Ruminant Disorders Associated with Pathogens Found within Ensiled Forages

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Abstract

Properly ensiled forages and grains are bio-secure when fed to dairy cows.[7] Silage management is, therefore, important to dairy health and profitability. Veterinarians offering production medicine consultation to dairy producers must be knowledgeable about ensilage. Pathogens are present in silage regardless of how well it is preserved. Problems that may occur include ensiling forages: 1) too wet, resulting in clostridial fermentation and causing reductions in voluntary feed intake, 2) that deteriorate upon extreme aerobic instability, resulting in growth of *Listeria monocytogenes,* and causing listeriosis, and 3) too dry, producing aerobic instability problems with molds and mycotoxins.

Fermentation Dynamics

A review of the fermentation process will help in the understanding of potential pathogens that may be present within ensilage. Aerobic and anaerobic microorganisms are involved in silage fermentation[2] (Figure 1).

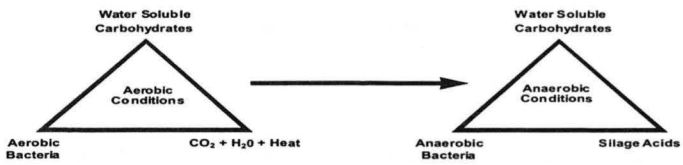

Figure 1. Fermentation dynamics

Aerobic activity occurs while the silo is being filled and at feedout. Good silo management minimizes aerobic activity, thus reducing dry-matter losses. Oxidation of energy-rich sugars produces excess heat, which can damage forage protein.[10] Good silo management also maximizes the anaerobic conversion of water-soluble carbohydrate to silage acids, thus reducing pH to a range that is inhospitable to spoilage organisms. This conversion results from anaerobic hetero- and homofermentation.

Heterofermentative anaerobes convert water-soluble carbohydrate into various fermentation end products at the expense of energy (because dry matter is decreased). Homofermentative bacteria (lactic-acid bacteria) convert water-soluble carbohydrate to lactic acid; little energy is consumed, and dry-matter loss is diminished. Hetero- and homofermentative anaerobes are both essential to silage fermentation; however, efficient fermentation minimizes heterofermentation and maximizes homofermentation.

The type of crop and its growing environment determine the mixture of aerobic and anaerobic microbial population that will become ensiled with forages. Four basic types of microbial populations exist on fresh crop forages: 1) lactic acid bacteria, 2) endospore-producing bacteria, 3) coliforms, and 4) aerobic spore formers (Table 1).[14]

The great majority of these microorganisms are the aerobic spore formers (yeast and mold) that are strict aerobes and contribute little or nothing to silage preservation. Their activity is minimized with the exclusion of oxygen.

Of those organisms capable of anaerobic growth, the coliforms from the *Enterobacteriaceae* family are most abundant. The lactic acid bacteria are found in lowest populations, but will dominate during the ensiling process. These bacteria commonly are found on the aerial parts of plants and will be more prevalent on outer leaf surfaces than on stems.

The endospore-producing bacterial population is comprised of clostridial and *Bacillus* organisms. They are not normal members of the forage crop microflora and are detected either in low numbers or cannot be detected at all on green plants. The presence of endospore-producing bacteria probably results from soil contamination and/or manure slurry application, which is a function of nitrogen content within the forage. [1,6]

Listeria monocytogenes can be found in silages that have undergone extreme aerobic decomposition. The organism also is soilborne and is dormant until proper conditions exist within silage to allow proliferation.

Table 1. Microorganisms found in fresh and ensiled forages

Group	Microorganisms	Oxygen Dependent	Incidence of microorganism	
			Fresh Crop	Stable Silage
Lactic Acid Bacteria	*Lactobacillus sp.*	Anaerobic	Low	High
	Streptococcus sp.		High	Low
	Leuconostoc sp.		High	Low
	Pediococcus sp.		High	Low
Endospore Forming Bacteria	*Clostridia spp.*	Anaerobic	Low to none	Low to none
	Bacillus spp.	Aerobic/Anaerobic		
Coliform Bacteria	*Enterobacteriaceae*	Aerobic to facultatively Anaerobic	High	Low
	E. coli			
	Klebsiella spp.			
	Pseudomonas spp			
Aerobic spore formers	Yeast	Aerobic	High	Low
	Torulopsis spp.			
	Candida spp.			
	Hansenula			
	Mold			
	Fusarium			
	Aspergillus spp.			
	Penicillium spp.			
	Mucor spp.			

