J. Dairy Sci. 101:4132–4142
https://doi.org/10.3168/jds.2017-13901

Silage review: Foodborne pathogens in silage and their mitigation by silage additives[1]

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ABSTRACT

Silage is one of the main ingredients in dairy cattle diets and it is an important source of nutrients, particularly energy and digestible fiber. Unlike properly made and managed silage, poorly made or contaminated silage can also be a source of pathogenic bacteria that may decrease dairy cow performance, reduce the safety and quality dairy products, and compromise animal and human health. Some of the pathogenic bacteria that are frequently or occasionally associated with silage are enterobacteria, *Listeria*, *Bacillus* spp., *Clostridium* spp., and *Salmonella*. The symptoms caused by these bacteria in dairy cows vary from mild diarrhea and reduced feed intake by *Clostridium* spp. to death and abortion by *Listeria*. Contamination of food products with pathogenic bacteria can cause losses of millions of dollars due to recalls of unsafe foods and decreases in the shelf life of dairy products. The presence of pathogenic bacteria in silage is usually due to contamination or poor management during the fermentation, aerobic exposure, or feed-out stages. Silage additives and inoculants can improve the safety of silage as well as the fermentation, nutrient recovery, quality, and shelf life. This review summarizes the literature on the main foodborne pathogens that occasionally infest silage and how additives can improve silage safety.
Key words: silage, pathogen, food safety, milk

INTRODUCTION

Silages are among the most common dietary ingredients used on modern dairy and beef operations but silage quality is often measured without assessment of the presence of pathogenic microorganisms and toxins. Yet poorly made or contaminated silages can harbor pathogens (Nightingale et al., 2004; Vilar et al., 2007) that reduce animal performance (Driehuis, 2013), cause diseases of cattle (Pedroso et al., 2010), and constitute a threat to human health (Ogunade et al., 2016; Driehuis et al., 2018).

Forages are typically contaminated with pathogens when slurry is spread on the fields as a fertilizer or when forages are contaminated with soil-borne pathogens during harvest (Davies et al., 1996; Russell et al., 2000). Cattle are the main reservoir of certain pathogenic microorganisms such as *Escherichia coli* O157:H7 (Chapman et al., 1997; Mechie et al., 1997), which can enter slurry lagoons via cattle manure and subsequently be irrigated on crops. Consequently, silage, like other livestock feeds, can be an important vehicle of transmission of pathogens on the farm (Lynn et al., 1998; Pedroso et al., 2010). Inadequate silage fermentation and poor silage feed-out management favor the proliferation of pathogens in silage (Pedroso et al., 2010). The most common pathogenic microorganisms that are found in silage are *Escherichia coli*, particularly *E. coli* O157:H7, *Listeria monocytogenes*, *Bacillus* spp., *Salmonella*, and *Clostridium* spp. (Wilkinson, 1999).

Silage bacterial inoculants and chemical additives are known for their positive effects including improving fermentation, increasing DM and nutrient recovery, and extending aerobic stability. In addition to these effects, some commercial additives have demonstrated the capacity to mitigate the pathogenicity of silage, and thereby preventing the spread of pathogens on the farm. The objective of the current review is to summarize the literature on the main foodborne pathogens in silage and their mitigation by the use of silage additives or inoculants.

ENTEROBACTERIA

Enterobacteria are gram-negative facultative anaerobic bacteria. Some species of enterobacteria can

Received September 26, 2017.
Accepted November 27, 2017.
[1]This article is part of a special issue on silage management.
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use nitrate as an electron aceptor in place of oxygen (Muck, 2010). Epiphytic enterobacteria, including *Erwinia herbicola* and *Rahnella aquitilis*, often dominate fresh crops, but others supersede these during ensiling such as *Escherichia coli*, *Hafnia alvei*, and *Serratia fonticola* (Driehuis and Elferink, 2000). Enterobacteria deaminate and decarboxylate AA in silages and reduce NO_3, thereby enhancing ammonia and biogenic amine production (Pahlow et al., 2003). Enterobacteria also compete with lactic acid bacteria (**LAB**) for nutrients during fermentation (Pahlow et al., 2003); however, their growth and viability decrease as the pH declines (Heron et al., 1993). Factors that impair silage fermentation or contamination of aerobically exposed silage can provide conducive conditions for growth of these bacteria (Ogunade et al., 2017).

