Silage pathogenicity and implications for the ruminant production chain

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The objective of this paper is to discuss pathogenic organisms and other harmful agents that may be present in silages, consider their implications for ruminant animal production, and to recommend management practices that are critical to ensuring the hygienic quality of silages.

Pathogens and harmful agents in silages

Yeasts

Unlike those on fresh crops that are nonfermentative, silage yeasts are facultative aerobic fungi that can tolerate oxygen deprivation and low pH for prolonged periods. Although, these conditions inhibit their growth, yeasts remain viable during the anaerobic fermentation phase by deriving energy from fermentation of plant sugars into ethanol and carbon–di-oxide predominantly, especially when small quantities of oxygen are present (McDonald et al., 1991). This fermentation depletes some of the energy and fermentable substrates that would have been available to ruminants feeding on the forage, but the main adverse effects of silage yeasts occur after the ensiling phase. Active yeast growth and respiration resumes when oxygen infiltrates the silage mass after silo opening. Pahlow et al. (2003) reported that yeasts of the genera *Candida* and *Hansenula* (*Pichia*) are particularly prolific during the aerobic phase because of their lactate assimilating ability and their strong affinity for glucose. These yeasts typically outcompete *Sachharomyces cerevisiae*, which does not assimilate lactate but is the dominant yeast at the end of ensiling in sorghum and corn silages (Sanderson, 1993). In most cases, yeasts are the initiators of aerobic deterioration (Pahlow et al., 2003). Lactate assimilation by yeasts increases the pH after silo opening, leading to a more conducive environment for the proliferation of pathogenic organisms like molds, *Listeria*, and *Bacilli* that worsen aerobic deterioration.

An additional major problem arising from aerobic spoilage is the development of inedible waste due to silage oxidation. McDonald et al. (1991) cited studies showing that up to 75% of the original forage DM was lost as waste; furthermore, waste-associated DM losses ranged from 3.4 to 51.5% across studies. Certain yeasts have also been associated with the incidence of mastitis in cattle. For instance, Elad et al. (1995) implicated yeasts from wheat silage as the cause of the onset of acute mastitis in cattle though the condition only persisted when milking hygiene was inadequate.

Molds and mycotoxins

Silage molds are filamentous aerobic fungi that promote silage aerobic deterioration thereby depleting nutrients and enhancing DM losses. Except for *Penicillium* spp, common silage molds (*Monascus, Fusarium, Aspergillus, Rhizopus,* and *Geotrichum* spp) are less tolerant of low oxygen and low pH conditions than silage yeasts (Pahlow et al., 2003). Consequently, in well-prepared intact silos, molds are found mainly in parts exposed to oxygen infiltration such as the top layer or sides; whereas in open silos, molds proliferate throughout the silage in areas where the pH is high (McDonald et al., 1991). Like yeasts, molds degrade sugars and lactic acid in silages but they also degrade cellulose, which is one of the most important energy sources for ruminants.

Although several molds produce mycotoxins, *Aspergillus, Penicillium* and *Fusarium* molds are the most frequent causes of decreased feed value and livestock poisoning. *Aspergillus fumigatus* molds are responsible for causing aspergillosis in livestock and man. This disease is characterized by inflammation and nodulation of various nasal and intestinal passages and it occurs in cattle, sheep, horses, pigs and man. Smith and Lynch (1973) reported that feeding moldy, *A. fumigatus*-contaminated corn to pregnant beef cows resulted in irritability, incoordination, and orange-colored livers and about 8% of cows died at parturition or shortly afterwards. Certain species of *Aspergillus* as well as thermophilic *Actinomyces* also cause Farmers Lung. This is a type of hypersensitivity pneumonitis resulting from inhalation of moldy forage or silage and it causes respiratory problems and occasional mortality (Wild and Chang, 2009).

