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Harmful Microorganisms in Silage and Their Inhibition Measures

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Abstract

Silage feed is prone to contamination by harmful microorganisms and their metabolic toxins during fermentation and storage, which degrades 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 strategies and methods for inhibiting harmful microorganisms during silage fermentation, thereby providing references for developing high-quality silage additives, optimizing fermentation environments, suppressing the growth of harmful microorganisms, and enhancing the nutritional quality of silage feed.

Full Text

Harmful Microorganisms in Silage and Their Inhibition Measures

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Abstract: During fermentation and storage, silage is readily contaminated by harmful microorganisms and their metabolic toxins, which reduces silage quality and ruminant productive performance and seriously endangers the health of animals and humans. This paper reviews the types of harmful microorganisms in silage and the mechanisms by which their metabolites affect the nutritional quality and aerobic stability of silage, as well as measures and methods for inhibiting harmful microorganisms during silage fermentation. It provides a reference for developing high-quality silage additives, optimizing the fermentation

environment, inhibiting the growth of harmful microorganisms, and improving the nutritional quality of silage.

Keywords: silage; spoilage microorganisms; pathogenic microorganisms; inhibition measures

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To ensure that forage grasses such as whole-plant corn and alfalfa are not affected by seasonality, silage microbial anaerobic fermentation technology is used to convert them into high-quality feed for ruminants throughout the year[^1-2]. The most commonly used raw materials for silage are corn and alfalfa; because they have relatively high nutritional value and high fiber content, they have become the most widely used feed components in dairy-cow production. In developed countries in Europe and North America in particular, approximately 60%–80% of dairy cows use corn silage feed[^3-4]. Globally, one dairy cow consumes about 26 kg of dry matter (DM) per day, of which corn silage accounts for 50%–75% of the feed ration[^5]. However, during fermentation and storage, silage is easily contaminated by harmful microorganisms, which then leads to the accumulation of toxic metabolites of harmful microorganisms and damages the health of animals and humans[^6]. In recent years, many studies abroad have successively identified harmful microorganisms in silage, including fungi [such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma*], bacteria (clostridia, botulinum bacilli, *Listeria*, and coliform bacteria, etc.), and changes in toxic and harmful metabolites before and after silage fermentation[^7]. In China, however, there have been relatively few reports on harmful microorganisms in silage.

Because the use of silage continues to increase worldwide, especially in developing countries[^8], reducing the risk of contamination of animal feed by harmful microorganisms, ensuring the excellent quality of silage, reducing the direct threat posed to animals by harmful microorganisms, guaranteeing the safety of meat and dairy products, and safeguarding human health are hot topics and key priorities of concern. This paper reviews recent progress concerning the types of harmful microorganisms in silage and the hazards of their metabolic toxins, as well as the use of silage additives to inhibit aerobic spoilage and pathogenic microorganisms, reduce losses of silage nutrients, and improve animal productive performance and health. It provides a scientific basis for studying the characteristics of harmful microorganisms in silage and their metabolites, developing high-quality silage additives, inhibiting the proliferation and growth of harmful microorganisms, reducing the formation of their metabolic toxins, and improving the nutritional quality of silage and animal health.

Fig. 1: Theoretical changes in oxygen content, pH, and different microbial populations during silage fermentation processing

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1. The silage fermentation process and the roles of its different microbial communities

The theoretical changes in the physicochemical properties of forage and in microbial communities during ensiling are shown in Fig. 1[^9].

Fig. 1. Theoretical changes in oxygen content, pH and different microbial populations during silage fermentation processing[^9]

