SILAGE FERMENTATION AND SILAGE ADDITIVES
- Review -

K. K. Bolsen[1,2], G. Ashbell and Z. G. Weinberg

Feed Conservation Laboratory, Agricultural Research Organization,
The Volcani Center, Bet Dagan 50250, Israel

Summary

Advances in silage technology, including precision chop forage harvesters, improved silos, polyethylene sheeting, shear cutting silo unloaders, and the introduction of total mixed rations, have made silage the principal method of forage preservation. A better understanding of the biochemistry and microbiology of the four phases of the ensiling process has also led to the development of numerous silage additives. Although acids and acid salts still are used to ensile low-DM forages in wet climates, bacterial inoculants have become the most widely used silage additives in the past decade. Commercial inoculants can assure a rapid and efficient fermentation phase; however, in the future, these products also must contribute to other areas of silage management, including the inhibition of enterobacteria, clostridia, and yeasts and molds. Nonprotein nitrogen additives have the problems of handling, application, and reduced preservation efficiency, which have limited their wide spread use. Aerobic deterioration in the feedout phase continues to be a serious problem, especially in high-DM silages. The introduction of competitive strains of propionic acid-producing bacteria, which could assure aerobically stable silages, would improve most commercial additives. New technologies are needed that would allow the farmer to assess the chemical and microbial status of the silage crop on a given day and then use the appropriate additive(s).

(**Key Words** : Ensiling Process, Fermentation, Silage Preservation, Additives, Bacterial Inoculants)

Introduction

Silage is the feedstuff produced by the fermentation of a crop, forage, or agricultural byproduct of generally greater than 50% moisture content. Ensiling is the name given to the process, and the container (if used) is called a silo. Silage dates back to about 2000 B.C. However, the modern era did not begin until 1877, when a farmer in France, A. Goffart, published a book based upon his own experiences with corn silage.

Since the 1950s, the amount of silage made in most developed countries has increased steadily and often at the expense of hay (Wilkinson and Stark, 1992). Silage-making is much less weather-dependent than hay-making, and silage is mechanized more easily, is suited better to large-scale livestock production, and is adapted to a wider range of crops, i.e., corn, sorghums, and winter or spring cereals (Bolsen, 1985).

A well-preserved silage of high nutritional value is achieved by harvesting the crop at the proper stage of maturity; minimizing the activities of plant enzymes and undesirable, epiphytic microorganisms (i.e., those naturally present on the plant); and encouraging the dominance of lactic acid bacteria (LAB) (McDonald, 1980). Two dominant features must be considered for every silage: 1) the crop and its stage of maturity and 2) the management and know-how imposed by the silage-maker.

The key "ensileability" criteria for a crop are : 1) dry matter (DM) content; 2) sugar content; and 3) buffering capacity (resistance to acidification). In these respects, corn is the "nearly perfect" crop, whereas alfalfa is at the other extreme and is the most difficult crop to preserve as silage. Grasses usually contain more water-soluble carbohydrates (WSC) and have less resistance to acidification than legumes.

[1] Address reprint requests to Dr. K. K. Bolsen, Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas 66506-0201, USA.

[2] Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas 66506-0201, USA.

Received October 28, 1995
Accepted May 1, 1996

The Ensiling Process

When making decisions about silage management techniques, it is important to have a good understanding of the events that occur during silage preservation. The major processes involved can be divided into four phases: 1) aerobic, 2) fermentation, 3) stable, and 4) feedout. Each phase has distinctive characteristics that must be controlled in order to maintain forage (silage) quality throughout the periods of harvesting, silo filling, and silage storing and feeding.

Aerobic phase

As the chopped forage enters the silo, two important plant enzyme activities occur: respiration and proteolysis. Respiration is the complete breakdown of plant sugars to carbon dioxide and water, using oxygen and releasing heat. Simultaneously, plant proteases degrade proteins to primarily amino acids and ammonia and, to a lesser extent, peptides and amides (i.e., asparagine and glutamine) (McDonald et al., 1991).