Another disease of immunocompromized dairy cattle often associated with silage contaminated with *Aspergillus fumigatus* is hemorrhagic bowel syndrome (HBS), also known as hemorrhagic jejunal syndrome (HJS) or bloody gut disease. This recently discovered syndrome causes sudden reductions in feed intake and milk production, intestinal hemorrhaging, and sudden death of cattle. It is responsible for the death of about 2% of dairy animals in the US (Baker, 2002). *Aspergillus fumigatus* has been proposed as the agent causing with HBS in dairy cattle because the incidence of the disease has been associated with ingestion of moldy hay containing the pathogen; also high populations of the fungus are often present in the blood of affected cows (Puntenney et al., 2003). However, others have implicated *Clostridium perfringes* Type A in the etiology of the syndrome because the bacteria has been found in tissues and feces of affected animals (Kirkpatrick et al., 2001; Dennison et al., 2002). Acidotic diets are thought to contribute to the problem, because acidic conditions predispose *C. perfringes* to toxin production and the classical symptoms. However, this pathogen is also found in the intestines of healthy animals therefore its role in the pathogenesis of the syndrome is unclear (Berghaus et al., 2005). Although, the exact cause of the disease remains unknown, suggestions that moldy or clostridial silages are involved in the etiology of the disease emphasize the importance of ensuring good silage management practices to minimize outbreaks.

The most notorious pathogenic effect of molds probably occurs via the mycotoxins they produce. Mycotoxins are poisonous secondary metabolites that can reduce feed intake, growth, and milk production and also cause diseases, reproductive problems and death in livestock. The most common and most problematic mycotoxins are those produced by Penicillium (PR toxin, mycophenolic acid, roquefortine C, patulin), Fusarium (deoxynivalenol, zearalenone, T-2 toxin), and Aspergillus (aflatoxin, gliotoxin, fumitremorgens, fumigaclavines), but others may also be

present (Whitlow, 2009). However, little is known about conditions that optimize mycotoxin production by molds.

Kiessling et al. (1984) showed that zearalanone, ochratoxin A, diacetoxyscirpenol, and T-2 toxin were rumen degradable but deoxynivalenol and aflatoxin B_1 were not. However, these authors noted that some of these were not completely degraded and some of the degradation products remained toxic. Therefore, the extent of ruminal degradation of such toxins appears to be variable; situations resulting in a faster rate of ruminal feed passage or a low population of protozoa in the rumen may reduce mycotoxin degradation in the rumen (Whitlow and Hagler, 2009).

Driehuis (2000) indicated that little or no dietary zearalenone, deoxynivalenol ochratoxin A is transmitted into the milk of dairy cows. In contrast, aflatoxin B_1, the most toxic (carcinogenic) and widespread of the aflatoxins, can be passed from contaminated silage into milk where it is excreted as aflatoxin M_1. On average, milk aflatoxin M_1 concentrations are approximately 1.7% of the aflatoxin B_1 concentration in the total ration dry matter (Whitlow, 2005). Dietary levels of B_1 above 100 ppb can compromise the performance of dairy cattle, and cause kidney damage in beef cattle (Garett et al., 1968; Whitlow, 2005). Aflatoxin is the only mycotoxin with Food and Drug Administration (FDA) action levels in the US. These are 20 ppb in most feeds and 0.5 ppb in milk.

Mycotoxin production can occur during plant growth in the field leading to uniform contamination of silage, or in hotspots where oxygen pockets allow mold growth during ensiling or on the silo face at feedout (Adesogan, 2006). Delayed harvesting, slow or delayed filling, inadequate packing and sealing, slow feedout rates and damaged bunker or bag plastic can lead to pockets of mycotoxin production (Whitlow and Hagler, 2009). Recent work also showed that aflatoxin levels in rust-infested corn silage exceeded actionable levels stipulated by the US Food and Drug Administration (Queiroz et al., 2009). Other factors such as insect damage may have similar effects. Therefore, it is imperative that management practices that avoid these predisposing factors are implemented to minimize the risk of mycotoxicoses in ruminants and humans.

Bacilli

Bacilli are gram positive, facultatively anaerobic, sporulating bacteria; and their endospores tolerate harsh environmental temperatures including milk pasteurization or boiling temperatures (Pahlow et al., 2003). *Bacilli* are probably the first groups of microorganisms to develop in deteriorating silages after yeasts initiate the process (Pahlow et al., 2003). However, *Bacillus* species 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).

Bacillus cereus is particularly notorious because 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 this bacteria cause foodborne illnesses, notably emesis and diarrhea (Ankolekar et al., 2008).