In general, epiphytic microbial communities on growing crops mainly include pseudomonads (*Pseudomonas*), actinomycetes (*Actinomycetes*), Listeria, and lactic acid bacteria (LAB), etc. (Table 1)[^{10}]. Because silage raw materials and climates differ among regions, the types and numbers of epiphytic bacteria on maize and pasture may vary. However, once these silage materials are chopped, compacted, and sealed in a silo, physicochemical and microbial community changes occur during fermentation and storage. Many studies have shown that, in well-prepared silage, LAB are the dominant fermentative bacteria; they rapidly lower pH, which facilitates silage preservation. Typical LAB in silage include the genera *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, and *Weissella*[^{11}]. If soil contaminates the silage, or if the silage remains aerobic for a prolonged period or undergoes slow acidification, the microbial community in the silage will be dominated by clostridia, yeasts, molds, and unintentionally introduced pathogenic microorganisms such as Listeria. When silage is opened for feeding to animals, air enters silage with a low degree of compaction, which can cause aerobic microorganisms that were inhibited during the early stage of silage fermentation to revive, grow, and multiply; pH rises, and these harmful silage microorganisms and their metabolic products pose serious threats to silage quality and animal production.

Table 1. Typical microbial composition on crops before ensiling (fresh weight basis)[^{10}]; CFU/g

Microbe	Population	Microbe	Population
Total aerobic bacteria	>10 000 000	*Clostridia*	100–1 000
LAB	10–1 000 000	*Bacilli*	100–1 000
Enterobacteria	1 000–1 000 000	Acetic acid bacteria	100–1 000

Yeasts	1,000–100,000	Propionic acid bacteria	10–1,000
Molds	1,000–10,000		

2. Harmful Microorganisms in Silage and Their Metabolic Products

During the preparation and use of silage, some undesirable and harmful microorganisms are inevitably introduced and produced, jeopardizing the long-term preservation of silage and animal production, and thereby affecting the health of animals and humans.

2.1 Yeasts and Molds

Yeasts are facultative anaerobic eukaryotes. During the initial stage of ensiling or after silo opening under aerobic conditions, they participate in aerobic spoilage of silage and are therefore the most important group of undesirable microorganisms in silage feed[^{12}]. Courtin et al.[^{13}] tested and validated mathematical models of aerobic spoilage caused by yeasts in grass and whole-crop corn silage. Yeasts can tolerate acids during silage fermentation; when oxygen is present after silo opening, they aerobically metabolize organic acids such as succinic acid, citric acid, and lactic acid in silage, raising the pH and slowing the growth of acid-tolerant microorganisms. Epiphytic yeasts on silage raw materials can convert water-soluble carbohydrates (WSC) into carbon dioxide (CO_2) and alcohols, affecting silage quality, damaging the liver of animals, and leading to reduced feed intake. Tao Ya et al.[^{14}] found that, among microorganisms attached to grain hulls, aerobic bacteria were the most abundant, followed by *Escherichia coli* and yeasts; lactic acid bacteria came next, and molds were the least abundant. Mixing in whole-crop corn for ensiling can increase the number of lactic acid bacteria; when the proportion of corn added exceeds 50%, silage quality is good.

Molds are strictly aerobic microorganisms and can be detected only at the very beginning of ensiling or after silo opening in an aerobic environment. Orsi et al.[^{15}] extracted 195 samples from Brazilian corn silage, detected and analyzed the types of molds, and found that *Fusarium* sp. was the most common, followed in order by *Penicillium*, *Paecilomyces*, *Trichosporon* sp., and *Cladosporium* sp. During ensiling, molds can produce many secondary metabolites, including mycotoxins. Even if molds disappear during silage fermentation, the mycotoxins they secrete remain toxic in the silage feed and do not disappear. Roigé et al.[^{16}] reported that the most common mycotoxins in corn silage were citrinin (70%), enniatins (47%), and moniliformin (34%). Niderkorn et al.[^{17}] showed that the genus *Fusarium* can produce more than 20 mycotoxins, mainly deoxynivalenol, zearalenone, and fumonisins. Cavallarin et al.[^{18}] first investigated changes in the accumulation of yellow *Aspergillus* toxin during 7 d of aerobic exposure of corn silage, and found that the production of aflatoxin

could be inhibited by adding *Lactobacillus buchneri* or by covering with a plastic film to prevent oxygen entry. After a silage silo is opened, prolonged feeding to animals will expose the silage to air to a greater or lesser extent, and low doses of mycotoxins may be produced. Ingestion of silage containing low doses of mycotoxins can lead to reduced immune-system function, dysregulation of hormones, and other nonspecific symptoms in animals. Myllykoski et al.[^{19}] added feed contaminated with three types of molds (*Fusarium*, *Penicillium*, and *Monascus*) to beef cattle diets, after which the cattle developed empty-gut hemorrhagic syndrome.