The loss of sugar is crucial from the standpoint of silage preservation. Sugars are the principal substrate for the lactic acid bacteria (LAB) to produce the acids to preserve the crop. Excessive heat production (i.e., temperatures above 42-44℃) can result in Maillard or browning reactions, which reduce the digestibility of both protein and fiber constituents. The main aerobic phase losses occur during exposure to air before a given layer of forage is covered by a sufficient quantity of additional forage to separate it from the atmosphere or before an impermeable cover (i.e., polyethylene sheeting) is applied.

Fermentation phase

Once anaerobic conditions are reached in the ensiled material, anaerobic microorganisms begin to grow. The LAB are the most important microflora, because forages are preserved by lactic acid. The other microorganisms, primarily members of the family *Enterobacteriaceae,* clostridial spores, and yeast and molds, have negative impacts on silage. They compete with the LAB for fermentable carbohydrates, and many of their end products have no preservative action.

The enterobacteria have an optimum pH of 6-7, and most strains will not grow below pH 5.0. Consequently, the population of enterobacteria, which is usually high in the pre-ensiled forage, is active only during the first 12-36 hours of ensiling (Lin et al., 1992). Then their numbers decline rapidly, so they are not a factor after the first few days of the fermentation phase.

Growth of clostridial spores can have a pronounced effect on silage quality. Clostridia can cause secondary fermentation, which converts sugars and organic acids to butyric acid and results in significant losses of DM and digestible energy. Proteolytic clostridia ferment amino acids to a variety of products, including ammonia, amines, and volatile organic acids. Like the enterobacteria, clostridial spores are sensitive to low pH, and clostridia require wet conditions for active development. Clostridial growth is rare in crops ensiled with less than 65% moisture, because sufficient sugars usually are present to reduce the pH quickly to a level below 4.6-4.8, at which point clostridia can not grow. For wetter forages (70% moisture or more), reducing the pH to less than 4.6 either by the production of lactic acid or by direct acidification with the addition of acids or acid salts is the only practical means of preventing the growth of these bacteria with today's technology.

The period of active fermentation lasts from 7-21 days. Forages ensiled wetter than 65% moisture usually ferment rapidly, whereas fermentation is quite slow when the moisture content is below 50%. For forages ensiled in the normal moisture range (55-75%), active fermentation is completed in 7-14 days. At this point, fermentation of sugars by LAB has ceased, either because the low pH (below 4.0-4.2) stopped their growth or there was a lack of sugars for fermentation.

The populations of epiphytic microorganisms on silage crops are quite variable and are affected by forage specie, stage of maturity, weather, mowing, field-wilting, and chopping (Fenton, 1987; Spoelstra and Hindle, 1989). Numerous studies have shown that the chopping process tends to increase the microflora numbers compared with those on the standing crops, and the LAB population is most enhanced (Muck, 1989; Lin et al., 1992). This phenomenon was explained earlier as inoculation from the harvesting machine and microbial multiplication in the plant juices liberated during harvest. However, recent findings of Pahlow (1990) demonstrated that these large increases in microflora numbers were impossible to achieve by microbial proliferation and growth, because the time involved was too short, or by contamination from harvesting equipment, which could occur in the first load but not in later loads. A new "somnicell" hypothesis proposes that bacteria assume a viable but unculturable stage on the surface of intact plants (Pahlow and Muller, 1990). The chopping process activates the previously dormant population by releasing plant enzymes (i.e., catalase and superoxide dismutase) and manganese compounds.

The LAB ferment WSC to primarily lactic acid, but also produce some acetic acid, ethanol, carbon dioxide,

and other minor products. This is a rather large group of bacteria, which includes species in six genera (table 1). They are divided into two categories; the homofermentative LAB produce only lactic acid from fermenting glucose and other six-carbon sugars, whereas heterofermentative LAB produce acetic acid, ethanol, and carbon dioxide in addition to lactic acid (McDonald et al., 1991). In the fermentation phase, competition between strains of LAB determines how homofermentative the ensiling process will be.