Environmental factors that determine epiphytic microflora population diversity on crops include plant maturity, weather, grazing, manuring, and pre-wilting of the crop.[14] Lactic acid bacteria and *Clostridia* increase on red clover in later cuttings, while meadow fescue has increased homofermentative *Lactobacillus* populations in April through September. Corn tends to become more populated with yeast as the crop matures. Lactic acid bacteria prefer moderate warm weather with high humidity while *Clostridia* are little affected by climatic factors. This probably is because the latter organisms are obligate anaerobes that occur on the growing plant in endospore form only. *Clostridia* and *Lactobacillus* are more prevalent on fields that were previously pastured. *Clostridia* are more numerous on forage with a history of liquid manure application 2-3 months prior to harvest.

Microbial populations increase dramatically in the period between harvest and when the material reaches the silo. Nutrient-rich substrate from mechanical treatment (chopping) of the plant is made available to the microbes, explaining their proliferation.

Clostridial Fermentation

There are 2 basic anaerobic fermentations that can occur in silage: 1) lactic fermentation and 2) clostridial (butyric acid) fermentation; these are often referred to as primary and secondary fermentation, respectively.[3,14] In the event of insufficient acid being formed by primary fermentation, clostridial fermentation of 2 types assume importance, depending upon the primary substrate requirement: 1) saccharolytic (lactate fermenting) and 2) proteolytic (putrefactive) activity. Initially, saccharolytic *Clostridia* utilize plant sugars and lactic acid as substrate to proliferate and produce butyric acid. Since butyrate is weaker than lactic, acetic, or propionic acid, silage pH rises and carbon dioxide is released. Likelihood of ketosis increases when dairy cows are fed ensilage with increased levels of butyric acid.[11]

Conditions may then become favorable for the proliferation of proteolytic *Clostridia*, which are less tolerant of acid than their saccharolytic counterparts, resulting in the formation of amines, amides, and ammonia from proteins and amino acids. This causes a further increase in pH. End-products of protein breakdown have been attributed by researchers as a reason why ruminant voluntary dry matter intakes of ensiled feedstuffs are typically 5%-20% less than non-ensiled crop.[9] Other reasons for dry-matter depression include the moisture content, free acidity, and osmolality of rumen liquor as a result of feeding ensilage versus fresh forage.

Forages ensiled beyond maximum recommended moisture levels may produce clostridial activity.[3] The precise level of acidity at which clostridial activity is

suppressed depends primarily on the dry matter content of the silage. Generally, ensilage with a dry matter content of about 30% will stabilize at a pH of around 4.0. In crops with lower dry matter content, the moisture tends to offset the preservative action of the primary fermentation acids. *Clostridia* can tolerate high concentrations of acid in environments where water is freely available and they may not, therefore, be inhibited at a pH as low as 4.0. Silages with dry matter contents that exceed 35% will inhibit *Clostridia* activity by a lack of moisture, whereas *Lactobacillus* organisms are able to proliferate. *Clostridia* are inhibited by a combined effect of acidity and moisture availability at intermediate levels of dry matter (30%-35%).

Wet, non-wilted, and overly mature forages have less soluble sugars for completion of fermentation. A terminal pH of less than 4.5 is not achieved, thus creating an environment suitable for clostridial activity.

A diverse species combination of clostridial organisms may be present and dictate how animals will respond to silages undergoing clostridial fermentation (Table 2).[3,13] For instance, *C. butyricum* produces only butyric acid, and by itself should not produce silage with depressed dry matter intake properties. However, silages fermented by *C. scatol* produce a combination of end products ranging from acetate to caproate that, when found together with skatol, produce a nauseating silage. The unpalatability of silage is further enhanced by proteolytic clostridial organisms that produce an assortment of nitrogenous end products.

Table 2. Classification of clostridial microorganisms

Saccharolytic	Proteolytic	Others
C. butyricum	*C. bifermentans*	*C. perfringens* (rare)
C. paraputrificum	*C. sporogens*	*C. sphenoides*
C. sphnoides	*C. botulinum (type B)*	
C. tyrobutyricum		
C. scatol (rare)		

Clinical indicators of ensiled forages that have gone clostridial include: 1) greenish/olive color, 2) wet and slimy feel, and 3) rancid and foul smell. Ruminants may display depressed or fluctuating dry matter intakes and production when offered clostridial silage because of unpalatable nitrogenous and butyric end products produced from the secondary fermentation.

