobacillus buchneri*, which is added to silage to reduce aerobic spoilage. This bacterium ferments lactic acid to acetic acid and 1, 2 propanediol (Oude Elferink et al., 2001). The 1,2 propanediol can be further converted to propionic acid by HeLab like *L. diolivorans* (Krooneman et al., 2002) or directly by a novel strain of *L buchneri* when glucose and cobalamine are present (*Lb. buchneri* A KKP 2047p; Zielinska et al., 2017; Muck et al., 2018). The acetic acid and propionic acid produced inhibit the growth of lactate-utilizing yeasts and molds that cause spoilage, thereby increasing silage aerobic stability. *Lactobacillus hilgardii*, has a similar mode of action *to L. buchneri* (Heinl et al., 2012) and some early studies suggest that it prevented DM losses (Avila et al., 2012) and increased aerobic stability relative to *L. buchneri* (Polukis et al., 2016). However, other studies have not shown clear and consistent differences in the aerobic stability response of both bacteria (Ferrero et al., 2018; Arriola et al., 2020a). Muck et al., 2018 cited several studies showing that antifungal compounds produced by *L. buchneri and* other HeLab may also contribute to improved aerobic stability.

The first meta-analysis on effects of *L. buchneri* alone on silage showed that the inoculant increased aerobic stability of silages in a dose-dependent manner (Kleinschmit and Kung, 2006). Applying *L. buchneri* at $\leq 10^5$ was less effective than $>10^5$ cfu/g at increasing the aerobic stability of corn, grass, and small grain silages. These authors confirmed that applying *L. buchneri* alone resulted in a small (1-1.8%) increase in DM loss. A recent meta-analysis by our group (Arriola et al., 2020b) confirmed that applying *L. buchneri* to various forages markedly increased aerobic stability (by 79%) except in tropical grasses and whole plant sorghum silages (Figure 2). This was confirmed in the meta-analysis of Blajman et al. (2018) on corn silage when *L. buchneri* or other unspecified individual obligate heterofermenters were examined. However, Rabelo et al.

(2018) showed that *L. buchneri* did not affect aerobic stability of sugarcane silage in their meta-analysis. The failure to increase aerobic stability of sorghum and sugarcane silages may reflect high residual WSC concentrations which led to ethanolic fermentations and the growth of lactate-assimilating yeasts that cause spoilage.

