Harmful Microorganisms in Silage and Their Control Measures: Postprint

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Abstract

Silage is susceptible to contamination by harmful microorganisms and their metabolic toxins during fermentation and storage, which reduces silage quality and ruminant production performance, and poses serious threats to animal and human health. This review summarizes the types of harmful microorganisms in silage and the mechanisms through which their metabolites affect silage nutritional quality and aerobic stability, as well as measures and methods for inhibiting harmful microorganisms during silage fermentation, providing a reference for developing high-quality silage additives, optimizing fermentation conditions, suppressing harmful microbial growth, and improving silage nutritional quality.

Full Text

Harmful Microorganisms in Silage and Their Suppression Measures

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Abstract

Silage is susceptible to contamination by harmful microorganisms and their metabolic toxins during fermentation and storage, which reduces silage quality and ruminant production performance while seriously endangering animal and human health. This paper reviews the types of harmful microorganisms in silage and the mechanisms by which their metabolites affect nutritional quality and aerobic stability, as well as measures and methods for suppressing harmful microbes during silage fermentation. The objective is to provide a reference

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Keywords: silage; spoilage microorganism; pathogenic microorganism; suppression measure

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To ensure that whole-plant corn, alfalfa, and other forages can be used year-round regardless of growing season, anaerobic fermentation technology utilizing silage microorganisms is employed to convert these crops into high-quality feed for ruminants [1-2]. The most commonly used silage materials are corn and alfalfa, which have high nutritional value and fiber content and have become the most widely used dietary components in dairy cattle farming, particularly in developed European and American countries where approximately 60%–80% of dairy cattle are fed corn silage [3-4]. Globally, a dairy cow consumes about 26 kg of dry matter (DM) daily, with corn silage accounting for 50%–75% of the diet composition [5].

However, silage is easily contaminated by harmful microorganisms during fermentation and storage, leading to accumulation of toxic metabolites that damage animal and human health [6]. In recent years, numerous foreign studies have identified various harmful microorganisms in silage, including fungi [*Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, etc.] and bacteria [butyric acid bacteria, *Clostridium botulinum*, *Listeria*, *Escherichia coli*, etc.], along with changes in toxic metabolites before and after fermentation [7]. In contrast, domestic research on harmful microorganisms in silage remains limited. With increasing silage usage worldwide, particularly in developing countries [8], reducing the risk of harmful microbial contamination in animal feed, ensuring silage quality, protecting animals from direct threats, and guaranteeing the safety of final meat and dairy products to safeguard human health have become critical priorities.

This review summarizes recent progress on harmful microorganisms and their metabolic toxins in silage, the use of additives to suppress aerobic spoilage and pathogenic microbes, and strategies to reduce nutrient losses and improve animal production performance and health. The aim is to provide a scientific basis for studying the characteristics of harmful silage microorganisms and their metabolites, developing high-quality additives to inhibit microbial proliferation and toxin production, and enhancing silage nutritional quality and animal health.

1. Silage Fermentation Process and the Role of Different Microbial Populations

Theoretical changes in feed physicochemical properties and microbial populations during the silage process are illustrated in Figure 1 [Figure 1: see original paper] [9]. Generally, the epiphytic microflora on growing crops includes *Pseudomonas*, Actinomycetes, *Listeria*, and lactic acid bacteria (LAB) (Table 1)

[10]. Due to variations in silage materials and climate across different regions, the types and quantities of epiphytic bacteria on corn and forage may differ. However, once these materials are chopped, compacted, and sealed in silos, physicochemical and microbial changes occur during fermentation and storage.

Numerous studies have shown that in well-prepared silage, LAB dominate the fermentation, rapidly reducing pH to facilitate preservation. Typical silage LAB include *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, and *Weissella* [11]. Under adverse conditions such as soil contamination, prolonged aerobic exposure, or slow acidification, the microbial community shifts to *Clostridia*, yeasts, molds, and accidentally introduced pathogens like *Listeria*. When silage is opened for feeding and air enters poorly compacted silage, aerobic microbes suppressed during initial fermentation can revive and proliferate, raising pH and posing serious threats to silage quality and animal production.

2. Harmful Microorganisms and Their Metabolites in Silage

Undesirable and harmful microorganisms are inevitably carried into and produced during silage production and use, jeopardizing long-term preservation and animal production and thereby affecting animal and human health.

2.1 Yeasts and Molds

Yeasts are facultative anaerobic eukaryotes that participate in aerobic spoilage during initial ensiling or after silo opening, making them the primary undesirable microbial group in silage [12]. Courtin et al. [13] tested and validated mathematical models for yeast-induced aerobic deterioration in grass and whole-plant corn silage. Yeasts can tolerate acidity during silage fermentation and, upon aerobic exposure, aerobically metabolize organic acids such as succinic acid, citric acid, and lactic acid, causing pH elevation and slowing the growth of acid-tolerant microorganisms. Epiphytic yeasts on silage materials can convert water-soluble carbohydrates (WSC) into carbon dioxide (CO_2) and alcohols, affecting silage quality, damaging animal livers, and reducing feed intake.

