Effects of microbial additives in silages: facts and perspectives

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Abstract

Silage inoculants have been used to maintain and (or) improve the nutritive value of forages stored as silage. Inoculants based on homolactic acid bacteria have been used to ensure a quick fermentation and rapid drop in pH based on the production of lactic acid. In turn, there is usually less production of acetic and butyric acids and lower concentrations of ammonia-N. Newer inoculants based on *Lactobacillus buchneri* have been developed to improve the aerobic stability of silages by increasing acetic acid and decreasing yeasts. Some inoculants have also resulted in improvements

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in fiber digestion via moderations of rumen pH and production of ferulic acid esterase. Silage inoculants work best when applied at correct application rates and when they are evenly distributed through the forage mass. They are also more effective when the management of the silage has been good.

Introduction

The primary goal of making silage is to maximize the preservation of original nutrients in the forage crop for feeding at a later date. However, fermentation in the silo is a very uncontrolled process can easily lead to less than optimal preservation of nutrients. In order to assist in the fermentation process, various silage additives have been used to improve the recovery of nutrients and energy in silage, often with subsequent improvements in animal performance. This review will focus on some practical aspects of the fermentation process and the uses of microbial inoculants. For a more in-depth review on all silage additives see the review by Kung et al., 2003.

The ensiling process

From a practical view, the three most important things that must occur in order to make good silage are 1) the rapid removal of air, 2) the rapid production of lactic acid that results in a rapid drop in pH, and 3) continued exclusion of air from the silage mass during storage and feedout.

Rapid removal of air is important because it prevents the growth of unwanted aerobic bacteria, yeasts, and molds that compete with beneficial bacteria for substrate. If air is not removed quickly, high temperatures and prolonged heating are commonly observed. Air can be eliminated by wilting plant material to recommended dry matters (DM) for the specific crop and storage structure, chopping forage to a correct length, quick packing, good compacting, even distribution of forage in the storage structure, and immediately sealing the silo. After chopping, plant respiration continues for several hours (and perhaps days if silage is poorly packed) and

plant enzymes (e.g., proteases) are active until air is used up. Air must be removed before optimal fermentation can take place.

Once air is removed, fermentation can begin. Lactic acid bacteria (LAB) utilize water-soluble carbohydrates to produce lactic acid, the primary acid, responsible for increasing the acidity and decreasing the pH in silage. The strength of silage acids can be determined by measuring silage pH. A pH above 7 is considered basic whereas a pH below 7 is acidic. A pH of 7 is neutral and means that a product is neither acidic nor basic. Depending on the crop, plant material in the field can range from a pH of about 5 to 6 and decrease to a pH of 3.6 to 4.5 after acid is produced. A quick reduction in silage pH will help to limit the breakdown of protein in the silo by inactivating plant proteases. In addition, a rapid decrease in pH will inhibit the growth of undesirable anaerobic microorganisms such as enterobacteria and clostridia. Eventually, continued production of lactic acid and a decrease in pH inhibits growth of all bacteria.

In general, once fermentation is complete, good silage will remain stable and not change in composition or heat. This is why filling silos quickly and sealing of silos immediately after filling is so important. However, depending on the mixture of fermentation end products, silage can spoil rapidly if exposed to air during storage and feed out. A common misconception is that molds are responsible for spoilage of silage when it is exposed to air. However, yeasts (not molds) are the primary microorganisms that cause aerobic spoilage and heating. When exposed to air, yeasts metabolizes lactic acid that causes the pH of the silage to increase, thus allowing bacteria that were inhibited by low pH to grow and further spoil the mass. Airtight silos and removal of sufficient silage during feed-out can help to prevent aerobic spoilage. Various silage additives (which will be discussed later in this paper) can also improve aerobic stability.

Although the ensiling process appears quite simple, many factors can affect what type of fermentation takes place in a silo and thus, the mixture of end products (Figure 1). For example, the buffering content of a forage mass can have an effect on silage fermentation. Alfalfa has a high buffering capacity in comparison to corn.