Transmission of toxins to humans through dairy products and meat is a potential risk and hazard. Compared with other animals, ruminant rumen microorganisms have a certain capacity for biotransformation of and resistance to mycotoxins. Mobashar et al.[^{20}] [[unclear: text continues on next page]]

produced patulin A and citrinin are harmful to the kidneys of many animals, whereas ruminants can degrade them, thereby reducing their toxicity. Rumen microorganisms in ruminants can also degrade part of the zearalenone and single-ended trichothecene toxins, but the capacity to metabolically degrade fumonisin B_1 in the rumen is weak. However, when ruminants consume, over long periods, diets based on a high proportion of contaminated feed, acidification of the rumen environment may increase the animals' sensitivity to mycotoxins and may reduce the detoxification capacity of microorganisms.

Yeasts and molds can contaminate and degrade silage and may have potentially negative effects on animal and human health. Therefore, research on inhibition strategies for yeasts and molds during ensiling and on the degradation of their toxins should not be neglected in the fields of ruminant feed and nutrition.

2.2 Bacteria

At present, harmful bacteria found in silage are more closely associated with the occurrence of disease in animals in the short term, whereas their effect on the degradation of silage is relatively small. The pathogenesis of disease in humans or animals may result from direct interactions between bacteria and the host (e.g., *Listeria*) or from toxic compounds produced by them (toxins or biogenic amines).

2.2.1 Butyric Acid Bacteria The butyric acid bacteria found in silage originate from soil bacteria introduced into the silo during the collection of silage raw materials. In a relatively low-pH environment, they can convert lactic acid into butyric acid, hydrogen, and CO_2. Therefore, the extensive growth of butyric acid bacteria can induce an increase in pH and promote the growth of acid-intolerant spoilage microorganisms. Grass and maize silages are the most important transmission media for butyric acid bacteria infecting animals. The principal butyric acid bacteria in silage belong to the genus *Clostridium*, especially the two butyric clostridia (*C. tyrobutyricum* and *C. butyricum*) and

bacilli (*Bacilli*), particularly waxy spore-forming bacilli; these species are the main spoilage microorganisms in silage[21].

2.2.2 *Clostridium botulinum* As is well known, *Clostridium botulinum* can produce extremely pathogenic toxins that may lead to death in animals and humans. Lindström et al.[22] reported that, after feeding poor-quality silage containing *Clostridium botulinum* and its toxins, dairy cows exhibited proliferation of *Clostridium botulinum* and toxin production in the gastrointestinal tract. Therefore, inhibition of the growth of *Clostridium botulinum* in silage and degradation of its toxins require further study.

2.2.3 *Listeria* *Listeria* is widely present in water, pasture, silage, organic matter, soil, feces, and other environments. The main source of infection of this bacterium in ruminants is spoiled silage. *Listeria* present in silage or feces increases the risk of its presence in milk and subsequent transmission to humans. The unicellular proliferative *Listeria* (*L. monocytogenes*) has been found in grass and maize silages. When pH exceeds 4.5, the risk of *Listeria* occurrence further increases. The incidence of infection with *L. monocytogenes* on farms feeding silage year-round is 3–7 times higher than on farms not feeding silage. Schocken-Iturrino et al.[23] found in a study in Brazil that 65.6% of opened silages contained *Listeria*,

among which 10% are unicellular proliferative listeriae. The survival, growth, and abundance of listeriae in silage depend on the pH and the degree of anaerobiosis of the silage.