Listeria

Listeria are opportunistic gram-positive aerobic or facultatively anaerobic bacteria that cause high mortality rates and a wide range of diseases in immunocompromized animals and humans including meningitis, encephalitis, septicicaemia, gastroenteritis, mastitis and abortions (McDonald et al., 1991). *Listeria monocytogenes* (formerly *Bacterium monocytogenes*) is the main causative agent and the main source of the pathogen in ruminants is spoiled silage (Wiedman, 2003). This facultatively anaerobic bacterium is ubiquitous as it can tolerate refrigeration temperatures, low water activity, and a wide range of pH. Previous studies indicated that the organism required pH values above 5 (McDonald et al., 1991) but Ryser et al. (1997) demonstrated that some strains survived pH values below 4. 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 low silage quality (as indicated by high pH) and presence of *Listeria* spp. in silage (29.5 vs. 6.2% for pH above or below 4.5, respectively). The pathogen is commonly found in baled silages because of their relatively low density, high pH and high surface area to mass ratio (McDonald et al., 1991). In well-prepared bunker silages, *L. monocytogens* only thrives in areas exposed to a prolonged low rate of oxygen infiltration because they are inhibited by low pH conditions. However, those that survive ensiling may experience a growth surge if the pH increases after silo opening (Figure 1). 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 (Villar et al., 2007). *Listeria* monocytogenes can be transmitted from contaminated silages into milk. Fortunately, the pathogen is destroyed by adequate pasteurization but it may survive in soft cheeses and dairy products that are not subjected to such treatments (Griffiths, 1989).

Clostridia

Clostrida are gram positive, mostly obligately anaerobic, sporulating bacteria that thrive in low-sugar silages particularly when plant moisture (>70%), pH (>4.6), temperature (>30^{o}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 forages (Adesogan et al., 2004). Clostridial presence in silage is mainly from soil contamination or slurry application and this can lead to contamination of animal products (Figure 2). Those most commonly found in silage include saccharolytic types that ferment sugars and organic acids (e.g. *C. butyricum* and *C. tyrobutyricum*) as well as others that ferment both sugars and amino acids (e.g. *C. sporogenes* and *C. perfringens*); however, those that ferment amino acids exclusively are uncommon in silages (Pahlow et al., 2003). Certain saccharolytic *Clostridia* 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 pungent, acrid odor typically depresses intake in ruminants.

Depletion of lactate at feedout by saccharolytic *Clostridia* in silage increases the pH thereby enhancing the growth of proteolytic *Clostridia* that deaminate and catabolize amino acids into fatty acids (McDonald et al., 1991). Consequent increases in the ammonia concentration and protein solubility of silages make the silage protein less ideal for highly productive 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), some are potentially toxic. Many of these putrefaction-associated compounds are malodorous and unpalatable, therefore they reduce feed intake by livestock (Table 1). Fusi et al. (2004) showed that oral administration of biogenic amines to kids reduced dry matter intake, growth rate, and body weight and adversely affected the 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 et al., 1992).

Although *Clostridia* 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, 2009). Enteric syndromes in cows, humans, lambs, and monogastric livestock are also common . *Clostridium perfringes* 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. This phenomenon is called late blowing of cheese and the large quantities of butyric acid produced by clostridial fermentation in the cheese result in a rancid odor and taint the flavor (Cocolin et al., 2004).

Enterobacteria

Enterobacteria are gram positive facultatively anaerobic bacteria. Epiphytic enterobacteria including *Erwinia herbicola* and *Rahnella aquitilis* often dominate fresh crops, but these are superseded by others like *Escherichia coli, Hafnia alvei,* and *Serratia fonticola* during ensiling (Driehuis and Elferink, 2000). Although enterobacteria actively compete with lactic acid bacteria in the early stages of ensiling, they are inhibited once the pH drops below 4.5 (Pahlow et al., 2003) but those that survive ensiling can start growing actively when the pH increases after aerobic deterioration (Driehuis and Elferink, 2000). Like *Clostridia, Enterobacteria* deaminate and decarboxylate amino acids in silages, thereby enhancing ammonia and biogenic amine production and increasing the risk of depressed intake and ineffiecient N utilization by livestock.

Escherichia coli O157:H7, a shigatoxin producing gram-negative bacteria is the most notorious of the enterobacteria. It has emerged as an important cause of food borne disease. In children and the elderly, it initially causes acute bloody diarrhea but this may evolve into hemolytic uremic syndrome, a severe illness characterized by anemia and kidney failure. In ruminants, the pathogen can cause intestinal disorders and mastitis (Weinberg et al., 2004). Cattle are the main

reservoir of *E. coli* O157:H7 and the pathogen may be present in feces, milk, and feed of dairy cows (Armstrong et al., 1996; Mechie et al., 1997; Chapman et al., 1997; Lynn et al., 1998). Silage can be contaminated with *E. coli* O157:H7 via manure or irrigation water (Weinberg et al., 2004) but the pathogen disappears from contaminated silages when the pH drops below 4 - 5 (Bach et al., 2002; Chen et al., 2005; Pedroso et al., 2009). However, the pathogen has been found in decaying commercial silages with relatively high pH values and it survived for three weeks in grass silages (pH 4 to 4.6) recontaminated with the pathogen (Reinders et al., 1999). Therefore, it is critical that silage pH is kept below 4 during and after ensiling to prevent the growth of the pathogen.