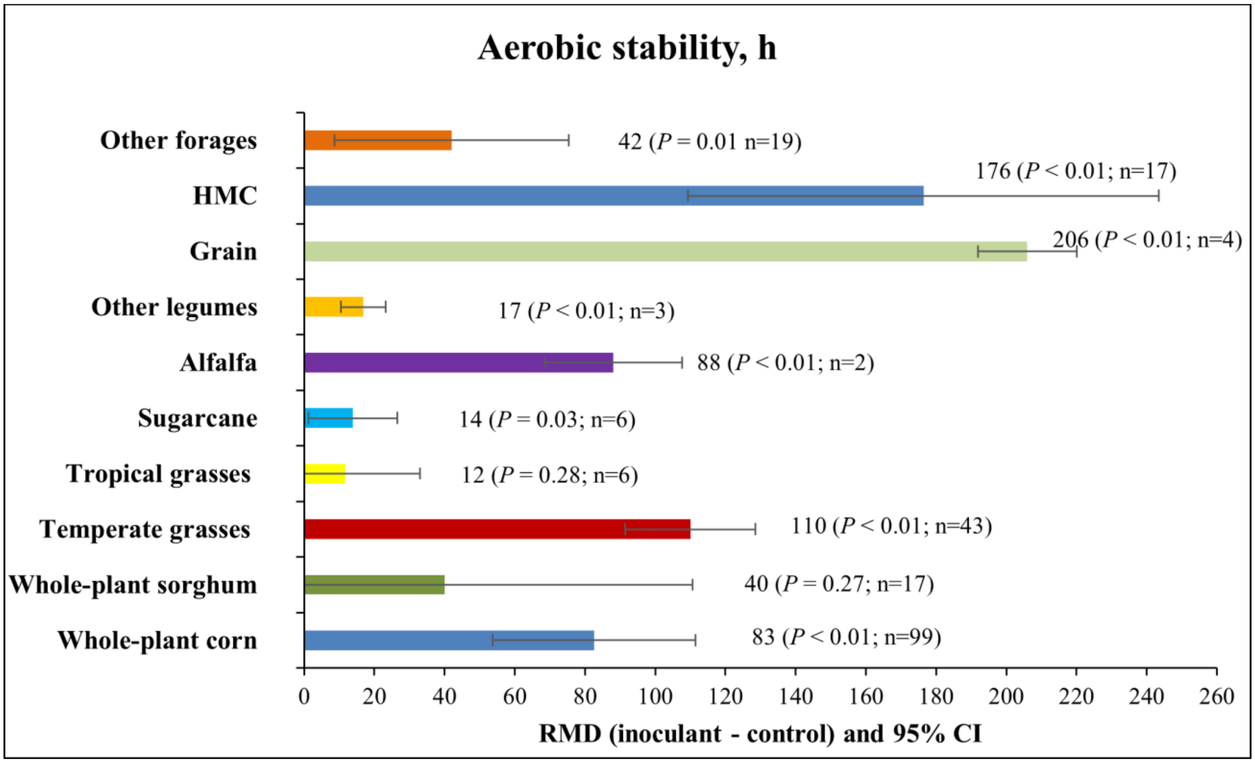

Figure 2. Aerobic stability responses to inoculation with *Lactobacillus buchneri* (LB)-based inoculants (LBB) with or without homofermentative or obligate heterofermentative bacteria as affected by forage type. RMD = raw mean differences between LB inoculated and uninoculated silage. HMC= high moisture corn; other forages= pea-wheat, rice, triticale, clover-ryegrass, alfalfa-ryegrass, oat, potato-wheat, sweet potato, potato hash. Adapted from Arriola et al. (2020b).

Lactobacillus buchneri increased DM losses by small amounts in meta analyses on corn and small grain silages (Kleinschmit and Kung, 2006; 1- 1.8%) and various forages (Arriola et al., 2020b; 0.3%; Figure 3). However, Rabelo et al. (2018) reported that *L. buchneri* application to sugarcane decreased ethanol production thereby reducing DM losses. Therefore, applying *L. buchneri* alone causes only small DM losses in most forages, and reduces them in sugarcane. There have been insufficient studies on obligate heterofermentative alternatives to *L. buchneri* like *L. hilgardii, L. brevis* (Danner et al., 2003), *L. kefiri* (Daniel et al., 2015) *or L. parafarraginis* (Liu et al., 2014) to determine their individual effects on aerobic stability with a meta-analysis.