Tao et al. [14] found that the most abundant microorganisms on amaranth were aerobic bacteria, followed by *E. coli* and yeasts, then LAB, with molds being the least numerous. Mixing amaranth with whole-plant corn for ensiling increased LAB numbers, and when corn exceeded 50% of the mixture, silage quality was good.

Molds are strict aerobes only found during initial ensiling or after aerobic exposure. Orsi et al. [15] analyzed 195 samples from Brazilian corn silage, identifying *Fusarium* sp. as the most common genus, followed by *Penicillium*, *Aspergillus*, *Trichosporon*, and *Cladosporium*. Molds produce numerous secondary metabolites, including mycotoxins, which retain their toxicity in silage even after molds disappear. Roigé et al. [16] reported that the most common mycotoxins in corn

silage were penicillium toxins (70%), fusarium toxins (47%), and aspergillus toxins (34%). Niderkorn et al. [17] demonstrated that *Fusarium* can produce over 20 mycotoxins, primarily dioxins, zearalenone, and fumonisins. Cavallarin et al. [18] first examined aflatoxin accumulation during 7 days of aerobic exposure in corn silage, showing that adding *Lactobacillus buchneri* or covering with plastic film to prevent oxygen entry could inhibit aflatoxin production.

After silo opening, prolonged feeding inevitably exposes silage to air, potentially generating low-dose mycotoxins. Ingesting silage containing low-dose mycotoxins can cause non-specific symptoms in animals such as immune dysfunction and hormonal imbalance. Myllykoski et al. [19] observed hemorrhagic jejunal syndrome in beef cattle fed diets contaminated with three mold genera (*Fusarium*, *Penicillium*, and *Aspergillus*). Transmission of toxins to humans through dairy products and meat represents a potential risk. Compared to other animals, rumen microorganisms in ruminants possess certain mycotoxin biotransformation and resistance capabilities. Mobashar et al. [20] reported that ochratoxin A and penicillin produced by *Aspergillus ochraceus* damage kidneys in many animals, but ruminants can degrade these toxins, reducing their toxicity. Rumen microbes can also degrade some zearalenone and trichothecenes, though fumonisin B1 is poorly metabolized in the rumen. However, long-term feeding of high-proportion silage-based diets may acidify the rumen environment, potentially increasing animal susceptibility to mycotoxins and reducing microbial detoxification capacity.

Given that yeasts and molds can contaminate and degrade silage while posing potential negative impacts on animal and human health, research on suppression strategies and toxin degradation during ensiling cannot be overlooked in ruminant nutrition.

2.2 Harmful Bacteria

Currently, harmful bacteria identified in silage are more closely associated with short-term disease occurrence in animals than with silage degradation. Disease onset in humans or animals may result from direct bacteria-host interactions (e.g., *Listeria monocytogenes*) or from toxic compounds produced (toxins or biogenic amines).

2.2.1 Butyric Acid Bacteria Butyric acid bacteria in silage originate from soil bacteria introduced during material collection and can convert lactic acid to butyric acid, hydrogen, and CO_2 under relatively low pH conditions. Extensive growth of butyric acid bacteria can induce pH increases, promoting the growth of non-acid-tolerant spoilage microorganisms. Forage and corn silage are the most important transmission vectors for butyric acid bacteria infection in animals. The main butyric acid bacteria in silage belong to *Clostridium*, particularly two species (*C. tyrobutyricum* and *C. butyricum*), and *Bacilli*, especially *Bacillus cereus*, which are primary spoilage microorganisms [21].

2.2.2 *Clostridium botulinum* *Clostridium botulinum* is well known for producing extremely pathogenic toxins that can cause death in animals and humans. Lindström et al. [22] reported that feeding poor-quality silage containing *C. botulinum* and its toxins can lead to bacterial proliferation and toxin production in dairy cattle intestines. Therefore, further research is needed on inhibiting *C. botulinum* growth and degrading its toxins in silage.

2.2.3 *Listeria* *Listeria* is widely present in water, forage, silage, organic matter, soil, and feces. The primary source of infection in ruminants is spoiled silage, and the presence of *Listeria* in silage or feces increases the risk of its presence in milk, subsequently transmitting to humans. *Listeria monocytogenes* has been detected in forage and corn silage, with risks increasing when pH exceeds 4.5. Farms feeding silage year-round have 3–7 times higher infection rates than those not feeding silage. Schocken-Iturrino et al. [23] found that 65.6% of opened silage samples in Brazil contained *Listeria*, with 10% being *L. monocytogenes*. The survival, growth, and abundance of *Listeria* in silage depend on pH and anaerobic conditions.