Escherichia coli O157:H7, a Shiga toxin producing gram-negative bacterium, is the most notorious of the enterobacteria. It is a foodborne pathogen associated with hemorrhagic colitis and hemolytic uremic syndrome, a severe illness characterized by anemia and kidney failure in children and the elderly (USDA-APHIS, 2001). The main source of milk contamination is undoubtedly fecal (Hussein and Sakuma, 2005; Farrokh et al., 2013) though an intramammary source due to pre- or subclinical mastitis is possible (Stephan and Kuhn, 1999), though controversial (Farrokh et al., 2013). Cattle are the main reservoir of *E. coli* O157:H7 and more than 30% of all cattle are asymptomatic carriers (Callaway et al., 2006; Reinstein et al., 2007). Forage and silage can be contaminated with *E. coli* O157:H7 via manure or irrigation water (Weinberg et al., 2004), and the pathogen has been commonly detected as part of the epiphytic microbial population of some forage crops (Driehuis, 2013; Ogunade et al., 2016). A rapid drop in pH has been shown to eliminate *E. coli* in silage (Bach et al., 2002; Byrne et al., 2002). Pedroso et al. (2010) evaluated the effectiveness of 3 commercial bacterial inoculants at controlling *E. coli* O157:H7 in corn silages. The pathogen was eliminated within 3 d of ensiling with or without silage inoculation when the pH dropped below 4.0. In a similar study, *E. coli* O157:H7 was eliminated from ensiled, artificially contaminated wheat and corn forages when the pH dropped below 5.0 (Chen et al., 2005). A similar result was observed for *E. coli* O26, a different pathogenic strain of *E. coli*, in corn silages (Duniere et al., 2011). The elimination of this pathogen in these studies was probably due to the inhibitory low pH, the enhanced antimicrobial activities of organic acids at low pH, or both (Bjornsdottir et al., 2006).

Pathogenic *E. coli* may persist during ensiling when the acidification rate is low (Weinberg et al., 2004; Ogunade et al., 2017). Chen et al. (2005) used an *E. coli* strain that was tagged with a green fluorescent protein and was resistant to kanamycin to inoculate wheat and corn forages, and reported that the strain survived longer in wilted wheat silages because the pH decreased more slowly than in direct-cut unwilted silages. Ogunade et al. (2016) demonstrated that compared with untreated samples, inoculation of alfalfa with *Lactobacillus plantarum* or *L. buchneri* increased the rate of pH decline, which led to earlier inhibition (7 vs. 16 d) and eventually elimination of *E. coli* O157:H7, which was added at ensiling. The slow rate of pH decline in the control alfalfa silage was attributed to the high buffering capacity, the low water-soluble carbohydrate concentration, or both. In a similar trial using corn silage, which has much lower buffering capacity than alfalfa, within 3 d of fermentation the pH had decreased below 4.0 and the pathogen had been eliminated from silages that were or were not inoculated with *L. plantarum* or *L. buchneri* (Ogunade et al., 2017). However, when all silages were subsequently reinoculated with *E. coli* after aerobic exposure, the *L. plantarum* and control silages had higher *E. coli* counts (5.39 and 5.30 log cfu/g, respectively) and higher pH values (5.67 and 6.13, respectively) compared with the *L. buchneri* silages, which had a pH value of 4.24 and an approximately 10,000-fold lower *E. coli* count.

Most of the experiments that studied the survival of pathogenic *E. coli* used laboratory silos, which are more controlled environments than farm silos. Farm silos are more prone to air penetration and soil contamination (Jonsson et al., 1990), which can enhance the growth of undesirable microbes. For instance, the presence of oxygen in the silo prolonged the survival of pathogenic *E. coli* during ensiling (Duniere et al., 2011; Driehuis, 2013). Under aerobic conditions that prevail after ensiling, factors that reduce silage acidity can increase the *E. coli* population (Donald et al., 1995). Substantial (up to 4,000 cfu/g of silage) *E. coli* populations were found in the upper corners or shoulders of commercial wheat and corn silages stored in aerobically exposed bunker silos (Weinberg et al., 2004) during the feed-out stage. The high population densities in these areas are due to the low density of the silage in the shoulders, which makes them more prone to air penetration with subsequent increased pH values and spoilage (Weinberg et al., 2004).

Pedroso et al. (2010) monitored the survival of *E. coli* O157:H7 in aerobically exposed corn silage samples experimentally inoculated with the pathogen after silo opening to mimic survival of the ensiling process by the pathogen, postensiling contamination, or both. The control silage or those treated with *E. coli* alone, or *E. coli* and a mixture of *P. pentosaceus* and *P. freudenreichii*, had high pH values (4.71, 5.67, and 6.09)

and high counts of the pathogen (2.87, 6.73, and 6.87 cfu/g), whereas those treated with *L. buchneri* alone or with *L. buchneri* and *P. pentosaceus* had lower pH values (<4) and low or no *E. coli* counts, respectively. This study showed that to prevent the growth of *E. coli* in silage, it is critical to maintain the pH below 4 during and after ensiling. This can only be achieved by good management practices such as an adequate feed-out rate as well as use of appropriate silage additives that increase the acidification rate during ensiling and prevent growth of lactate-utilizing yeasts, which increase the silage pH during the feed-out stage.