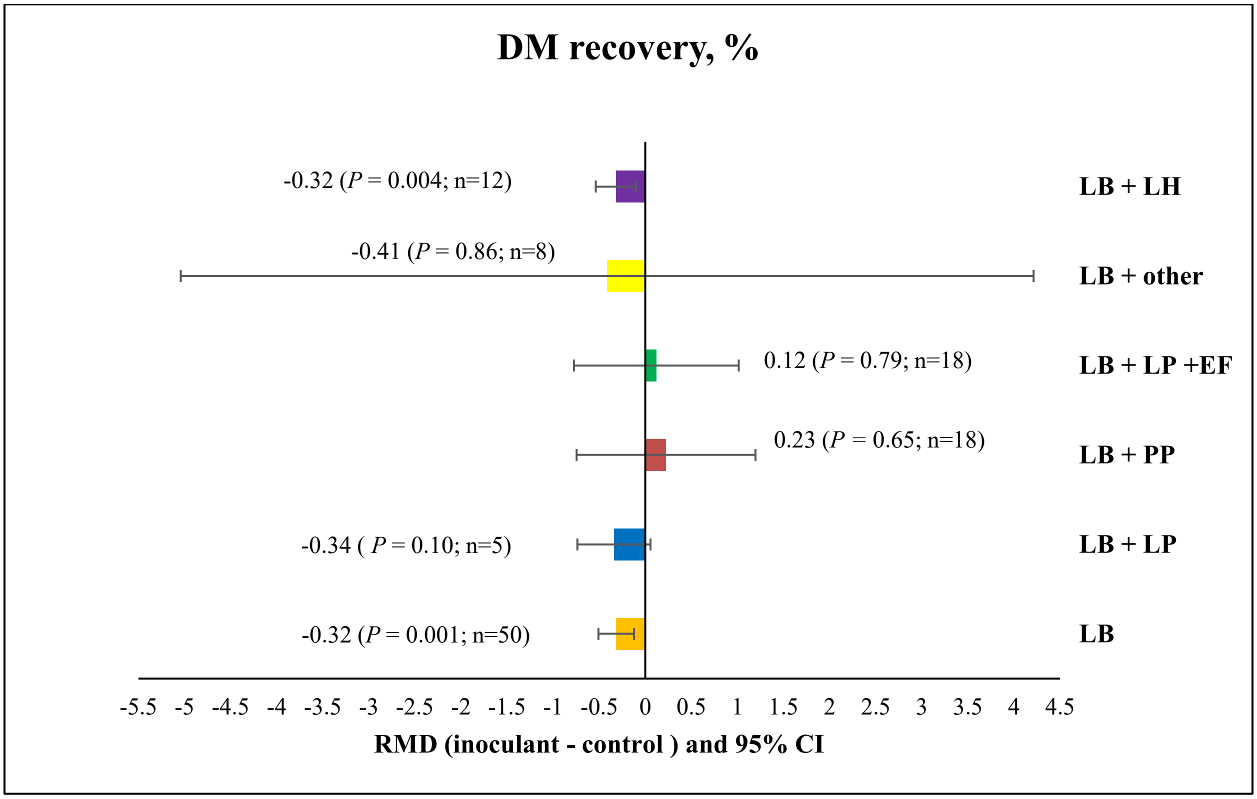

Figure 3. Lactic acid bacteria type effects on silage DM recovery responses to inoculation with *Lactobacillus buchneri* (LB)-based inoculants (LBB) with or without homofermentative or obligate heterofermentative bacteria. LB = *Lactobacillus buchneri* alone; LB+LP = *L. buchneri* with *Lactobacillus plantarum,* LB+PP = *L. buchneri* with *Pediococcous pentosaceus;* LB+LP+EF = *L. buchneri* with *L. plantarum and Enterococcus faecium;* LB+LP+PP = *L. buchneri with L. plantarum and P. pentosaceous;* LB+LH = *L. buchneri* with *Lactobacillus hilgardii*; LB+other = *L. buchneri* with other species like *Lactococcus lactis, Lactobacillus casei, Pediococcus acidilactici*. Adapted from Arriola et al. (2020b).

Obligate Heterofermentative LAB Mixtures

In the last decade, a few studies have examined combinations of *L. buchneri* with either *L. brevis*, *L. parafarraginis* or *L. hilgardii* (Avila et al., 2012, Liu et al., 2014*;* Ferrero et al., 2018, Arriola et al., 2020a). The purposes were to prevent DM losses and to achieve a greater aerobic stability than *L. buchneri.* A third purpose is to achieve aerobic stability earlier than *L. buchneri*, which requires 30 to 60 d to convert lactate to 1, 2 propanediol, and subsequently to increase aerobic stability (Muck et al., 2018). However, the results of applying mixtures of HeLab have been inconsistent. Our recent meta-analysis (Arriola et al., 2020b) showed that when a mixture of *L. buchneri and L. hilgardii* were applied to various forages, like *L. buchneri alone,* it markedly increased aerobic stability (79%) and slightly increased pH (0.7%) and DM losses (0.34%). These effects were achieved by increases in acetate (47%), 1, 2 propanediol (269%) and propionate concentrations (35%), which decreased yeast counts as well as mold counts in most cases. Similar, reductions in yeast and mold counts and increases in acetate and aerobic stability were evident in the meta-analysis of HeLAB (*L. buchneri and L. brevis*) effects on corn silage but DM losses were surprisingly increased by 50% (Bernardi et al., 2019) partly due to greater losses in farm vs. laboratory silos.