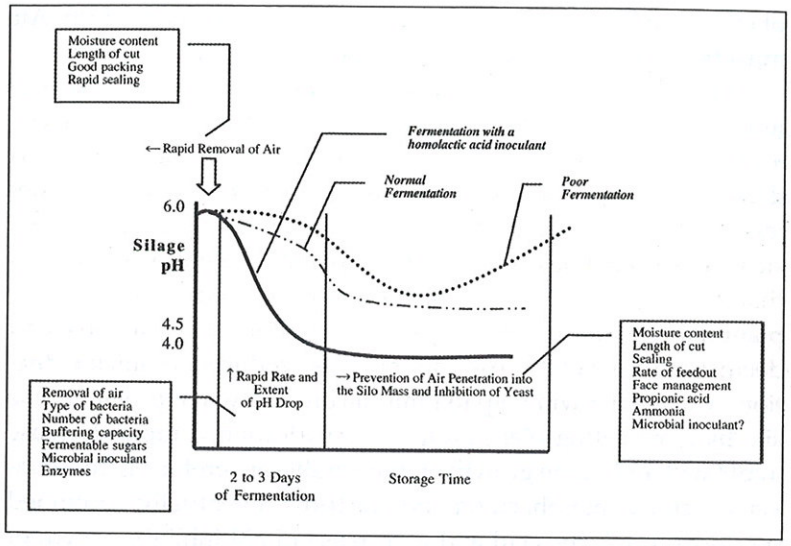

Figure 1. The three major events that make good silage and factors that can affect the silage fermentation process.

Thus, it takes more acid production to lower the pH in alfalfa than in corn silage, resulting in the former being more difficult to make. The dry matter content of the forage can also have major effects on the ensiling process via a number of different mechanisms. First, drier silages do not pack well and thus it is difficult to exclude all of the air from the forage mass. Second, as the dry matter content increases, growth of lactic acid bacteria is curtailed and the rate and extent of fermentation is reduced. (For example, acidification occurs at a slower rate and the amount of total acid produced is less). Thirdly, undesirable bacteria called clostridia tend to thrive in very wet silages and can result in excessive protein degradation, DM loss, and production of toxins. Where weather permits, wilting forage above 30-35% DM prior to ensiling can reduce the incidence of clostridia because these organisms are not very osmotolerant (they do not like dry conditions). Delayed filling results in excessive amounts of air trapped in the forage mass that can result in a decrease of fermentable water-soluble carbohydrates.

The types and numbers of bacteria on the plant also have profound effects on silage fermentation. Natural populations of lactic acid bacteria (LAB) on plant material are often low in number and heterofermentative (produce end products other than lactic acid). In addition, if air is not removed from the silage mass, other types of fermentation can occur.

Silage inoculants

As shown in Table 1 many end products are commonly produced during the fermentation process but many of these end products are associated with less than desirable fermentations. Of the several types of acids produced during, lactic acid is the strongest acid (stronger than the other acids) and preferred end product of silage fermentation. In fact, homolactic acid fermentation that produces only lactic acid is a desirable fermentation because of the high energy and dry matter recoveries (Table 2). Note that in the undesirable fermentations, large amounts of carbon dioxide (CO_2) are produced. Because CO_2 is a gas, the carbon (or dry matter) is lost to the environment. This explains why these fermentations have low DM recoveries.

Table 1. Common end products of silage fermentation.

Item	Positive or negative	Action(s)
pH	+	Low pH inhibits bacterial activity
Lactic acid	+	Inhibits bacterial activity by lowering pH
Acetic acid	–	Associated with undesirable fermentations
	+	Inhibits yeasts responsible for aerobic spoilage
Butyric acid	–	Associated with protein degradation, toxin formation, and large losses of DM and energy
Ethanol	–	Indicator of undesirable yeast fermentation and high DM losses
Ammonia	–	High levels indicate excessive protein breakdown
Acid detergent insoluble nitrogen (ADIN)	–	High levels indicate heat-damaged protein and low energy content

Table 2. Predominant fermentation pathways in silage.

Type of fermentation	End-products	Theoretical DM recovery %	Theoretical energy recovery %
Homolactic (glucose)	lactic acid	100	99
Heterolactic (glucose)	lactic acid, ethanol, CO_2	76	98
Heterolactic (fructose)	lactic acid, acetate, mannitol, CO_2	95	99
Yeast (glucose)	ethanol, CO_2	51	99
Clostridia (glucose and lactate)	butyric acid, CO_2	49	82

Homolactic acid bacteria

Because forage often naturally contains many detrimental types of bacteria, the concept of adding a microbial inoculant to silage was to add fast growing homofermentative lactic acid bacteria (^{ho}LAB) in order to dominate the fermentation resulting in a higher quality silage. Some of the more common homolactic acid bacteria (^{ho}LAB) used in silage inoculants include *Lactobacillus plantarum* (note: this organism is now officially classified as a heterolactic acid bacteria), *L. acidophilus, Pediococcus acidilactici, P. pentacaceus,* and *Enterococcus faecium.* Microbial inoculants contain one or more of these bacteria, which have been selected for their ability to dominate the fermentation. The rationale for multiple organisms comes from potential synergistic actions. For example, growth rate is faster in *enterococci* > *pediococci* > *lactobacilli.* Some *pediococci* strains are more tolerant of high DM conditions than are *lactobacilli* and have a wider range of optimal temperature and pH for growth (they grow better in cool conditions found in late Fall and early Spring). Table 3 lists several common microbes that have been studied as silage inoculants.