TABLE 1. LACTIC ACID BACTERIA OF IMPORTANCE IN THE ENSILING PROCESS AND THEIR FERMENTATION PRODUCTS

Genus	Species	Glucose fermentation
Lactobacillus	*acidophilus* *casei* *comyiformis* *curvatus* *plantarum* *salivarus*	Homofermentative[1]
	brevis *buchneri* *fermentum* *viridescens*	Heterofermentative[2]
Pediococcus	*acidilactici* *cerevisiae* *pentosaceus*	Homofermentative
Enterococcus	*faecalis* *faecium*	Homofermentative
Lactococcus	*lactis*	Homofermentative
Streptococcus	*bovis*	Homofermentative
Leuconostoc	*mesenteroides*	Heterofermentative

Source: McDonald et al. (1991).

[1] Microorganisms that ferment sugars to predominantly lactic acid.

[2] Microorganisms that ferment sugars to a variety of organic acids, ethanol, and carbon dioxide.

Stable phase

Following the active growth of LAB, the ensiled material enters the stable phase. If the silo is properly sealed and the pH has been reduced to a low level, little biological activity occurs in this phase. However, very slow rates of chemical breakdown of hemicellulose can occur, releasing some sugars. If active fermentation ceased because of a lack of WSC, the LAB might ferment the sugars released by hemicellulose breakdown, causing a further slow rate of pH decline.

Another major factor affecting silage quality during the stable phase is the permeability of the silo to air (i.e., oxygen). Oxygen entering the silo is used by aerobic microorganisms (via microbial respiration), causing increases in yeast and mold populations, losses of silage DM, and heating of the ensiled mass. Pathogens, such as *Listeria monocytogenes*, have been found to proliferate in silages exposed to oxygen infiltration at low levels. The risk of *L. monocytogenes* is greater in low-DM silages and at high levels of oxygen ingress into the silo (Donald et al., 1993).

The amount of aerobic loss in this phase is related not only to the permeability of the silo but also to the density of the silage. If the silage is left unsealed, substantial DM losses can occur at the exposed surface (Bolsen et al., 1993). These losses can be reduced by covering the surface of the ensiled material with polyethylene sheeting, whether in vertical tower or horizontal bunker, trench, or stack silos (Dickerson et al., 1992). Oxygen can pass through polyethylene, but at a very slow rate. Cracks in the silo wall or holes in the polyethylene seal obviously increase the rate at which oxygen can penetrate the silage mass.

Feedout phase

When the silo is opened, oxygen usually has unrestricted access to the silage at the face. During this phase, the largest losses of DM and nutrients can occur because of aerobic microorganisms consuming sugars; fermentation products (i.e., lactic and acetic acids); and other soluble nutrients in the silage. These soluble components are respired to carbon dioxide and water, producing heat. Yeasts and molds are the most common microorganisms involved in the aerobic deterioration of the silage, but bacteria, such as *Enterobacteriaceae* and *Bacillus* spp., also have been shown to be important in some circumstances (Woolford, 1984; Muck and Pitt, 1993). Besides the loss of highly digestible nutrients in the silage, some species of molds can produce mycotoxins and/or other toxic compounds that can affect livestock and human health.

The microbial activity in the feedout phase is the same as that occurring because of oxygen infiltration during the stable phase. The major difference is the amount of oxygen available to the microorganisms. At feedout, the microorganisms at the silage face have unlimited quantities of oxygen, allowing them to grow rapidly. Once yeasts or bacteria reach a population of 10^7-10^8 colony-forming units (cfu) per g of silage or molds reach 10^6-10^7 cfu per g, the silage will begin to heat, and digestible

components, like sugars and fermentation products, will be lost quickly. The time required for heating to occur is dependent of several factors including: 1) numbers of aerobic microorganisms in the silage, 2) time exposed to oxygen prior to feeding, 3) silage fermentation characteristics, and 4) ambient temperature.