In addition to pH-mediated inhibition, bacterial metabolites may also directly inhibit the growth of pathogens. Pure cultures of *L. buchneri* and *L. plantarum* have exhibited pH-independent antibacterial activity against *E. coli* O157:H7 (Pedroso et al., 2010; Ogunade et al., 2017). Similar results on *Micrococcus luteus* and *Pseudomonas aeruginosa* were observed by Gollop et al. (2005) who studied the antibacterial effects of 10 silage inoculants (3 strains of *L. plantarum*, 2 strains of *P. pentosaceus*, 1 strain of *L. pentosus*, 3 strains of *E. faecium*, and 2 strains of *L. buchneri*). However, the specific antibacterial compounds that inhibited the growth of the pathogens were not identified in these studies. Some studies have shown that LAB including those used in silage inoculants such as *L. lactis*, *L. plantarum*, and *L. buchneri* can produce bacteriocins (Yildirim et al., 2002; Field et al., 2012). These antimicrobial peptides can eliminate or reduce the growth of other bacteria related to the strain producing them (Yang et al., 2014). *Lactococcus lactis*, for example, is a nisin-producing bacteria that has reduced butyrate and ammonia production by *Clostridium* spp. (Jatkauskas et al., 2015), but the relative contribution of nisin to the reduction is unknown. In fact, to our knowledge, no silage inoculants rely primarily on bacteriocin activity for their beneficial effects on silage quality and preservation.

Lactobacillus species have been used commercially, as an orally ingested probiotic, to reduce pathogenic *E. coli* shedding in feedlot operations in the United States. A direct-fed microbial (**DFM**) combination of *L. acidophilus* (LA51) and *P. freudenreichii* (NP24) reduced the prevalence of *E. coli* shedding by finishing steers and also improved their feed efficiency by approximately 2% (Elam et al., 2003). Peterson et al. (2007) evaluated the effect of supplementing *L. acidophilus* NP51 on the performance and reduction of *E. coli* shedding by finishing steers in a 2-yr study. No treatment effect was found on daily gain (1.67 vs. 1.69 kg/d), feed efficiency (0.147 vs. 0.150), or DMI (11.42 vs. 11.31 kg/d). However, DFM-treated steers were 35% less likely to shed *E. coli* O157:H7 than those fed the control diet. Due to the high cost of vaccinating ruminants to reduce shedding of pathogenic *E. coli* (Lueger et al., 2012), and the potential benefits of *Lactobacillus*-based DFM products (Lema et al., 2001; Brashears and Galyean, 2002), future studies should evaluate the effects of other *Lactobacillus*-based silage inoculants on fecal shedding of pathogenic *E. coli*.

LISTERIA

Listeria are opportunistic gram-positive facultative anaerobic bacteria that cause high mortality and a wide range of diseases in immune-compromised animals and humans including meningitis, encephalitis, septicemia, gastroenteritis, mastitis, and late-term abortions (McDonald et al., 1991; Wesley, 2007). It is estimated that in the United States annually, clinical cases of listeriosis can be as many as 2,500 and up to 500 deaths can result (Mead et al., 1999). Whereas the fatality rate in humans is around 20%, that of livestock can be as high as 100% (Schukken et al., 2003). *Listeria monocytogenes* (formerly *Bacterium monocytogenes*) is the main causative agent, and spoiled silage is considered to be the main source of this pathogen in ruminants (Wiedmann, 2003). This pathogen is ubiquitous and seems to be more prevalent in dairy operations (Nightingale et al., 2004, 2005; Ho et al., 2007a,b). The capacity of *L. monocytogenes* to survive in different environments and hosts is unique to non-spore-forming bacteria and is related to the fact that it can tolerate refrigeration temperatures, low water activity, and a wide range of pH. Previous studies indicated that the organism requires pH values above 5 (McDonald et al., 1991), but Ryser and Marth (2007) reported that some strains survived pH values below 4.0. The presence of *L. monocytogenes* in silages is frequently associated with poor fermentation. Vilar et al. (2007) conducted a cross-sectional study on the prevalence and source of *L. monocytogenes* in milk from 98 dairies in Spain and statistically verified the relationship between presence of *Listeria* spp. in silage and low silage quality as indicated by a high pH. Silages samples with pH equal to or above 4.5 had more samples that had *L. monocytogenes* than silages with lower pH (29.5 vs. 6.2%, respectively).

The pathogen thrives in parts of the silage where oxygen is not properly excluded or in others exposed to oxygen infiltration, because they are inhibited by low pH conditions. However, those that survive ensiling may experience a growth surge if the pH increases beyond 4.5 after silo opening. The pathogen is commonly found in baled silages because of their relatively low density, high pH, high surface area to mass ratio, and presence of aerobic pockets due to plastic damage or insufficient number of plastic layers in baled silage (McDonald et

al., 1991; Nucera et al., 2016). In Britain, outbreaks of listeriosis are more commonly associated with sheep because cattle are more resistant and sheep are more commonly fed baled silages (McDonald et al., 1991). Nevertheless, cattle are often asymptomatic carriers of the pathogen (Vilar et al., 2007).