2.2.4 *Escherichia coli* Ruminants are considered primary carriers of Shiga toxin-producing *E. coli* (STEC), recognized as foodborne pathogens. Humans become infected through contaminated food or water or direct contact with contaminated animals or environments. Silage serves as a vector for transmitting pathogenic *E. coli* in ruminants. Cernicchiaro et al. [24] reported high prevalence of *E. coli* O157 and O157:H7 in corn silage fed to cattle. STEC can survive in the low pH and fermentation environment of poor-quality silage. Large quantities of *E. coli* are detected in spoiled silage, and insufficient anaerobic conditions can delay lactic acid fermentation, slow pH reduction, and increase pathogenic *E. coli* survival. Dunière et al. [25] detected 10^3 CFU/g of STEC and *E. coli* O26 in corn silage after aerobic exposure. Therefore, silage is vulnerable to STEC contamination upon opening, and suppression measures should be implemented.

2.2.5 Other Harmful Bacteria Reilly et al. [26] indicated that cattle fed corn silage, grass silage, or molasses are more susceptible to bovine tuberculosis (bTB), an infectious disease caused by *Mycobacterium bovis*. Grant et al. [27] detected *Yersinia enterocolitica* in silage fermented for over 20 months, with 6.5% of 46 silage samples testing positive. Pathogen presence correlated with high silage pH and other indicators. Nam et al. [28] found that *Campylobacter* sp. occasionally present in silage is also an important foodborne pathogen. *Salmonella*, a pathogen causing severe diarrhea and routinely monitored as a hygiene indicator in feed, has not been reported in silage.

2.3 Biogenic Amines

The main biogenic amines (BAs) in silage are putrescine, cadaverine, and tyramine, derived from arginine, lysine, and tyrosine, respectively, with smaller

amounts of tryptamine, histamine, spermidine, and spermine. BAs are formed from free amino acids or small peptides through the action of microbial amino acid decarboxylases produced by certain LAB such as *Lactobacillus*, *Leuconostoc*, *Enterococcus*, and *Pediococcus*, as well as many other bacterial genera present in silage including *Clostridium*, *Bacillus*, *Klebsiella*, *E. coli*, and *Pseudomonas*. Steidlová et al. [29] investigated BAs in 51 corn silage samples, finding tyramine, putrescine, cadaverine, spermidine, histamine, spermine, and tryptamine at concentrations of 145.0, 136.0, 96.2, 37.9, 3.0, 2.8, and 2.5 mg/kg, respectively. Due to bacterial proteolysis, BAs in silage are associated with protein degradation and reduced nutritional value. BAs can cause rumen metabolic disorders and acidosis in ruminants. BA formation may be influenced by temperature, initial pH reduction rate, and oxygen, though the mechanisms underlying these effects require further investigation.

3. Strategies for Suppressing Harmful Microorganisms in Silage

Currently, no methods exist to treat and improve spoiled silage; the only option is disposal and cessation of feeding. The primary problems in silage fermentation are the use of poor-quality or immature forage materials, failure to rapidly establish anaerobic conditions for acidification, and contamination with pathogens and spoilage microorganisms. Researching strategies to suppress harmful microorganisms during silage fermentation is essential for extending shelf life, improving nutritional value, and enhancing quality. Preventive strategies applied at different stages—from preparation to aerobic exposure after opening—can prevent pathogen development and silage spoilage.

3.1 Reducing Pathogens in Forage at Harvest

To prevent silage degradation, pathogen entry into the silage ecosystem must be minimized. Johansson et al. [30] demonstrated that measures such as harvesting forage four weeks after fertilization, cutting at appropriate heights above soil to avoid contamination, and other practices can reduce *Clostridia*, *Listeria*, clostridial spores, and *E. coli* in silage. Growth conditions and harvest timing are also important; late harvest with high DM content (>50%) increases susceptibility to self-heating and mycotoxin infection. Additionally, contamination from pathogens carried by raw materials, silos, and equipment should be avoided.

3.2 Rapidly Establishing Anaerobic Conditions

Rapid anaerobic establishment prevents effluent production and promotes rapid LAB growth and pH decline. Silage effluent originates from plant respiration and aerobic microbial activity, with volume depending on DM content, silo type, compaction, chop length, and additive use. Effluent causes DM and carbohydrate losses while diluting additives. Therefore, selecting appropriate DM

content (30%–40%), chop length (2–6 cm), and compaction density (600 kg/m^3) is necessary to rapidly establish anaerobic conditions, prevent effluent, and ensure nutritional quality.