2.2.4; *Escherichia coli*

Ruminants are considered the main carriers of Shiga toxin-producing *Escherichia coli* (STEC). STEC is recognized as a foodborne pathogen; humans become infected by ingesting contaminated food or water, or through direct contact with contaminated animals or environments. Silage is a vehicle by which ruminants transmit pathogenic *E. coli*. Cernicchiaro et al.[1] reported that *E. coli* O157 and *E. coli* O157:H7, with relatively high morbidity, were found in corn silage fed to cattle. STEC can survive in the low-pH and fermentative environment of poor-quality silage. Large numbers of *E. coli* have been detected in spoiled silage. Insufficient anaerobic duration during ensiling can delay lactic acid fermentation, slow the decrease in pH, and increase the survival of pathogenic *E. coli*. Dunière et al.[2] found that, after aerobic exposure of corn silage, (10^3) CFU/g STEC and *E. coli* O26 were detected. Therefore, silage is susceptible to STEC contamination after opening the silo, and inhibitory measures should be properly implemented.

[1]24
[2]25

2.2.5; Other harmful bacteria

Reilly et al.[3] showed that cattle fed corn silage, grass silage forage, or sugar beet pulp are more prone to develop bovine tuberculosis (bTB). bTB is an infectious disease caused by *Mycobacterium bovis*. Grant et al.[4] detected *Yersinia enterocolitica* in silage fermented for more than 20 months; 6.5% of 46 silage samples were positive, and the presence of the pathogen was associated with indicators such as the high pH of the silage. Nam et al.[5] found that *Campylobacter* sp. occasionally occurs in silage and is also an important foodborne pathogen. *Salmonella* is a pathogen that causes severe diarrhea and is also a hygiene indicator commonly monitored in feed, but no related reports have yet been made for silage.

2.3; Biogenic amines

The main biogenic amines (BA) in silage are putrescine, cadaverine, and tyramine, which are derived respectively from ornithine, lysine, and tyrosine; small amounts of histamine, tryptamine, spermidine, and spermine are also present. BA are formed through the amino acid decarboxylase activity produced by microorganisms such as free amino acids or small peptides; amino acid decarboxylases are produced by certain LAB, such as *Lactobacillus*, *Leuconostoc*, *Enterococcus*, and *Pediococcus*, as well as by many genera occurring in silage, such as *Bacillus*, *Clostridium*, *Klebsiella*, *E. coli*, and *Pseudomonas aeruginosa*. Steidlová et al.[6] investigated and measured BA in 51 corn silage samples; tyramine, putrescine, cadaverine, spermidine, tryptamine, spermine, and histamine were 145.0, 136.0, 96.2, 37.9, 3.0, 2.8, and 2.5 mg/kg, respectively. Owing to the hydrolysis of bacterial proteins, BA in silage is associated with the degradation of silage protein and the reduction in nutritive value. BA can lead to health problems in ruminants, such as rumen metabolic disorders and ruminal acidosis. The formation of BA in silage may be affected by factors such as temperature, the rate of pH decline at the initial stage of ensiling, and oxygen; however, the mechanisms by which these factors affect the BA content and composition of silage are···

···remains to be further studied.

3; Strategies for inhibiting harmful microorganisms in silage

To date, there is no method, either in China or abroad, for treating and improving spoiled silage; it can only be discarded and feeding must be stopped. The main problems in the fermentation process of silage are the use of poor-quality or immature green forage as raw material, the inability to rapidly establish an

[3]26
[4]27
[5]28
[6]29

anaerobic environment so that the silage becomes acidified, and the simultaneous introduction of contaminating pathogens and spoilage microorganisms. To prolong the shelf life of silage and improve its nutritional value and quality, it is highly necessary to study strategies and methods for inhibiting harmful microorganisms during silage fermentation. Throughout the entire ensiling process, from preparation to opening of the silo, adopting different preventive strategies at different stages can prevent the generation of pathogens and silage spoilage.

3.1; Reducing forage pathogens at harvest

To avoid silage degradation, pathogen entry into the silage-feed ecosystem should be prevented as much as possible. Johansson et al.[30] reported that measures such as fertilizing 4 weeks later, harvesting forage, and cutting at an appropriate height above the soil to avoid soil contamination can reduce butyric acid bacteria, *Listeria*, clostridial spore-forming bacilli, and coliform bacteria in silage. The growth conditions of silage raw materials and the choice of harvest time are also very important. Selecting raw materials that are harvested relatively late and have a relatively high DM content (>50%) for ensiling makes them more susceptible to the effects of natural maturation and mycotoxin contamination. In addition, the pathogens carried by the raw materials, silos, tools, and machinery used for harvesting should be prevented from entering the silage.