Heterofermentative with Homofermentative LAB Mixtures

Most of the inoculant studies in the last decade have examined if applying HoLAB and HeLAB together (MixLAB) would capture the benefits of both types of bacteria while overcoming their drawbacks (Filya, 2003; Arriola et al., 2011). Specifically, the aim is to exploit the improvement in fermentation and reduction in DM losses by HoLAB, while overcoming their failure to improve aerobic stability by adding HeLAB. While these combination inoculants have included various HoLAB like *L. plantarum, E. Faecium, P. acidilactici, L. lactis*, almost all have included *L. buchneri* as the HeLAB, though *L. hilgardii or L. brevis* have been used in some instances.

Various meta analyses have examined effects of MixLAB on preservation of specific forages. Our recent meta-analysis (Arriola et al. 2020b) confirmed that across several forage types, they improved aerobic stability except in whole-plant sorghum and tropical grass silages, by reducing yeast and mold counts. In addition, they improved fermentation and prevented slight increases in DM losses and pH caused by applying HeLAB alone. Therefore, our study confirmed the multiple benefits of applying MixLAB cocktails.

Other meta analyses have also shown that aerobic stability was improved by applying MixLAB to corn (Blajman et al., 2018; Zhang et al., 2018; Bernardi et al., 2019) and alfalfa (Blajman et al., 2020) silage. This was attributed to acetate-mediated reductions in yeast and mold counts. Among the latter studies, MixLAB effects on DM losses were only reported by Bernardi et al. (2019), who noted that they were increased by 50% and by 23% by HeLAB and MixLAB, respectively. These increases are much greater than the slight increases reported by Kleinschmit and Kung (2006; 1-1.8%) and Arriola et al. (2020b; 0.3%) who only analyzed studies published in English. In contrast, the study of Bernardi et al. (2019) included older studies (1980 to 2017) published in English as well as Portuguese, and Spanish. Therefore, the higher DM losses in the Bernardi et al. (2019) study may partly reflect older responses as well as different management practices and forage species used in South America versus those in North America and Europe.

Inoculants Containing other Microbes

Studies have shown some promise in using alternatives to LAB for improving silage attributes such as *Propionibacteria* for improving aerobic bacteria (Filya et al., 2004), *Streptococcus bovis* for improving the fermentation (Ferreira et al. (2013) and *Bacillus* spp. for improving aerobic stability (Lara et al. (2016), etc. Studies have also successfully inoculated forages with yeasts to preserve, multiply and deliver them to ruminants (Savage et al., 2014; Duniere et al., 2015). However, the number of studies using these LAB alternatives have been insufficient to verify their effects through a meta-analysis.

Inoculants Containing Digestibility Enhancers

Various enzymes have been added to certain inoculants to increase digestibility measures. Most of such studies have investigated effects of LAB inoculants containing "cellulase" or "hemicellulase" enzymes. These generic names do not specify the precise activities added and many studies have neither independently verified the enzyme activities nor examined effects of the bacteria with and without the enzyme. Thus, it is often impossible to ascertain if the enzyme improved digestibility. In the meta-analyses of Oliveira et al. (2017) and Bernardi et al. (2019), only 2.4% and 13% of 130 and 140 studies involved combinations of inoculants with enzymes, and enzyme inclusion had no effect on silage digestibility.

A few studies have examined the potential to use *L. buchneri* or *L. brevis* strains that improve aerobic stability but also improve secrete digestibility-enhancing esterase enzymes (Nsereko et al., 2008). However, while some of such attempts have shown promise, the results have not been consistent in corn (Kang et al., 2009; Lynch et al., 2015) or alfalfa silage (Lynch et al., 2014).