3.3 Measures for Establishing Acidification

Acidification is the primary preservation mechanism, depending on anaerobic LAB fermentation, buffering capacity, and DM content. Soil incorporation increases buffering capacity; if high, aerobic microorganisms persist longer, reducing hexose and pentose availability and limiting LAB fermentation. This leads to secondary clostridial fermentation, converting lactic acid to butyric acid, raising pH, and causing further spoilage. For years, chemicals, sugars, and enzymes have been added to promote acidification and limit pathogen growth [31].

Beyond chemicals and enzymes, microbial inoculants are increasingly used to promote rapid organic acid accumulation, reducing fermentation and DM losses. The main organic acid produced by epiphytic LAB or additives is lactic acid, which promotes pH decline. Most commercial inoculants are homofermentative LAB due to high lactic acid production efficiency, with *Lactobacillus plantarum* being the most common. Generally, 1$×10^{6}$ CFU/g of microbial inoculant is sufficient to outcompete epiphytic LAB and become dominant. Other commonly used species include *Lactobacillus*, *Pediococcus*, and *Enterococcus*. Heterofermentative LAB, typified by *Lactobacillus buchneri*, produce high acetic acid concentrations that inhibit fungi and improve aerobic stability, gaining increasing application in recent years.

3.4 Preventing Air Entry and Improving Aerobic Stability

From silo filling to storage, contamination and air entry must be avoided to obtain high-quality silage. For long-term sealed storage, polyethylene films and two-sided black-white composite oxygen barrier films (125 μm) are commonly used, with the latter proven to reduce spoilage and DM losses. Compared to polyethylene films, oxygen barrier films delay yeast and mold growth during aerobic exposure, resist bird and rodent damage, and protect against UV radiation.

Silage fermentation represents competition between aerobic and anaerobic processes; when silage is opened, air entry degrades nutrients. Therefore, silo capacity should match herd size and feeding requirements to ensure sufficient daily removal depth, minimizing aerobic exposure. Clean cuts also limit air penetration and spoilage. Additives such as formic acid compounds and microbial inoculants enhance aerobic stability, with inoculants widely used in bunker silos. While homofermentative inoculants are effective, some researchers suggest lactic acid can serve as a substrate for lactate-assimilating yeasts, causing spoilage under insufficient anaerobic conditions. Heterofermentative fermentation is preferred for improving aerobic stability at feed-out. Numerous studies have shown that the heterofermentative inoculant *Lactobacillus buchneri* improves aerobic stabil-

ity and reduces fermentation losses through increased acetic and propionic acid concentrations, pH decline, and production of antimicrobial compounds such as n-propanol, propyl acetate, and isobutanol that inhibit yeast and mold growth [32].

3.5 Direct Inhibition of Harmful Microorganisms

Additives are commonly used to suppress harmful microorganisms and ensure silage quality. Sodium nitrite combined with hexamethylenetetramine effectively inhibits clostridial growth, while sodium benzoate restricts yeast proliferation. Adding calcium formate, sodium benzoate, and sodium nitrite to corn silage significantly reduces concentrations of zearalenone, deoxynivalenol, ochratoxin, and fumonisin.

Microbial inoculants also inhibit pathogen growth. In addition to organic acids, beneficial silage bacteria produce other antimicrobial substances such as hydrogen peroxide (H_2O_2), ethanol, ketones, exopolysaccharides, and antimicrobial peptides. Corn also contains phenolic compounds like ferulic and coumaric acids with natural antimicrobial properties, specifically proven to inhibit *E. coli* O157:H7, O111, *Listeria*, *Salmonella*, and *Yersinia*. Ferulic acid esterase-producing LAB represent third-generation silage inoculants that improve rumen fiber digestion and may influence antimicrobial effects through ferulic acid activity [33]. However, the antimicrobial mechanisms of silage inoculants require further investigation, as species including *Lactobacillus*, *Lactococcus*, *Propionibacterium*, and *Enterococcus* possess esterase genes with potential antimicrobial effects against silage pathogens.

4. Summary and Outlook

In recent years, rapid development of ruminant production in China—including dairy cattle, beef cattle, and sheep—requires high-quality silage as material support. However, spoilage microorganisms severely impact silage, causing spoilage, significant economic losses, and restricting widespread application in ruminant farming. Research on silage in China started relatively late, with insufficient attention to controlling harmful microorganisms.

To prevent harmful microorganisms from transmitting to animals and humans through the silage vector and food chain, threatening health, research must be strengthened on suppressing harmful microbes and eliminating or reducing their metabolic toxins. We need to employ continuously updated biotechnology to discover new microbial strains from silage resources and isolate excellent antimicrobial inoculants, providing theoretical references for further improving silage quality and ruminant production performance.

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Note: Figure translations are in progress. See original paper for figures.