The laboratory indicators of clostridial fermentation include high silage moisture, pH, butyric acid, and ammonia nitrogen levels.[2,12] The normal silage acid levels for ensiled forages and grains are listed in Table 3. Lactic acid is the strongest organic acid and is most efficient in energy conservation. Acetic and propionic acids are weaker than lactic acid, requiring more sugar for acid production to achieve terminal pH. Butyric and valeric acids are the weakest acids and their production is least energy efficient.

Table 3. Silage acid values for ensiled forages

Volatile Fatty Acid	Ideal Fermentation	Average Fermentation
Lactic Acid	>3 %	2-3 %
Acetic Acid	< 2 %	2-3 %
Propionic Acid	< 1 %	< 1 %
Butyric Acid	0	< 0.1%
Valeric Acid	0	<0.1 %

Besides destroying the nutritional value of silage, *C. tyrobutyricum* spores from silages may contaminate milk and cause "late blow" in hard cheeses such as Gouda and Edam cheeses.[14] Clostridial silages also may present a health hazard to cattle.

Documented studies indicate that botulism in horses and cattle may occur from consuming silage.[3,13] *C. botulinum* is widely distributed in nature and there exists no way of destroying the organism in the soil. Therefore, its presence inevitably occurs in forages. The problem is more prevalent in round bale silage because substrate availability is less for primary fermentation, resulting in terminal pH being greater than 4.5 and conducive to clostridial fermentation. The organism is uncommon in soils of the United Kingdom, compared to that of the Baltic regions and parts of the United States. No denaturation technique of the botulism toxin is known to exist in silages diagnosed with the organism.

An association is sometimes made by veterinarians between the presence of enterotoxemia in dairy cattle and clostridial silages, inferring that *C. perfringens* is one of the clostridial organisms in the ensilage. Laboratory diagnostics indicating the presence of clostridial activity do not verify that a toxic species is being fed to cattle.

Twelve silage samples from Pioneer Technical Services were submitted to University of Arizona's clostridial enterotoxemia laboratory to determine if *C. perfringens* was present. Analysis of the 12 samples revealed that 4 of the silages had elevated butyric acid and ammonia nitrogen levels, while the other 8 were normal. The Arizona laboratory found *C. perfringens*

Type A present in 3 samples that were considered normal and well-fermented silages. Conversely, those silages diagnosed as clostridial silages by Pioneer Technical Services failed to display *C. perfringens* upon Arizona's laboratory analysis. These observations lead one to speculate that well-preserved silages may harbor *C. perfringens,* yet not cause enterotoxemia symptoms within the herd.

A short-term voluntary intake solution when feeding clostridial silage includes feeding at lower inclusion levels with other normal forages. The butyric acid in clostridial silage produces enhanced silage stability properties, allowing the forage to be fed at minimal feed-out rates. The problem silage may be allocated to other livestock groups, such as heifers. Molasses may be incorporated into the ration to stimulate palatability. Removal of forage for next day's feeding from the silo and spreading it out on a clean surface may allow some of the undesirable volatile fatty acids to dissipate into the atmosphere and may help stimulate voluntary intake.

Long-term crop planning to minimize clostridial activity can be achieved through observance of proper harvest moistures between 65-70%. Corn and sorghum forages should be direct-cut and ensiled based upon moisture and maturity of the live plant. Legume and grass forages should have adequate wilt-time after cutting to allow the crop to enter proper moisture levels through moisture evaporation. Legumes should be harvested at proper maturity to ensure adequate plant sugar availability for complete fermentation. Alfalfa maturity should be 30% ADF and 40% NDF.

Listeriosis (Circling Disease)

Listeria monocytogenes is a saprophytic soilborne bacteria that causes listeriosis. It is primarily a result of ruminants ingesting spoiled, decaying poor-quality silage.[14] The organism survives well at low temperatures and at pH greater than 5.5. Listeria will not grow in well-managed silos where primary fermentation has produced a pH less than 5.5 and air has been totally excluded. The organism prefers aerobic conditions and survives well in a low-dry matter environment. Later cuttings of grasses for silage are more likely to produce *Listeria* because of lower soluble sugar levels and higher proportion of relatively inert fiber, resulting in poor fermentation.

