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]

O. C. M. Queiroz,* I. M. Ogunade,† Z. Weinberg,‡ and A. T. Adesogan§[2]
*Animal Health and Nutrition, Ch. Hansen, Buenos Aires, 1107, Argentina
†Division of Food and Animal Science, College of Agriculture, Food Science and Sustainable Systems, Kentucky State University, Frankfort 40601
‡Department of Food Science, Agricultural Research Organization, the Volcani Center, Bet Dagan, 50250, Israel
§Department of Animal Sciences, Institute of Food and Agricultural Science, University of Florida, Gainesville 32606

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.
[2]Corresponding author: adesogan@ufl.edu

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)