Listeria monocytogenes can be transmitted from contaminated silages into milk. Fortunately, the pathogen is destroyed by adequate pasteurization but may survive in soft cheeses and dairy products that are not subjected to such treatments (Griffiths, 1989). Most of the research on the presence of *Listeria* in silages has focused on the fermentation characteristics of the silage. Pauly and Tham (2003) found high correlations between silage pH ($r = 0.92$), the concentration of lactic acid ($r = -0.80$), and the pooled amount of undissociated acids ($r = -0.83$) with the presence of *Listeria monocytogenes* in grass silage. The authors also demonstrated that ensiling wilted silages with high DM content (540 g/kg) did not reduce *Listeria* counts, indicating that low moisture conditions did not inhibit the pathogen. In the same experiment, *L. plantarum* inoculation reduced the *Listeria* population in direct cut (200 g/kg of DM) and wilted grass silage (430 g/kg of DM) by 2.2 and 7.9 log cfu/g. Wilson et al. (2005) confirmed the anti-listerial activity of lactic acid by using cell-free culture filtrates of MRS broth to grow a specific strain of *L. plantarum* SK1. They demonstrated that lactic acid alone reduced *Listeria* counts by 3.0 log cfu/mL of de Man, Rogosa, and Sharpe broth during the late log or early stationary phase of growth.

Bacteriocins also have been identified as agents that control *Listeria*. Mantovani and Russell (2003) demonstrated that bovicin HC5, a bacteriocin produced by *Streptococcus bovis* HC5, which causes efflux of intracellular potassium at pH below 6.0, has a bactericidal effect on *L. monocytogenes* and is capable of decreasing viability by 5 to 7 logs after 2 h of exposure. Acid-adapted *L. monocytogenes* (pH 4.6) was as sensitive to bovicin as non-acid-adapted cells. Sparo et al. (2006) characterized enterocin MR99, a bacteriocin produced by *Enterococcus faecalis* isolated from early-fermented corn silage (less than 15 d), and showed that the protein is stable over a wide range of pH (4.0–8.0) and heat resistant with proven bactericidal effect on *Listeria*. Amado et al. (2012) used pour plating to show that a combination of nisin-producing *Lactococcus lactis* CECT 539 and pediocin-producing *Pediococcus acidilactici* NRRL B-5627 with *L. plantarum* prevented survival of *L. monocytogenes* in corn and ryegrass silages within 24 h of fermentation. The authors also used the PCR–degrading gradient gel electrophoresis (**DGGE**) technique to determine if *L. monocytogenes* had become viable but not culturable during the fermentation. The DGGE profiles revealed the DNA band for the pathogen disappeared after 16 d of fermentation in untreated ryegrass silages, but it had disappeared within 2 d in inoculated silages. In the same trial, when *L. plantarum* was applied to ryegrass in combination with either *L. lactis* or *P. acidilactici*, the DNA band for the pathogen disappeared after 5 d of fermentation, which indicates a synergistic inhibitory effect when both bacteriocin-producing bacteria were inoculated with *L. plantarum*. This synergistic effect is partly attributable to the low pH achieved (<4.0) within 2 d of fermentation when the combination inoculant was used. Low pH values promote the release of nisin and pediocin from cell surfaces of *L. lactis* and *P. acidilactici*, respectively (Guerra at al., 2001), and at pH of 3.5, both bacteriocins exhibit their maximum antibacterial activity (Guerra and Pastrana, 2002).

Amado et al. (2016) evaluated the effect on *L. monocytogenes* in corn silage of pediocin alone or combined with an inoculant containing a mixture of *L. plantarum*, *E. faecium*, and *L. buchneri* (1×10^6 cfu/g). The DGGE profiles of the PCR products targeting the *iap* gene of *L. monocytogenes* disappeared within 8 d in silages treated with pediocin with or without the inoculant, but that for the untreated silage persisted until the last silo opening time on d 30 of fermentation. These results confirm that bacteriocins or bacteriocin-producing bacteria can be effective additives to control *Listeria* in silages. Follow-up studies are needed to ensure that these additives decrease shedding of *Listeria* by dairy or beef cattle and have no negative effects on their health and performance.