Situations where *Listeria* will thrive are: 1) waste silage from silos being incorporated into the ration, 2) frozen silage on stave silo walls which thaws and falls onto good silage, and 3) balage systems where limited fermentation and poor management have caused spoilage of the feedstuff. Corn silage has been implicated as more likely to cause the disease than other silages, but this is more true of sheep than cattle.

Cattle consuming *Listeria* contaminated silage will produce a latent or apparent infection in a high proportion of animals fed, but only a few will develop clinical signs of "circling disease". Symptoms will start at more than 10 days after initial ingestion of the spoiled silage.[5]

Besides treating for symptoms of listeriosis, managers and veterinarians should identify silages that display decomposition and eliminate them from the ration. Precautions should be taken during the silo unloading process to keep silo walls clean and promptly discard aerobically unstable silages to avoid contact with well preserved ensilage. Spoiled silage and frozen silage should be removed to prevent contamination of good feed.

Yeast, Molds and Mycotoxins

Molds have no significant beneficial purpose to the ensiling process, and their ability to proliferate results from silage environments that are aerobically unstable.[13] There have been numerous syndromes in ruminants supposedly due to the ingestion of fungi or their toxins from spoiled silages.

Yeast and mold activity results from ensiling crops with less than recommended moisture levels, less than adequate compaction, no cover, and slow feedout rates. These conditions permit air penetration and/or entrapment into the silage, thus permitting the sporulation of aerobic yeast and mold organisms that produce heating, depressed palatability and mycotoxin problems.

Mold activity in silage is initiated from an elevated pH due to yeast that utilizes lactic acid as substrate. It becomes active from the introduction of oxygen into the silo. *Candida* and *Hansula* are lactate consumers, and these organisms usually preclude mold activity when their population counts exceed 100,000 colony forming units per gram of forage (CFU/Gm).[14] Yeast aerobic instability by itself may not result in ruminant disorders and cause depressed feed intakes.[11] The mold activity that follows yeast propagation is what causes palatability problems with cattle.

A multitude of different genera and species of fungi may colonize silage. The growth parameters of molds vary in that some proliferate while the crop is growing in the field, while others propagate during storage. Table 4 lists commonly found mold organisms that exist in silages and high-moisture grains.[8]

Field fungi conditions that contribute to their activity include high humidity (>70%) and temperatures that fluctuate between hot days and cool nights. Field molds usually do not grow in stored ensilage because the low pH and oxygen silage environment is not conducive to their survival. The most common field fungi that produce mycotoxins are *Fusarium gramminearum* and *moniliforme* and *Aspirgillus flavus*.

Storage fungi usually do not invade the crop prior to harvest. Soilborne mold spores are brought into storage structures with the crop. Up to 24 molds have been identified in ensilage, but most are considered non-pro-

Table 4. Key to silage molds[2]

Mold	Characteristics	Toxin and Problems
White to Pinkish-White		
Fusarium tricincturn (Gibberella zeae)	Appears fluffy or powdery; prolific spore production may also be reddish color in grains	T-2; Dysentery, poor gains/ milk yield depression
Fusarium graminearum	found in cereal grains	Zearalonone -reproductive DON - Feed refusal, depressed gains mainly in swine
Fusarium moniliforme	found primarily in corn grain	Fumonisin—horses primarily; hogs; humans
Mucor	white-gray fluffy hyphae with black spores; common soil and manure contaminant	None
Monilia	Similar to Mucor; white-yellow	None
Brown to Black		
Rhizoctonia	found especially in clover	Slaframine (slobber factor). Salivation, diarrhea, bloat
Claviceps purpurea	toxin exists within mold mass rather than secreted into environment. Most common in grasses including wheat, rye, and barley	Ergot alkaloids that cause necrosis of hooves, tremors, and convulsions
Yellow to Yellow-Green		
Aspirgillus flavus	vegetative growth not visible; powdery spore production	Aflatoxins B1, B2, G1, G2. Carcinogenic toxin causing hemorrhaging, poor intakes, and diarrhea
Aspergillus fumigatus	found in corn silage	Unknown toxin that causes lung damage, reduced intakes, diarrhea, and abortions
Aspirgillus ochraceus	spore mass is yellow	Ochratoxin-Kidney damage in swine, little effect on adult ruminants
Green to Green-Blue		
Penicillium viradicatum	found in corn and small grains	Ochratoxin-see Aspirgillus ochraceus
Penicillium citrinin	found in corn and small grains	Citrinin-Kidney damage, weight loss, reduced intakes
Penicillium urticae	found in corn and small grains	Patulin-Antibiotic substance causing hemorrhaging of lung and brain tissue
Penicillium rouqueforti	corn silage and grains	PR toxin-Reproductive