Nitrates and nitrites

Plants transport nitrogen from roots to leaves in the form of nitrates but toxic levels can accumulate due to manure fertilization, plant photorespiration under high temperatures and moisture, injury from herbicides, and environmental stressors such as drought (Provin and Pitt, 2009). Nitrate toxicity causes reproductive problems and can be fatal to cattle or humans (Hill, 1999; Weinberg et al., 2004). Normally, ingested nitrates are converted to nitrite and then ammonia, such that they can be used to meet tissue N requirements. However, when ingested excessively, nitrites accumulate in the blood and bind to hemoglobin forming methmoglobin, which has a low oxygen carrying capacity (Provin and Pitt, 2009). Silage enterobacteria are usually effective at degrading nitrates (McDonald et al., 1991), but the product, nitrous oxide is also hazardous as it causes a respiratory problem in farm workers known as 'silo fillers disease' (Weinberg, 2004).

Strategies for ensuring the hygienic quality of silage

Preventing contamination of silage and animal products with pathogens requires the identification of critical control points (HACCP) 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 *Clostridia and Enterobacteria,* which cause proteolysis and secondary butyric fermentation. Specific measures include:

1. Choosing forages with a high WSC 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 reduces moisture concentrations to about 65% for grasses and 55% 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 WSC concentrations or high moisture concentrations. Additives containing molasses, nitrate, inorganic and formic acids, buffered acids or least 10^5 cfu/g of specific lactic acid bacteria (*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) 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 feedout 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; feedout 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 US, whereas in Israel rates of 20 to 30 cm/d are recommended (Muck et al., 2003). Feedout rates in tropical areas should be at least 15 cm/d (Whitlow and Hagler, 2009) because warm humid conditions enhance the growth of spoilage organisms.

4. Silage aerobic stability can be been enhanced with propionic, acetic and sorbic acids and to a lesser extent 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. (2009) 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 (Figure 3). All of these additives should be uniformly distributed in the silage for maximum efficacy.

5. Antioxidants like vitamin E and selenium, mold inhibitors like 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.

6. When animals show symptoms of mycotoxicoses, mycotoxins binders should be added to the diet and the suspect silage should be withdrawn from the diet. Regular use of mycotoxins binders in healthy animal diets as a preventative measure may be cost prohibitive. The literature suggests that mycotoxin binders are specific in their activity and none binds all mycotoxins. Therefore, where possible, rapid identification of specific problematic mycotoxins should be ensured before addition of a binder.

7. Moldy samples of forage should be discarded and it is important to note that mold counts often do not correlate with mycotoxins levels in forages.

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Table 1. Effect of intake of high levels of biogenic amines from alfalfa silage on DMI, ruminal DM digestibility (RDMD), total tract DM digestibility (TTDMD), and ruminal outflow (RDMOF) in steers (Phuntsok et al., 1998)

	Alfalfa silage level				
Item	0%	33%	67%	100%	SE
Biogenic amine intake, g/d					
Putrescine[a]	1.10	3.10	4.91	6.45	0.16
Cadaverine[a]	1.22	4.01	6.39	8.52	0.21
Histamine[a]	1.32	3.40	5.31	6.16	0.20
Performance indices					
DMI,[a] kg/d	8.18	7.13	7.19	6.07	0.30
RDMD,[b] %	48.50	45.86	46.67	43.61	1.36
TTDMD,[c] %	67.14	73.46	71.21	66.75	2.00
RDMOF,[b] kg/d	4.25	3.89	3.84	3.41	0.22

[a]Linear effect caused by treatment ($P < 0.01$).
[b]Linear effect caused by treatment ($P < 0.05$).
[c]Quadratic effect caused by treatment ($P < 0.05$).