BACILLI

Bacillus spp. are gram-positive, aerobic or facultative anaerobic, sporulating bacteria, and their endospores are able to tolerate harsh environmental temperatures, including milk pasteurization or boiling temperatures (Pahlow et al., 2003; Masiello et al., 2014). *Bacillus* spp. and other aerobic, less acid-tolerant microorganisms, such as molds and *Listeria*, usually grow in the silage after the oxidation of lactic acid by yeast under aerobic conditions (te Giffel et al., 2002; Vissers et al., 2007a). Therefore, preventing aerobic spoilage of silage can reduce the risk of silage contamination with *Bacillus* spp. However, *Bacillus* spp. may initiate spoilage in certain instances such as under high temperatures, in big bale silages, or after treatment with formaldehyde or antibiotics (McDonald et al., 1991). Though no supporting studies were found, it is likely that more *Bacillus* spores will be present on outer layers of bales than in central, tightly packed layers due to the greater exposure to oxygen of the former. Warm summer condi-

tions also favor the increase of spore counts of thermoduric *Bacillus* spp. in corn silage and bulk tank milk samples. Buehner et al. (2014) reported that the spore counts in corn silage samples increased from 3.60 log in winter to 6.33 log during summer, and spore counts in milk samples also doubled.

Contamination of milk with *Bacillus* spores is often due to soil contamination with the pathogen, but in many cases the diet is the inoculum. *Bacillus cereus* is particularly notorious because, when ingested, its spores can pass through the digestive tract intact, contaminate the milk of dairy cows, survive pasteurization temperatures, and decrease the shelf life of milk and cream (Christiansson et al., 1999; Pahlow et al., 2003). Furthermore, enterotoxins produced by these bacteria cause foodborne illnesses, notably emesis and diarrhea (Bennett et al., 2013). A positive relationship between the number of spores in the feces and milk ($r = 0.78$, $P < 0.001$) was observed when cows were fed increasing doses of *B. cereus* spores (Magnusson et al., 2007). The authors suggested that there was an elevated risk of having increased spore levels in milk (>100/L) if the spore content in the feces exceeded 10,000 to 100,000 spores/g. That level of shedding was obtained when cows were fed 9×10^8 to 9×10^9 spores of *B. cereus*/d.

Mitigation strategies to reduce *Bacillus* spp. contamination in silage should aim to control aerobic and facultative anaerobic bacteria that are not necessarily suppressed by fermentation products or low pH (Woolford, 1975; Pahlow et al., 2003). Under proper ensiling conditions, *Bacillus* spp. should not be able to compete for carbohydrates with LAB or cause significant changes in total acid concentration or pH (Moran et al., 1993). Homofermentative LAB inoculants could be used to dominate *Bacillus* spp. and other undesirable bacteria and promote an efficient homolactic fermentation. Aerobic *Bacillus* spp. are more prone to develop on the surface and side walls of silos due to oxygen penetration and low silage density, respectively. However *B. cereus* occurs in silage, it does not increase in numbers to the same extent as other *Bacillus* spp. or aerobic spore-formers (Driehuis, 2013). Vissers et al. (2007b) quantified *B. cereus* spores in feed, feces, bedding, and raw milk on Dutch farms and concluded that the population of *B. cereus* (maximum 4.0 $\log_{10}$) in silage samples was not critical for milk contamination. Nevertheless, the number of *B. cereus* spores can vary in silages; thus, it should always be considered as a possible source of contamination.

Sorbate and benzoate are effective preservatives to minimize the growth of *Bacillus* spp. (Woolford, 1975). Thomas et al. (1993) used a gradient gel plate technique to study the effect of preservatives (sodium benzoate, sodium nitrite, and potassium sorbate), pH, temperature, and sodium chloride concentration on the growth of *B. cereus*, *E. coli*, and *S. aureus*. Potassium sorbate was the most effective preservative to control *B. cereus* at pH 6.7. The higher effectiveness of sorbate versus benzoate is mainly because the undissociated form has the antimicrobial effect and the pKa of sorbate is higher (4.75) than that of benzoate (4.2; Kung et al., 2003).

Most of the foodborne pathogen mitigation methods used in the food industry are inadequate to treat silage because they are based on factors that cannot be applied to large silage quantities in farm-scale silos. These include application of heat (75°C to kill vegetative cells and 121°C to inactivate *Bacillus* spores), UV radiation, high pressure, and pulsed electric fields (Soni et al., 2016). However, nisin is promising for controlling *Bacillus* spores in contaminated food (Kuwano et al., 2005: Bermudez-Aguirre et al., 2012). Nisin may also have a role in controlling *Bacillus* spores in silage because nisin-producing bacteria have decreased silage contamination with other pathogenic bacteria such as *L. monocytogenes* (Amado et al., 2016). Future research should examine the potential of bacteriocins and bacteriocin-producing silage inoculants to inhibit the growth of *Bacillus* spp. in silage.