3.2; Rapid establishment of an anaerobic environment in silage

Rapid establishment of an anaerobic environment prevents the production of silage effluent, promotes rapid growth of LAB, and quickly lowers pH. Silage effluent mainly comes from plant respiration and the activity of aerobic microorganisms. The amount of effluent depends on the DM content, the type of silo, the packing density of the raw material, the chopping length of the raw material, and the use of silage additives. Effluent can cause losses of DM and carbohydrates and, at the same time, dilute silage additives. Therefore, selecting silage raw materials with an appropriate DM content (30%–40%), chopping length (2–6 cm), and packing density (600 kg/m^3), and rapidly establishing an anaerobic environment in the silo for silage production, are necessary measures to avoid the production of silage effluent and to ensure the nutritional quality of silage.

3.3; Measures for establishing an acidified environment

Acidification is the principal mechanism of silage preservation and depends on anaerobiosis-promoted LAB fermentation, buffering capacity, and the DM content of the raw material. Soil introduced into silage increases its buffering capacity. If the buffering capacity is high, aerobic microorganisms at the beginning of ensiling survive for a relatively long time, reducing the contents of hexoses and pentoses and limiting further LAB fermentation, which may lead to secondary fermentation by clostridial spore-forming bacilli, conversion of lactic acid into butyric acid, an increase in pH, and further spoilage. For many years, measures

such as adding chemical agents, sugars, and enzyme preparations to silage have been used to promote silage acidification and limit the growth of pathogenic microorganisms[31].

In addition to chemical and enzyme preparations, microbial inoculants have increasingly been applied to silage preservation. Their purpose is to promote the rapid accumulation of organic acids during ensiling, thereby reducing fermentation and DM losses. Epiphytic LAB or silage additives···

The principal organic acid produced by inoculants is lactic acid, which has a certain promoting effect on the reduction of pH. Most commercial microbial inoculants are homofermentative lactic acid bacteria, because they produce lactic acid with high efficiency. The most common are *Lactobacillus plantarum* fermentation inoculants. It is generally believed that microbial inoculants at 1×10^6 CFU/g are sufficient to overwhelm epiphytic LAB and become the dominant bacteria in silage; other commonly used bacteria include lactobacilli, pediococci, and enterococci. Another category is heterofermentative lactic acid bacteria, represented typically by *Lactobacillus buchneri*. It can produce high concentrations of acetic acid to inhibit fungi in silage, making silage less prone to spoilage when exposed to air, and in recent years it has gradually been widely applied.

3.4; Preventing the Entry of Air and Improving Aerobic Stability

To obtain good-quality silage, from the filling of raw materials into the silo to sealed storage during the ensiling period, it is necessary to prevent sources of contamination and air from entering the silage. At present, for long-term sealed ensiling, polyethylene film and two-sided black–white composite oxygen-barrier film (125 μm) are generally used; the latter has been shown to inhibit silage spoilage and reduce DM loss. Compared with polyethylene film, when silage is exposed to oxygen, it can delay the growth of yeasts and molds, and can also resist damage by birds and rodents as well as ultraviolet irradiation. The ensiling process is a competition between aerobic and anaerobic conditions. When the silo is opened after silage fermentation, the entry of air can cause the degradation of nutrients in the silage. Therefore, the capacity of the silo should be determined according to the scale of animal production and feeding requirements, ensuring that the daily removal depth is sufficient to minimize exposure of silage to air; neat cutting can also limit air penetration and spoilage by harmful bacteria.