Fermentation responses to homolactic acid bacteria

Alfalfa, grass, and small cereal grain crops have responded well to microbial inoculation with ^{ho}LAB. The fermentation of high moisture corn has also been improved with ^{ho}LAB. However, ^{ho}LAB microbial inoculation of corn silage has resulted in less consistent results. For example, I found 14 published (peer reviewed) studies in North America where corn silage was treated with a ^{ho}LAB microbial inoculant. Improvements in animal performance where found in only 3 instances and changes in fermentation end products were small. However, Bolsen et al. (1992) reported that in 19 studies conducted at Kansas State University, with corn silage, silages inoculated with ^{ho}LAB had 1.3 percentage units higher DM recovery, supported 1.8% more efficient gains, and produced 1.64 kg more gain per ton of crop ensiled with beef cattle. Similar results were found with treated sorghum silages. In certain instances, significant animal responses have been observed with inoculation although there was little effect on traditional end products of fermentation (Gordon, 1989; Kung et al., 1993). These data suggest that lack of detectable changes in classically measured fermentation end products is not a good indicator of the effectiveness of an inoculant.

Table 3. Some of the more common bacteria used as silage inoculants and some reasons for their use.

Organism	Type of organism	General reasons for addition	Primary end products
Lactobacillus plantarum	Lactic acid bacteria, traditionally considered homolactic but now classified as heterolactic	Rapid production of lactic acid Relatively acid tolerant	Lactic acid
Pediococcus sp.	Lactic acid bacteria, homolactic	Fapid production of lactic acid Faster growing than lactobacilli Some strains show good growth at cooler temperatures Some strains have good osmotolerance	Lactic acid
Enterococcus faecium	Lactic acid bacteria, homolactic	Rapid production of lactic acid Faster growing than lactobacilli	Lactic acid
Propionibacterium sp.	Propionibacteria	Production of antifungal compounds	Propionic and acetic acids, CO_2
Lactobacillus buchneri	Lactic acid bacteria, heterolactic	Production of antifungal compounds Ferulic acid esterase for improved fiber digestion	Lactic and acetic acids, propanediol, CO_2

When compared to untreated silages, silages treated with adequate numbers of a viable ^{ho}LAB should be lower in pH, acetic acid, butyric acid and ammonia-N but higher in lactic acid content (Table 4). In a review of the literature between 1990-95, Muck and Kung (1997) reported that microbial inoculation lowered pH, improved the lactic: acetic ratio, and lowered ammonia nitrogen content in more than 60% of studies. Dry matter recovery was improved in 35% of the studies. Dry matter digestibility was also improved in about one third of the cases. Microbial inoculation usually has little or no effect on the fiber content of silages because most lactic acid bacteria contain little or no ability to degrade plant cell walls. Decreases in fiber content may be due to partial acid hydrolysis of hemicellulose. Some data suggests that certain microbial inoculants can increase fiber digestion (Rice et al., 1990; Weinberg et al., 2007).

Effects of homolactic acid bacteria on animal performance

Relative to animal responses, Kung and Muck (1997) reported positive responses to microbial inoculants on intake, gain, and milk production (Table 5). The average response in milk production was a +1.36 kg per day in studies where milk production was statistically improved. Although literature summaries are encouraging,

Table 4. Theoretical effect of adding a microbial inoculant containing homolactic lactic acid bacteria on the end products of silage fermentation.

Item	Theoretical effect
DM recovery	Greater recovery
Rate of pH decline and final pH	Faster decline and lower final pH
Ammonia nitrogen	Lower content
Lactic acid	Greater content
Acetic acid	Lower content
Butyric acid	Lower Content
Ethanol	Lower content
Fiber (NDF/ADF)	Increased
DM digestibility	Increased
Animal performance	Increased

Table 5. A summary of animal responses to microbial inoculants between 1990 and 1995.