CLOSTRIDIA

Clostridium spp. are gram-positive, mostly obligate anaerobic, sporulating bacteria that thrive in low-sugar silages particularly when plant moisture (>70%), pH (>4.6), temperature (>30°C), and buffering capacity are high. Consequently, they often dominate the fermentation of unwilted legumes ensiled without additives (McDonald and Whittenbury, 1973) and can also be common in unwilted tropical grass silages (Adesogan et al., 2004). The presence of *Clostridium* spp. in silage is mainly from soil contamination or slurry application and this can lead to contamination of animal feeds and products. The outer layer of baled silos and top layer of bunker silos are usually more prone to clostridial spore formation. For instance, Borreani et al. (2013) reported that the central part of a corn silage bunker silo had less than 10^3 spores of *Clostridium* spp. per gram of silage, whereas the peripheral area had more than 10^6 spores per gram. Spores present in the feed can survive digestion and get into manure or dirt from which they get onto the exterior of teats and contaminate milk (Vissers et al., 2007b). *Clostridium* spp. most commonly found in silage include saccharolytic types that ferment several sugars (e.g., *C. butyricum*), few sugars and lactic acid (e.g., *C. tyrobutyricum*), and others that ferment both sugars and AA (e.g., *C. sporogenes* and *C. perfringens*); however, those that ferment AA exclusively are uncommon in silages (Pahlow et al., 2003).

Certain saccharolytic *Clostridium* spp. derive energy for growth by fermenting sugars and lactate into butyric acid, CO_2, and H_2. Although the antifungal properties of butyric acid can enhance aerobic stability (Adesogan et al., 2004), its presence in silages can increase problems with ketosis within the herd (Andersson and Lundstrom, 1985).

Depletion of lactate at feed-out by yeast and saccharolytic *Clostridium* spp. in silage increases the pH, leading to increased growth of proteolytic *Clostridium* spp. that deaminate and catabolize AA into fatty acids (McDonald et al., 1991). Consequent increases in the ammonia concentration and protein solubility make silage protein less ideal for high-producing cattle and enhance nitrogen pollution from cows fed such silages. Furthermore, biogenic amines such as cadaverine, glucosamine, histamine, putrescine, and tyramine can be produced during clostridial proteolysis in silages. Although they are present in small quantities in all cells and can be ruminally degraded to a large extent (Van Os et al., 1995; Phuntsok et al., 1998), biogenic amines are potentially toxic. Many of these putrefaction-associated compounds are malodorous and unpalatable; therefore, they reduce feed intake by livestock. Fusi et al. (2004) showed that oral administration of biogenic amines to kids reduced DMI, growth rate, and BW and adversely affected histological characteristics and carcass quality. Furthermore, histamine is lethal at high doses, and when injected intravenously at low doses, it stopped ruminal motility and eructation (Dain et al., 1955). Intake of putrescine has also been associated with ketonemia and depressed milk production in cattle (Lingaas and Tveit, 1992).

Although *Clostridium* spp. are normal flora of ruminant digestive tracts, dietary stress, injury, management changes, and parasitism can make them produce potent toxins that cause sudden bouts of abdominal pain, diarrhea, ulceration, and even death in calves (McGuirk, 2008). Enteric syndromes in cows, humans, lambs, and monogastric livestock are also common. *Clostridium perfringens* type A is frequently found in most cows with hemorrhagic bowel syndrome, consequently it is thought to be involved in the etiology of the syndrome. Rings (2004) cited studies in which outbreaks of botulism B in cattle were reported after wrapped small-grain haylages and ryegrass silage were fed and noted that *C. botulinum* grows and produces the neurotoxin when silage fermentation fails to achieve a pH less than 5.3. However, occurrence of *C. botulinum* in silages is rare (Driehuis and Elferink, 2000). An added clostridial problem is that spores transmitted from silage into milk can form outgrowths or gas pockets that double the size of cheese due to butyric fermentation. The resulting phenomenon, late blowing of cheese, and the large quantities of butyric acid produced by clostridial fermentation result in a rancid odor and tainted flavor of cheese (Cocolin et al., 2004).

Clostridium spp. are known to be intolerant of low (4.5) pH (Kaiser and Weiss, 1997; Kaiser et al., 1997); therefore, any additive, such as homofermentative bacteria, that can increase lactate concentration and hasten the pH drop during fermentation should inhibit clostridial activity in silage. When crops low in clostridia-inhibiting nitrates, such as grasses and immature whole crop cereals, are ensiled, LAB additives that ensure rapid acidification can reduce clostridial spores in the silage (Pahlow et al., 2003). The minimum number of epiphytic or added LAB (or both) required to inhibit clostridial growth is at least 100,000 cfu/g on a fresh weight basis (Weissbach and Honig, 1996; Kaiser et al., 1997). In contrast, silage additives that reduce acidification and increase the pH can lead to a clostridial fermentation. Custódio et al. (2016) reported that treating sugarcane silage with 15 g/kg of lime resulted in higher pH (3.62 vs. 4.8) and higher *Clostridium* spp. count (3.3 vs. 6.7 log cfu/g) than the untreated silage. Various doses of sodium nitrite (0, 0.5, 1.0, or 1.5 g/kg as fed) did not reduce the clostridial growth in the lime-treated silage.