Figure 1. The effect of aerobic deterioration on silage pH and the survival of L. monocytogenes at two sites in laboratory-scale bagged silage (McDonald et al., 1991)

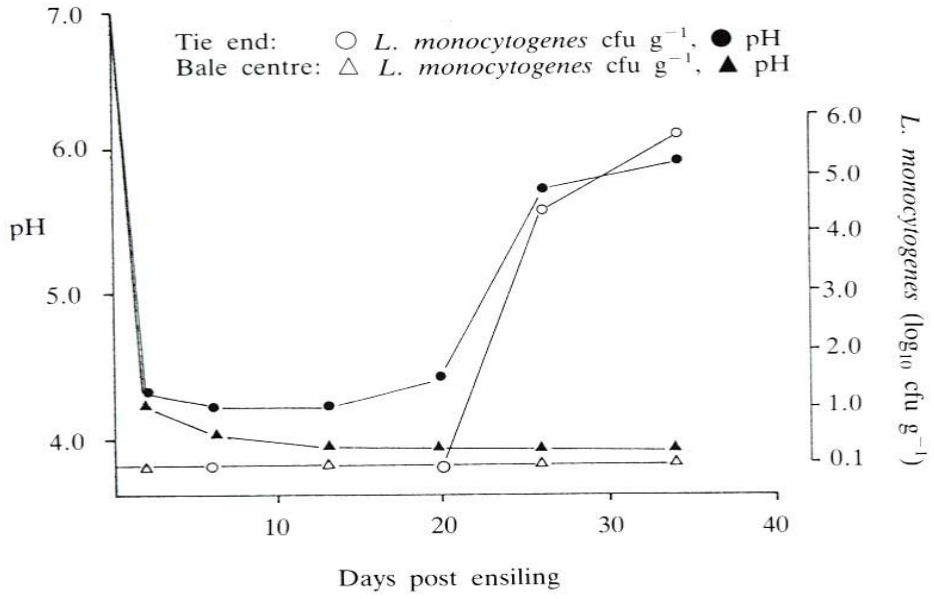

Figure 2. Production chain of a dairy farm with silage-based feeding; Main risk factors for Clostridia spore contamination (Weissbach, 2006)

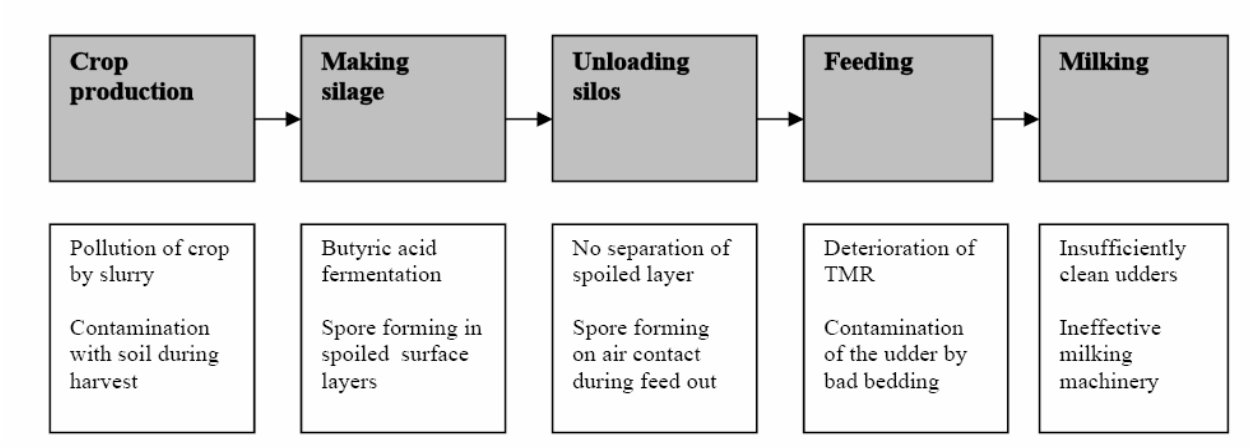

Figure 3. Effect of reinoculation of corn silages with 1×10^6 cfu/g of *E. coli* O157:H7 (EC) 144 h after silo opening (d 82) on pH and *E. coli* counts (EC; log cfu/g) 24 h later.
[1]BII = 1×10^6 cfu/g of *Pediococcus pentosaceus* and *Propionibacterium freudenreichii*; LB = 1×10^6 cfu/g of *Lactobacillus buchneri*; B500 = 1×10^6 cfu/g of *P. pentosaceus* and *L. buchneri*. S.E. values for pH and *E. coli* data were 0.41 and 1.04, respectively.