Aerobic stability can be enhanced by adding formic acid and its compounds as well as microbial inoculants, and microbial inoculants are widely used in open silos to maintain aerobic stability. Homofermentative inoculants are considered effective inoculants for silage, but some researchers believe that lactic acid can serve as a substrate for lactate-assimilating yeasts and, under insufficient anaerobic conditions, may lead to silage spoilage and deterioration. Heterofermentative lactic acid fermentation is the preferred option for improving the aerobic stability of silage at feed-out. Many studies have shown that the heterofermenta-

tive inoculant *Lactobacillus buchneri* has been demonstrated to increase aerobic stability and reduce silage fermentation losses. Its preservative effect is due to increased concentrations of acetic acid and propionic acid, which lower silage pH, as well as increased antimicrobial substances such as 1-propanol, propyl acetate, and isobutanol, thereby inhibiting or reducing the growth and survival of yeasts and molds[^{32}].

3.5; Direct Inhibition of Harmful Microorganisms

To ensure the quality of silage, additives that inhibit harmful microorganisms are usually added to silage. A mixture of sodium nitrite and hexamethylenetetramine can effectively prevent the growth of *Clostridium*; sodium benzoate can limit the growth of yeasts. Adding calcium formate, sodium benzoate, and sodium nitrite to corn silage can significantly reduce the concentrations of zearalenone, deoxynivalenol, ochratoxin, and fumonisin.

Microbial inoculants have a certain inhibitory effect on the growth of silage pathogens. In addition to organic acids, beneficial silage bacteria also produce other substances with antibacterial potential, such as hydrogen peroxide (H_2O_2), ethanol, ketones, extracellular polysaccharides, and bacteriocins. Corn also contains phenolic compounds such as ferulic acid and coumaric acid, which are natural antibacterial components present in plants. More importantly,

In addition, phenolic compounds have been clearly shown to inhibit the growth of *Escherichia coli* O157:H7, *E. coli* O111, *Listeria*, *Salmonella*, and *Yersinia enterocolitica*. LAB that produce ferulate esterase represent a third-generation silage inoculant; they are mainly used to improve fiber digestion in the rumen, and they may also influence the antibacterial effect in silage feed by regulating ferulic acid activity[^{33}]. However, at present, the antibacterial mechanisms of silage inoculants have not received sufficient attention or study. Bacterial species such as *Lactobacillus*, *Lactococcus*, *Propionibacterium*, and *Enterococcus* all possess genes for ferulate esterase and may have potential antibacterial effects against silage pathogens; their mechanisms of action require further investigation.

4; Conclusions and Prospects

In recent years, the rapid development of production of large ruminants such as dairy cattle, beef cattle, and mutton sheep in China has required high-quality silage feed as a material guarantee. However, spoilage microorganisms have serious negative effects on silage feed, causing deterioration and substantial economic losses, and restricting the broad application of silage feed in ruminant farming. Research on silage feed in China started relatively late, and insufficient attention has been paid in particular to the control of harmful microorganisms in silage. To prevent harmful microorganisms from being transmitted to animals and humans through silage feed carriers and the food chain, thereby threatening animal and human health, it is necessary to strengthen research on inhibiting

the occurrence of harmful microorganisms in silage feed and on eliminating or reducing their metabolic toxins. We need to adopt increasingly updated biotechnologies, continuously explore new bacterial species in silage-feed resources, and isolate and obtain superior microbial inoculants with antibacterial activity, so as to provide theoretical references for further improving the quality of silage feed and enhancing the productive performance of ruminants.

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Harmful Microorganisms in Silage and Their Suppression Measures

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Abstract: During the fermentation and storage of silage, it is easily contaminated by harmful microorganisms and their metabolic toxins, which can reduce the quality of silage and the production performance of ruminants, and seriously endanger the health of animals and humans. This paper reviewed the types of harmful microorganisms in silage, the mechanisms by which their metabolites affect the nutritional quality and aerobic stability of silage, and the measures and methods for inhibiting harmful microorganisms during silage fermentation, with the aim of providing a theoretical basis for the development of high-quality additives, optimization of the fermentation environment, inhibition of the growth of harmful microorganisms, and improvement of the nutritional

quality of silage.

Key words: silage; spoilage microorganism; pathogenic microorganism; suppression measure

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

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