Type of study	Intake	Gain	Milk production
Number of studies	67	15	36
Studies with positive responses	28%	53%	47%

(Kung and Muck, 1997)

caution should be used when interpreting such data because all inoculants are not equal and the conditions (e.g. rate of application, inoculant viability, species of bacteria, crop, and moisture levels) varied markedly among the studies. As many have pointed out in the past, products with organisms with the same name are not necessarily the same organism and may not have the same effectiveness (Dennis, 1992). For example, Rooke and Kafilzadeh (1994) reported that various strains of ^{ho}LAB improved silage fermentation but animal performance was improved by only 1 strain of organism. Probably the most impressive data set for a single inoculant is that of animal experiments conducted using *Lactobacillus plantarum* MTD1. A summary of 14 lactation studies conducted in University and government research institutes in North America and Europe using resulted in an average increase of 4.6% (Moran and Owen, 1994). Improvements in milk yield were obtained with a variety of crops (grass, corn, and alfalfa) across a wide spectrum of DM contents (15 to 46% DM). Similarly, 19 comparisons among untreated silages and Moran and Owen (1995) summarized silages treated with MTD1 for beef cattle. Across all studies and types of forage, cattle fed inoculated silage inoculated with MTD1 ate 7.5% more DM and gained 11.1% more weight.

Unfortunately, there is no good way to predict the effectiveness of microbial inoculants. A model developed by Pitt (1990) suggested that inoculants would be most effective on alfalfa during cool conditions of first, third and fourth cuttings. However, there are numerous products that have little or no research to support claims of improved fermentation or animal performance. Another factor, which complicates the evaluating process, is that the majority of bacterial inoculants are repackaged for distribution under

private label and numbers of bacteria may be low and/or other additives (e.g., enzymes, fermentation extracts, minerals) are included in the formulations.

Effects of homolactic acid bacteria on bunk life

Bunk life or aerobic stability improved in only 33% of the studies and in fact inoculation with ^{ho}LAB has, in many instances, made aerobic stability worse (Muck and Kung, 1997). This is probably due to a lower content of acetic acid and other potential antifungal end products. This finding is extremely ironic because, many producers buy microbial inoculants because they perceive an improvement in aerobic stability. The most recent thought from silage researchers suggest that there needs to be a compromise in silage fermentation end products such that recovery of nutrients in maximized that results in silages that are stable when exposed to air.

Propionibacteria

Several microorganisms that are not ^{ho}LAB have been used as silage inoculants specifically for the purpose of improving aerobic stability. For example, the *Propionibacteria* are able to convert lactic acid and glucose to acetic and propionic acids that are more antifungal than lactic acid. Florez-Galaraza et al. (1985) reported that addition of *P. shermanii* prevented the growth of molds and markedly reduced the initial population of yeast in high moisture corn where the final pH was greater than 4.5. Weinberg et al. (1995) saw little benefit from adding *Propionibacteria* to pearl millet and corn silage (final pH < 4.0) but reported improvements in the aerobic stability of wheat silage when the decline in pH was slow. Similarly, in 3 studies using laboratory silos, we (Kung et al., unpublished data) did not observed beneficial effects of *Propionibacteria* in corn silage (final pH 3.6 to 3.8). However, Bolsen et al. (1996) reported more propionic acid, lower yeasts and molds, and greater aerobic stability in corn silage (pH of 3.6) treated with *Propionibacteria*. Some concerns relative to the use of *Propionibacteria* that have not been adequately addressed are the loss of DM (from CO_2 production) and the fact that *Propionibacteria* have proteolytic activity. The primary reasons for the ineffectiveness of these organisms include

the facts that they are strict anaerobes, they are slow growing, and they are relatively acid intolerant.

Heterolactic *lactobacilli*

Contrary to past thinking, new research suggests that heterolactic acid bacteria may also be useful as silage inoculants when aerobic stability is a problem. Muck (1996) first suggested that *Lactobacillus buchneri* could improve the aerobic stability of silage. Driehuis et al. (1996) reported that corn silage treated with *L. buchneri* was more stable than untreated silage. They suggest that improved aerobic stability was due to the ability of *L. buchneri* to ferment lactic acid to acetic acid and 1,2 propanediol (Oude-Elferink et al., 1999). In a meta analysis, Kleinschmit and Kung (2006) reported significant improvements in aerobic stability when silages were treated with *L. buchneri*. Mari et al. (2009) collected samples from farm silos and showed that corn silages treated with *L. buchneri* had greater populations of this organism, fewer yeasts and greater aerobic stability over corn silages that had not been treated. Recently, Nsereko et al. (2008) developed a strain of *L. buchneri* capable of producing ferulic acid esterase which when used as a silage inoculant has the potential to improve fiber digestion in silages. However, digestion of NDF was not been consistently improved in several studies (Kang et al., 2009; Hofherr et al., 2008).

Inoculation rate, use, and storage of inoculants

The organism(s) from microbial inoculants must be present in sufficient numbers to effectively dominate the fermentation. The most commonly recommended inoculation rate for homolactic acid based-inoculant results in a final concentration of 100,000 (or 1×10^5) colony-forming units of this organism per gm of wet forage. There is limited evidence to support the suggestion of some that doubling or tripling this amount (e.g. 200,000-300,000 cfu) is more beneficial.