Combining HoLAB with sodium dodecyl sulphate (SDS), a surfactant, improved NDF degradability of barley silage whereas the inoculant alone only improved the fermentation (Baah et a., 2011). However, only a few of such studies exist and therefore their complementary effects on LAB have not been summarized through a meta-analysis.

Inoculant Effects on Animal Performance

Only three studies seem to have used a meta analytical approach to examine effects of inoculants on animal performance. We (Oliveira et al. (2017) examined effects of HoLAB on the performance of dairy cows and reported that across 31 studies, when at least 10^5 cfu/g were applied, milk production by dairy cattle was increased by HoLAB, regardless of the type of ensiled forage (Figure 4), LAB species and diet type but DM intake and DM digestibility were not affected. The increase in milk production was not evident when lower doses of HoLAB. In a subsequent meta-analysis of 12 studies, Arriola et al., (2020b) examined effects of HeLAB (*L. buchneri alone*) or MixLAB on the performance of dairy cows. Milk production, DM intake, DM digestibility and feed efficiency were not affected. In contrast, a meta-analysis of 35 studies on effects of applying different types of LAB to corn silage on the performance of sheep (16 studies) and beef and dairy cattle (25 studies) (Bernardi et al., 2019) reported that HoLAB or HeLAB did not affect milk yield ($P > 0.05$) but MixLAB decreased milk yield. They also reported that HoLAB increased DM intake in sheep, decreased it in beef cattle and increased in vitro DM digestibility and in vivo DM and NDF digestibility in sheep.