ducers of mycotoxins.[3] The most prevalent molds isolated from most North American feeds are *Mucor, Penicillium, Aspergillus,* and *Monilia*. *Aspergillus flavus* may produce aflatoxin as a field mold, although it is classified as a storage organism.

Some silage molds can grow within low oxygen and moderately low pH environments, but their survivability is limited to competition with anaerobic bacteria. Therefore, more stable silages are less prone to become moldy.[4]

Common diagnosed mycotoxins are aflatoxin produced by *Aspergillus flavus*, and DON (vomitoxin), zearalonone, T-2, and fumonisin that is produced by *Fusarium spp.* DON, zearalonone, and T-2 fit into a class of toxins known as the "trichothecenes".[8] However, mycotoxins may exist that are not included in routine diagnostics that include PR toxin (*Penicillium rouquefortine*) from *Penicillium rouquefortii*, AAL (*Alternaria alternia)* from *Alternaria*, and fusaric acid from *Fusarium spp.*[9]

DON is the most commonly diagnosed mycotoxin that dairy managers and professionals associate with dairy herd problems. However, dairy feeding studies that incorporate high concentrations of pure DON reveal no negative effects on dry matter intakes or milk yield.[8] Contrary to this research, field studies conducted in North Carolina correlate DON with dairy production and health problems. These studies conclude that other *Fusarium* secondary metabolites probably are causing problems and that DON should be considered a "marker".

DON may be present at various levels on farms with or without dairy production and health problems if a favorable fungal environment exists with a geographical area. In 1997, a collaborative pilot forage mycotoxin epidemiology study between Pioneer, University of Wisconsin and University of Vermont was conducted to determine levels of mycotoxins present in various feedstuffs on "sick" and "healthy" dairies as determined by cooperating field veterinarians and nutritionists.

The surveyed herds were determined "sick" or "healthy" through assessment of a 100-question survey regarding the status of vaccination history, infectious disease, mastitis, reproduction, nutrition and production. Feed samples were collected and analyzed for routinely field-produced mycotoxins: DON (vomitoxin), zearalonone, and fumonisin (all produced by *Fusarium spp.*). In addition, non-routinely tested mold storage-produced mycotoxins were analyzed for PR, CPA, and AAL toxins.

The sampling indicated that fumonisin, zearalonone, and CPA were minimal to negligible in the tested feeds. Therefore, these reported mycotoxin levels were not used in herd comparisons.

Findings indicated that DON was present on sick and healthy farms, although the study indicated methods used to identify problem and non-problem herds needed to be refined for a follow-up study.[9] A more extensive and better-defined survey and sampling is being repeated in 1999 on 400 dairy herds in Vermont and New York, looking primarily at possible toxins being produced from storage molds.

Additional data from the forage mycotoxin epidemiology study found the presence of non-diagnosed spoilage mold toxins. The AAL toxin from *Alternaria* and PR toxin from *Penicillium rouquefortine* was higher in sick herds than healthy herds.

DON level comparisons between herds classified as "truly problem herds and "truly healthy herds" indicated minimal differences exist between these herds. The PR and AAL level comparisons indicate that sick herds tend to have higher levels than healthy herds. The results are illustrated in the following graphs (Figures 2, 3, and 4).