Under farm conditions, DM losses in the feedout phase are largely a function of silage management practices. Few data are available to quantitate feedout losses in farm-scale silos, but laboratory studies indicate that DM losses are about 1.5-3.0% per day for each 8-12°C rise in the silage temperature above ambient (Woolford, 1984). A fast filling rate and tight sealing of the silo minimize the build up of aerobic microorganisms in the silage and maximize the production of fermentation products that will inhibit their growth. Adequate packing of the ensiled material reduces the distance that oxygen can penetrate the exposed silage face. Finally, feeding rate and silage density determine the length of time the silage is exposed to oxygen prior to feedout, and the shorter the exposure time, the less likely a silage is to heat during the feedout phase.

Categories of Silage Additives

In the early years of silage production, the reason for applying an additive was to prevent secondary fermentation and a butyric acid silage. As a result, the efficacy of the additive usually was judged by its effect on typical fermentation criteria, i.e., pH and contents of ammonia-nitrogen and lactic, acetic, and butyric acids (Spoelstra, 1991). This orientation on fermentation was reflected in the traditional division of additives into categories of fermentation inhibitors, fermentation stimulants, and substrate or nutrient sources (table 2).

TABLE 2. CATEGORIES OF SILAGE ADDITIVES AND ADDITIVE INGREDIENTS[1]

Inhibitors[2]		Stimulants			
Acids	Others	Bacterial inoculants[3]	Enzymes[4]	Substrate sources[5]	Nutrient sources
Formic	Ammonia	Lactic acid	Amylases	Molasses	Ammonia
Propionic	Urea	bacteria	Cellulases	Glucose	Urea
Acetic	Sodium chloride		Hemicellulases	Sucrose	Limestone
Lactic	Sodium nitrite		Pectinases	Dextrose	Other minerals
Caproic	Sodium sulfate		Proteases	Whey	
Sorbic	Sodium sulfate		Xylanases	Cereal grains	
Benzoic	Sodium hydroxide			Beet pulp	
Acrylic	Sulfur dioxide			Citrus pulp	
Hydrochloric	Formaldehyde			Rice bran	
Sulfuric	Paraformaldehyde			Wheat bran	

Source: Pitt (1990) and McDonald et al. (1991).

[1] Not all additives or ingredients used for silage are listed, not all listed are always effective, and not all listed are approved for use on ensiled material intended for livestock feed in all countries.

[2] Some inhibitors work aerobically, suppressing the growth of yeast, molds, and aerobic bacteria; others work anaerobically, restricting undesirable bacteria (i.e., clostridia and enterobacteria), plant enzymes, and possibly LAB.

[3] Most contain live cultures of LAB from the genera *Lactobacillus, Pediococcus, Enterococcus,* or *Streptococcus.*

[4] Most enzymes are microbial byproducts, which have enzymatic activity.

[5] Most ingredients also can be listed under nutrient sources.

In a comprehensive guide for silage additives available in the USA, Bolsen and Heidker (1985) included information on over 150 products, and a more recent guide (Anonymous, 1992) contained nearly 80 bacterial inoculants and about 10 acid, enzyme, or nonprotein nitrogen (NPN) additives. A guide for silage products used in the UK contained over 100 additives, including 62 inoculants and 33 acid-based (Wilkinson, 1990). Spoelstra (1991) reported the results of a 1988 survey of silage additives marketed in the 12 countries of the European Community. Of the 203 additives identified, 87 were inoculants, and 83 were acid-based or salts of acids.

Efficacy of Silage Additives

Bacterial inoculants

The first known use of LAB cultures was with ensiled sugar beet pulp in France at the beginning of this century (Watson and Nash, 1960). Kuchler (1926) (cited by Spoelstra, 1991) described an inoculant system developed in Germany, which included the growing of bacteria on the farm. Many of these earlier attempts to inoculate silage crops were not successful because: 1) the strains of LAB were not adapted to a silage environment or 2) the bacterial cultures were not viable at the time of use (Spoelstra, 1991).