Most microbial inoculants are available in powder or granular form. Inoculants applied in the dry form are often mixed with calcium carbonate (limestone), dried skim milk, sucrose or other carriers. These products can be applied by hand or by solid meter-

ing devices as per manufacturer's recommendations. Inoculants to be applied in the liquid form come as dried powders and are mixed with water just prior to use. (Use of chlorinated water may be detrimental to the inoculant if levels exceed more than 1.5 to 2 ppm.) Application can be with a simple watering can by weighing the incoming forage load and adjusting application based on the average unloading time. A better method is to use a metered liquid sprayer to evenly disperse the inoculant on the forage. Unused liquids should be discarded after a period of 24 to 48 h because bacterial numbers begin to decline. Water in inoculant tanks should not be allowed to increase over about 37°C because high heat will kill the inoculants (Mulrooney and Kung, 2008).

Kung (2009) reviewed a wide variety of causes that might cause a microbial inoculant to fail. Correct application rate and adequate distribution throughout the forage mass was highlighted as major factors that could cause failure of a microbial inoculant. However, application to forage at the chopper is highly recommended in order to maximize the time that microorganisms have in contact with fermentable substrates. Application at the chopper is more important if silage is being stored in a bunk or pile because it is difficult to achieve good distribution onto silage from a forage wagon. Distribution of the inoculant is less of a problem if it is applied at the blower of an upright silo or at the bagger. Throwing a can of dry inoculant onto a load of forage and hoping for even distribution is not an acceptable practice! Inoculants can be applied in a liquid or solid form. Data from our lab (Whiter and Kung, 2001) suggests that on higher DM silages (greater than about 45% DM), using a liquid based inoculant is preferable because the low moisture in these silages limits fermentation. Inoculants applied in a liquid form may be more advantageous because the bacteria are added with their own moisture to help speed up fermentation.

Storage is an important aspect of a high quality inoculant that contains live microorganisms. Some inoculants require refrigeration or freezing for optimum storage. Those that do not require cold temperatures for storage should still be kept in cool, dry areas away from direct sunlight. Moisture, oxygen and sunlight can decrease the stability of inoculants resulting in lower viable counts

and a product that does not meet label guarantees. Opened bags of inoculants should be used as soon as possible and, if not completely used, probably not carried over into the next season.

Conclusions

Silage additives can be useful tools to improve silage quality and animal performance; however, they are not replacements for good management practices. The question of which additive to use can sometimes be a difficult one. Table 6 shows some suggestions for use of silage additives. Cost of the product should not be the most important factor when choosing an additive! Proof of efficacy and cost should be considered together. Why buy a cheap additive that is ineffective? In contrast, the most expensive additive might not be the best either. How should one evaluate a silage additive?

Table 6. Some suggestions for use of silage additives.

Item	Additive of choice
1) Consistently make good quality silage. 2) No significant heating problems.	1) Homolactic acid based inoculant.
1) Consistently make good quality silage. 2) Some heating problems during warm weather.	1) Homolactic acid based inoculant and 2) Buffered propionic acid preservative added to TMR at feeding.
1) Usually make good quality silage. 2) Spoiled or hot silage usually only at silo opening and when feeding out the last silage from silo.	1) Homolactic acid based inoculant and 2) Buffered propionic acid preservative or new microbial inoculants designed to improve aerobic stability on several first and last loads into silo.
1) Consistently have problems with heating silage. 2) Inadequate daily removal of silage leading to hot feed.	1) Buffered propionic acid preservative at ensiling or 2) New microbial inoculants designed to improve aerobic stability.
1) For bunk, pit, or drive-over silos, significant spoilage on top layer even after covering.	1) Buffered propionic acid preservative or 2) New microbial inoculants designed to improve aerobic stability only on last loads into silo.
1) Extremely dry forage or forage chopped too long	1) Buffered propionic acid preservative or 2) New microbial inoculants designed to improve aerobic stability.
1) Silage that is moved, silage for selling, silage fed from intermediate feeding piles	1) Buffered propionic acid preservative or 2) New microbial inoculants designed to improve aerobic stability.

In my opinion, the three major issues that are relevant in North America for choosing an additive include a broad and extensive data base (proving efficacy under a broad range of conditions, crops, moistures, etc.) that 1) supports improvements in animal production, 2) supports improvements in DM or nutrient recovery, or 3) supports improved aerobic stability. Finally, choose an additive from a reputable company that stands behinds their products and offers excellent technical service support.

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