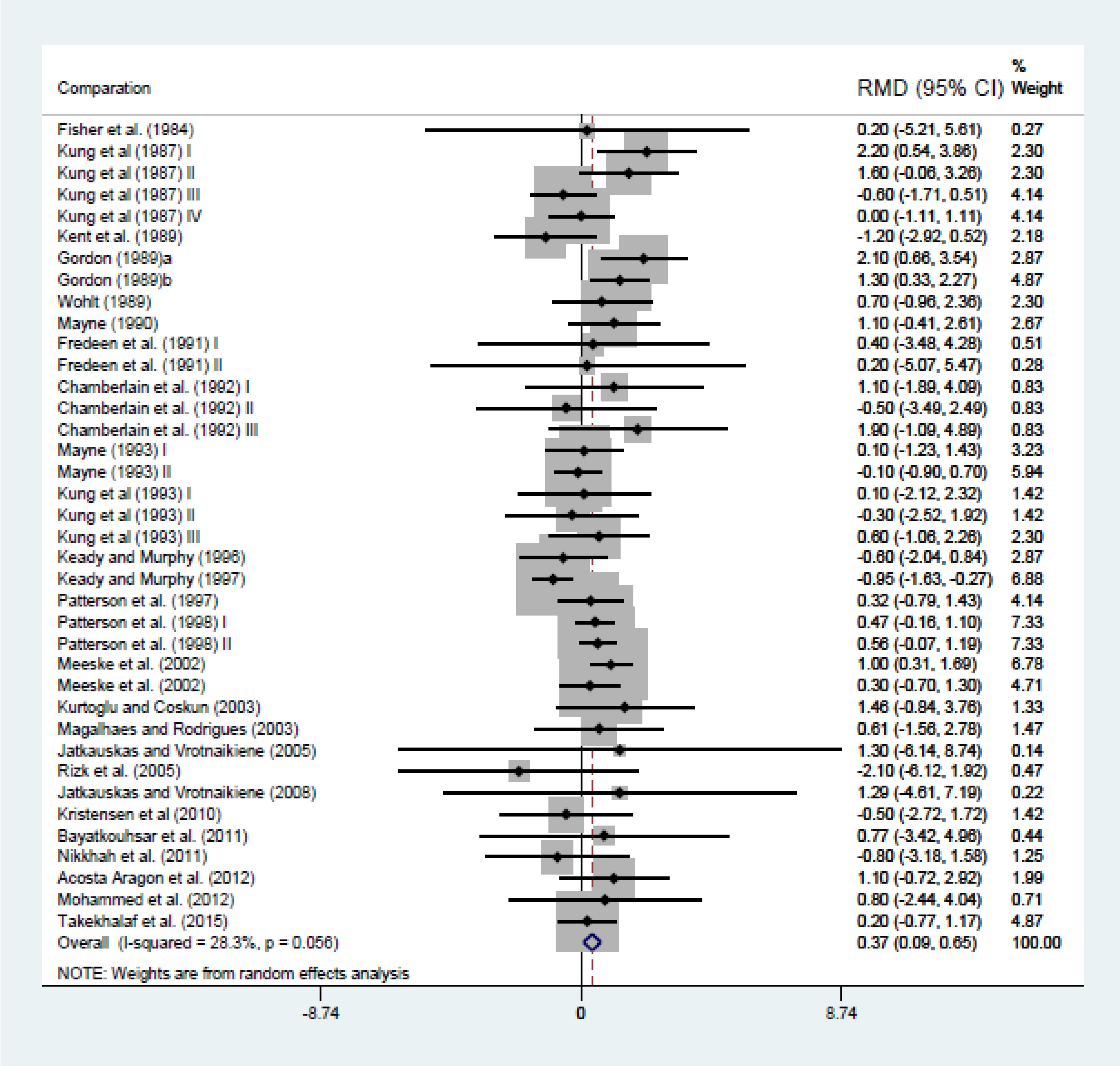

Figure 4. Forest plot showing the effect of silage inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on milk yield (kg/d) of dairy cows. The x-axis shows the raw mean difference (RMD); diamonds to the left of the solid line represent a reduction in the measure, 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 (Oliveira et al. (2017).

Differences between the dairy cow responses in our meta-analysis and that of Bernardi et al. (2019) may be due to the small number of studies involved as well as various factors pertaining to study inclusion and exclusion criteria. For instance, they included studies that were older (1983 – 2017), published in more languages (Portuguese, Spanish and English), and involved a wider range of HoLAB rates (10^4 to 10^{11} cfu/g) and more exotic cattle breeds. It can be surmised that based on the more recent studies (>1997) included in our meta-analysis, milk production is increased by HoLAB application but not by HeLAB or MixLAB. However, these trends may not be evident if older and non-English studies are included. Clearly more research is needed in this area to show if and how inoculation effects have change with time and geographical region.

Factors Affecting the Efficacy of Silage Inoculants

Several factors affect the efficacy of silage inoculants and thus influence the outcome of silage inoculant trials.

Inoculant Bacterial Composition

The bacterial composition is perhaps the most important factor influencing the efficacy of inoculants. The previous sections described the how silage fermentation pathways and products differ depending on the bacterial inoculant composition. Homofermentative bacteria should be added to improve the fermentation and DM recovery, not to improve aerobic stability. Heterofermentative bacteria be added to improve the aerobic stability not the fermentation. In addition, bacteria with the same fermentation pathway but with complementary pH niches may be combined in an inoculant. For instance, certain HoLAB combine *E. faecium or P. pentosaceus* as a starter culture with *L. plantarum* due to their ability to ferment hexoses at higher pH than *L. plantarum* (Kung, 2018). Bacteria strains also vary in efficacy; hence it is critical to select strains that have been proven in research studies.

Epiphytic Bacterial Population

Inoculants need to dominate the epiphytic bacterial population to shift the fermentation in the desired direction. McDonald et al. (1991) reported that a 10-fold domination of the epiphytic population by inoculant bacteria is required for their efficacy. Addah et al. (2014) noted that the population of inoculant LAB applied should be at least 10% greater than the natural bacteria that are on the forage. However, Lin et al. (1991) suggested that there was no relationship between epiphytic LAB numbers or species on adequacy of fermentation of alfalfa or corn silage. This disagreement may reflect the considerable variation in the types and numbers of epiphytic bacteria on different forages (100 to 1,000,000 cfu/g for alfalfa and barley and up to 1,000,000,000 cfu/g for corn; Bolsen et al., 1992; Merry and Davis, 1999; Addah et al., 2014). More research is needed to clarify the role of the species and population of epiphytic bacteria in the inoculant response.