Wilting also depresses clostridial growth (van Schooten et al., 1989) because of the affinity of *Clostridium* spp. for moisture. Clostridial spore counts decreased as DM content of grass at ensiling increased from 30 to 45% (Hengeveld, 1983).

Chemical additives can also reduce clostridial spores in silage. Application of a mixture of sodium benzoate, sodium nitrite, hexamine, and sodium propionate inhibited clostridial growth in low-wilted forages (Lättemäe and Lingvall, 1996). Similar results were obtained by Jonsson et al. (1990). Combinations of sodium nitrite and hexamine have also been effective at inhibiting *Clostridium* spp. (Lättemäe and Lingvall, 1996) as well as nitrite alone (Spoelstra, 1985).

SALMONELLA

Salmonella are gram-negative, facultative aerobic, motile rods, and enteric bacteria (Madigan et al., 2009). *Salmonella enterica* is an important pathogen that causes about 42,363 cases of foodborne illness per annum in the United States (Scallan et al., 2011). Peaks in the number of salmonellosis outbreaks are frequently associated with intake of raw or improperly pasteurized milk. *Salmonella enterica* is relatively common on and is represented by diverse serotypes on dairy farms (Callaway et al., 2005). Common symptoms of salmonellosis in cattle are watery or bloody diarrhea, dehydration, fever, depression, and poor performance.

A survey evaluating the prevalence of salmonella and other pathogenic bacteria in US dairies demonstrated that 22 out of 861 bulk tank milk samples were contaminated with 9 different types of *Salmonella* serotypes, including the Newport serotype, which is known to exhibit resistance to multiple antimicrobials (multidrug resistant; Van Kessel et al., 2004). An observational study in Washington State determined that the rate of new multidrug-resistant *Salmonella* strain introduction was 0.9 per herd-year (Adhikari et al.,2009), whereas in Wales and England a similar study reported an incidence rate of 0.43 cases of salmonellosis per farm-year for any serovar of *Salmonella enterica* (Davison et al., 2006). The transmission of *Salmonella* to herds seems to be more associated with management as the introduction is typically via contaminated animals, tools, and people, and less related to feed source (Langvad et al., 2006; Nielsen et al., 2007; Adhikari et al., 2009). In fact, Hanson et al. (2016) provide evidence of vertical transmission from a contaminated dam to her fetus such that dairy calves were born with the infection.

In the Washington State survey, from a total of 4,582 pooled feed samples from 59 herds and 665 samples from feed mills, only 3.5% were positive for *Salmonella* spp., including one corn silage sample that was positive for *Salmonella typhimurium* (Adhikari et al., 2009). The use of sewage water or slurry to irrigate fields dedicated to silage making is an important risk factor for silage contamination with *Salmonella*. Weinberg et al. (2004) evaluated fresh plant and silage samples of sewage-irrigated sorghum and corn fields in Israel and found only 1 silage sample was positive for *Salmonella* out of 5 corn silage samples. The contaminated sample was from a field were the fresh plant samples were also positive for *Salmonella*. Natural disasters such as storms, hurricanes, flooding, hailstorms or lodging can cause soil sediment contamination of forages and increase epiphytic counts of *Salmonella* and other pathogens. Nevertheless, if the fermentation is well established and a low pH (<4) is achieved, pathogens that require high pH for growth such as *Salmonella* will not survive. Therefore, the appearance of the pathogen in silage reflects poor silage-making practices or contamination after ensiling.

Ensiling duration seems to be an important factor affecting the population of *Salmonella*, especially in silages with low rates of pH decline. Johansson et al. (2005) examined the presence of *Salmonella* in artificially contaminated (1×10^8 cfu/g) ryegrass ensiled at 39 and 61% of DM for 0, 7, and 60 d of fermentation. All the 20 silage samples from d 0 and 7, were positive for *Salmonella*, whereas only 1 out of 5 samples from the wetter silage was positive after 60 d.

In general, the incidence of *Salmonella* contamination of silages samples is low and contamination is usually only a problem if the silage fermentation is inadequate. Consequently, no studies on inhibition of *Salmonella* by silage additives or inoculants were found. Nevertheless, studies on additive inhibition of the growth of *Salmonella* that contaminates aerobically exposed silages are needed to avoid entry of the pathogen into the food chain. Silage inoculants and organic acids such as propionic and acetic acid may be effective based on their use to control the pathogen in feed ingredients (Carrique-Mas et al., 2007; Jones, 2011).