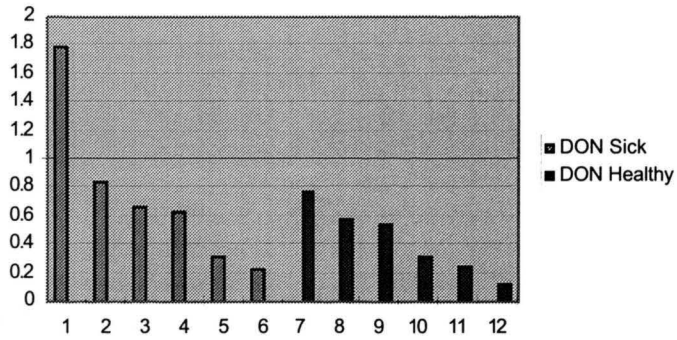

Figure 2. DON comparisons of truly sick to healthy herds

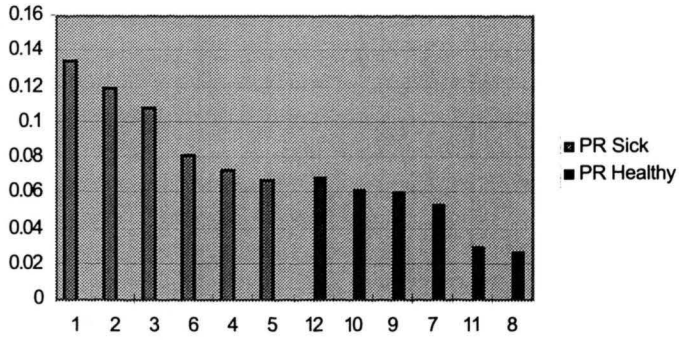

Figure 3. PR comparisons of truly sick to healthy herds

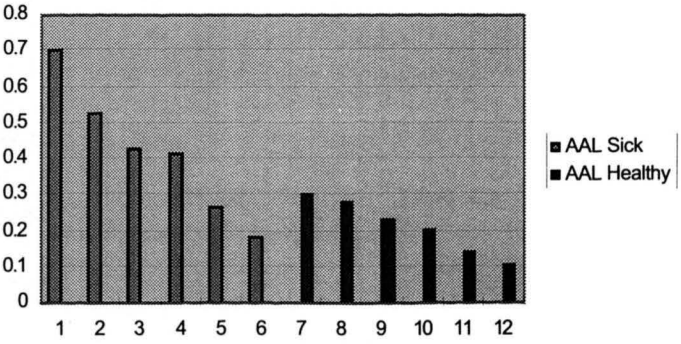

Figure 4. AAL comparisons of truly sick to healthy herds

Spoilage mold research is limited. University of Wisconsin research indicates that PR toxin from *Penicillium rouqueforti* caused abortions and retained placenta problems in dairy cattle.[9] In addition, Japanese silage researchers screened for mold prevalence in silages and demonstrated that *Penicillium rouqueforti* and the PR toxin is most commonly found in silages.

Dairy practitioners must be prudent when considering silage mycotoxins as the cause of dairy health and production problems. Mycotoxins in dairy herds may be a secondary contributor or have no bearing at all on herd problems. A complete differential diagnosis is essential to rule out nutritional disorders, infectious disease, rumen acidosis and other reasons why a dairy has impaired health and production problems. Forage mycotoxin diagnostics often utilize ELISA (Enzyme-linked immunosorbent assay) technology, resulting in unreliable, "false-positive" results. Diagnosis of forage mycotoxins should involve sample clean-up procedures to produce a clean extract. Such procedures exist with HPLC (high-pressure liquid chromatography) and GC (gas chromatography) tests.

There are several key points to remember when dealing with potential mycotoxin problems in animal feeds:

- Most molds are harmless and do not produce known mycotoxins.
- The majority of known mycotoxins are produced in the field prior to harvest, although unknown mycotoxins may be produced by storage molds within the silo.
- Vomitoxin (DON) should be considered a "marker"; if it is present, conditions exist for the field mold to produce other unidentified toxins.
- HPLC or GC tests should back up ELISA tests since current ELISA tests give many false positives when used in ensiled forages and grains.
- A comprehensive differential diagnosis is essential when non-healthy herds have mycotoxins present in feeds (many herd problems blamed on mycotoxins turn out to be nutritional).
- Proper crop management, from field to feedout, can reduce opportunities for mold growth and subsequent toxin production.

Summary

Fermented feeds that are properly managed display ideal fermentation properties and assure bio-security when fed to dairy cows. All silages probably contain pathogenic spores. However, well-fermented ensilage produces an environment that is inhospitable to undesirable pathogens such as *Clostridia, Listeria,* and yeast/mold microorganisms. Management practices that provide bio-security include: 1) proper harvest moisture, 2) proper harvest maturity, 3) fast filling of the silo, 4) proper chop length, 5) packing of bunkers, piles and pits, 6) sealing bunkers, piles and pits, 7) utilization of a quality forage inoculant, and 8) maintaining proper feedout rates.

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