Dose

Several authors have shown that inoculant effects on aerobic stability and DM losses, are dose dependent. Although doses examined in studies have ranged from < 10^4 to 10^{11} cfu/g, in practice the 10^4 to 10^6 cfu/g doses are most common. Most studies have shown that a dose of at least 10^5 cfu/g is necessary to reliably dominate the epiphytic bacterial population and produce the desired improvements in fermentation or aerobic stability (Kleinschmit and Kung, 2006; Oliveira et al., 2017). Higher doses ($\geq 10^6$ cfu/g) are sometimes more effective but may be uneconomical and lower doses are often less effective.

Bacterial Viability

Bacterial viability varies with prevailing conditions such as the moisture, temperature, acidity, etc. of the environment. Consequently, some manufacturers sell desiccants and oxygen scavengers with inoculants and recommend storing them in refrigerators prior to use. When testing inoculants, it is critical to verify the LAB counts and if necessary, adjust the dose, before inoculation. This is because some manufacturers add more bacteria than the recommended dose per bag to compensate for loss of viability prior to inoculation, while others add fewer. Exposure for several hours to the heat of the sun or to heat from the chopper engine can reduce viability of or kill inoculant bacteria, particularly after the inoculant is dissolved in water (Windle and Kung, 2016). This problem is more likely common when inoculant applicator tanks are close to the exhaust or engine of a chopper. Furthermore, inoculant viability may be reduced after 24 h of dissolution in water or by contaminants like chlorine or hydrogen peroxide in the water.

Mode of Application

Improper methods of inoculant application include those that involve manual application, shower-based methods, or application. These are all unlikely to be effective. Rather inoculants should be applied in a fine spray during chopping in the field to ensure uniform distribution throughout the forage mass. Proper calibration of inoculant applicators several times on the day of application critical.

Forage Type and Characteristics

The meta-analyses of Kleinschmit and Kung (2006), Oliveira et al. (2017) and Arriola et al. (2020b) revealed forage-specific effects of HoLAB and HeLAB on silage DM losses and or aerobic stability. HoLAB are more likely to be effective in forages that have high buffering capacities, high DM concentrations and low WSC concentrations and less effective in those that are too wet or immature at harvest. Fermentation is less likely to be improved by HoLAB application to well managed whole plant corn, sugarcane, or sorghum silage.

Silage Management

Delayed sealing may also reduce the rate of acidification of silage and it increases DM and energy losses. In addition, other poor silage management practices like poor sealing, low packing density, and low feedout rate can allow proliferation of spoilage yeasts and molds that increase aerobic stability (Borreani et al., 2018). Inoculant application may be more effective with these scenarios, but this should not be considered as an excuse for bad management.

Take Home Messages

1. Bacterial inoculants can be used to improve the fermentation, DM recovery, and aerobic stability of silage and the performance of dairy cows.

2. Inoculant effects vary with the species, strain and inoculation rate of the bacteria, the forage type and attributes and the storage and application method.

3. Homofermentative inoculants should be selected to improve the fermentation and reduce losses of DM, energy, and nutrients. They are particularly effective in forages with high buffering capacity or low WSC concentration and have improved milk production by dairy cows when applied at 10^5 cfu/g or greater.

4. Heterofermentative inoculants inhibit yeast and mold growth thereby increasing aerobic stability. They may cause a small increase in DM losses that is often offset by the improved aerobic stability, but they do not typically affect animal performance.

5. Combinations of homofermentative and heterofermentative bacteria may improve the fermentation, avoid, or minimize DM losses and improve aerobic stability.

6. To ensure efficacy, research-proven inoculants should be selected. They should be stored in a cool dry location and applied as recommended by the manufacturer at $\geq 10^5$ cfu/g.

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