STRATEGIES FOR ENSURING THE HYGIENIC QUALITY OF SILAGE

Preventing contamination of silage and animal products with pathogens requires the identification of critical control points (Hazard Analysis and Critical Control Points) that are related to contamination and replication of pathogenic silage organisms on the farm (Lynn et al., 1998). Silage contamination with pathogenic microorganisms can occur before, during, or after ensiling, and it is critical that adequate control measures are used at each of these stages to prevent contamination.

Before and during ensiling, management practices that favor rapid homolactic fermentations should be ensured because a rapid pH drop is critical to inhibiting *Clostridium* spp. and enterobacteria, which cause proteolysis and secondary butyric fermentation. Specific measures include

1. Choosing forages with a high water-soluble carbohydrate–to–buffering capacity ratio, where available hybrids resistant to fungi should be used.
2. Harvesting at appropriate moisture concentrations for ensiling and optimizing nutritive value and biomass yield.
3. Wilting in a way that prevents proteolysis but increases DM concentrations to about 35% for grasses and 45% for legumes.
4. Chopping forages to lengths that facilitate compaction but retain the physical effectiveness of the fiber.
5. Unloading forages promptly into silos lined with appropriate plastic sheets.
6. Compacting forages to a density of about 240 kg of DM/m^3 in the silo.
7. Sealing the silo promptly with appropriate sheets and maintaining anaerobic conditions for

the duration of ensiling by regularly sealing any holes that develop in the plastic cover.

8. Additives are not always necessary for good fermentation, but they are particularly useful for enhancing the fermentation of crops with high buffering capacities, low water-soluble carbohydrate concentrations, or high moisture concentrations. Additives containing molasses, nitrate, inorganic and formic acids, buffered acids, or least 10^5 cfu/g of specific LAB (*L. plantarum*, *Pediococcus acidilacti*, *P. pentosaceous*, and *Enterococcus faecium*) have enhanced homolactic fermentation by inhibiting undesirable bacteria, or increasing the rate of acidification, or dominating the flora. However, various additive and management-related factors determine additive efficacy. Therefore, more detailed reviews on the subject, such as that of Kung et al. (2003) and Muck et al. (2018), should be consulted before choosing an additive. Additives have also had secondary benefits for instance, inoculation with *L. casei* has successfully reduced the biogenic amine concentration of different silages, but *L. buchneri* inoculation had inconsistent effects (Nishino et al., 2007).

Yeasts, molds, *Listeria*, and enterobacteria that survive anaerobic fermentation grow rapidly when the pH is elevated during aerobic spoilage. Therefore, management practices that ensure the aerobic stability of silages and prevent increases in pH at feed-out are critical. To ensure aerobic stability, the following steps should be implemented:

1. Silo design should minimize the size of the silo face as wider faces facilitate oxygen ingress.
2. Where appropriate, shavers should be used to ensure smooth silo faces to minimize the surface area exposed and reduce oxygen ingress into the silage.
3. Silages should be fed out at rates that minimize the length of time the face is exposed to the air; feed-out rates of 5 to 10 cm/d from tower silos, 10 to 15 cm/d from bunker silos, and 30 or more cm/d from bag silos have been recommended in the United States, whereas in Israel rates of 20 to 30 cm/d are recommended (Muck et al., 2003). Feed-out rates in tropical areas should be at least 30 cm/d (Whitlow and Hagler, 2009) because warm humid conditions enhance the growth of spoilage organisms.
4. Silage aerobic stability can be enhanced with propionic, acetic, sorbic, and benzoic acid because of their antifungal nature. These compounds are also sold as mold inhibitors. *Lactobacillus buchneri* degrade lactate to acetate, which inhibits the growth of yeasts and molds, thereby improving aerobic stability (Driehuis et al., 1999). Consequently, *L. buchneri* inoculants have been successfully used to improve the aerobic stability of several forages. Pedroso et al. (2010) reported that *L. buchneri* inoculants enhanced the aerobic stability of corn silages by increasing acetate production to levels that inhibited yeasts and minimized or prevented the attendant increases in pH. Therefore, these *L. buchneri* inoculants curtailed the growth of *E. coli* O157:H7 in silages contaminated with the pathogen after silo opening. All of these additives should be uniformly distributed in the silage for maximum efficacy.
5. Antioxidants such as vitamin E and selenium; mold inhibitors such as propionic, sorbic, acetic, and benzoic acids; and mycotoxin binding adsorbents have been successfully used to reduce the risk of mycotoxicoses and prevent the transmission of aflatoxins into milk (Diaz et al., 2004; Whitlow and Hagler, 2009). *Lactobacillus buchneri* inoculation has also prevented aflatoxin synthesis in silages produced from corn plants infested with high levels of Southern rust (Queiroz et al., 2012).

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