Whittenbury (1961) defined the criteria that a LAB should satisfy for use in silage, and additional characteristics were cited by Woolford (1984) and Lindgren (1984). Woolford and Sawczyc (1984) screened 21 strains of LAB and found that none of them satisfied all criteria. *Lactobacillus plantarum* has been identified as one of the best suited LAB for inoculation of a silage crop, and single or multiple strains of this bacterium are included in virtually every commercial bacterial inoculant (Bolsen and Heidker, 1985; Wilkinson, 1990). Although *L. plantarum* satisfies most of the desired criteria, some strains are slow to produce lactic acid until the pH of the ensiled material falls below 5.0. Therefore, many commercial inoculants also contain species of *Pediococcus* and/or *Enterococcus*, which are active within the pH range of 5.0-6.5 and capable of dominating in the early stages of the fermentation phase (McDonald et al., 1991).

Modern technology developed over the past three decades has greatly improved the commercial production of the bacterial cultures used in silage inoculants. An overview of the procedures was presented by Aimutis and Bolsen (1988), and a summary of the fermentation and stabilization techniques was reported by Risley (1992). Perhaps no other area of silage management has received as much attention among both researchers and practitioners in the past decade as bacterial inoculants. It is beyond the scope of this paper to site all of the recently published scientific data. Summaries of several reviews are presented, as well as results from selected studies to document the effect of inoculants on silage fermentation, preservation, and nutritive value.

Muck (1993) compiled data from over 250 studies conducted between 1985 and 1992, and most were with alfalfa, cool-season grasses, or corn in North America and Europe. Inoculants significantly improved silage fermentation (i.e., by decreasing pH and ammonia-nitrogen and increasing lactic : acetic acid ratio) in 65% of the studies. When the results were separated by crop, pH was lowered by the inoculants in 75% of the alfalfa, 77% of the grass, but only 40% of the corn studies. In an earlier review that included studies conducted between 1985 and 1990, Muck and Bolsen (1991) reported improved fermentation efficiency in over 70% of the studies. At feedout, DM recovery was improved by the inoculants in 74% of the studies (25 out of 34), but aerobic stability was improved in only 42% of the studies (8 out of 19). The average increase in DM recovery in the studies that observed benefits from the inoculants was 2.5 percentage units. The authors stated that this was somewhat greater than would be expected from simply a more efficient fermentation phase alone, and that some improvement in aerobic stability during the feedout phase also must have occurred in many of the inoculated silages.

In the early 1980s, inoculants were being used to a limited extent in Europe, but results on farms were variable and scientific information was lacking on most aspects of bacterial inoculants (Castle, 1990). An international collaboration, EUROBAC, began in November, 1983, and joint studies on inoculants were conducted throughout Europe and Scandinavia. Results were obtained from 17 research institutes or universities in 11 countries, and data were available from 86 separate trials carried out both in the laboratory and on the farm. Classification of the grass silages based on DM content was: 1) direct-cut, $< 18\%$; 2) dry harvest conditions or "slightly wilted", 18-25%; 3) "moderately wilted", 25-35 %; and 4) "extensively wilted", $> 35\%$. It was the aim in these trials to compare the inoculant treatments to controls (i.e., the negative control was no additive, and the positive control was the most widely used chemical additive, formic acid).

In a summary of the fermentation results, only the positive control was consistently effective in preserving the direct-cut silages (Zimmer, 1990). However, some results indicated that inoculants containing strains of the genera *Lactobacillus* and *Pediococcus* gave a more homolactic fermentation and a nearly 4 percentage unit lower DM loss compared to the negative control, provided the grass had at least 1.5% WSC on a fresh basis. In the 18-25% DM silages, inoculants containing strains of *Lactobacillus* or *Lactobacillus* and *Pediococcus* were more effective than products that also contained a high proportion of *Streptococcus* (*Enterococcus*) *faecium*. Weather conditions during the wilting and harvesting periods produced a wide range in WSC content in the pre-ensiled grasses and more variable responses to the inoculants. In the moderately wilted silages (25-35% DM), inoculants that contained *Lactobacillus* alone or in combination with *Pediococcus* markedly improved the fermentation process compared to the negative control, and they were as effective as formic acid. For the grasses

ensiled above 35% DM (mean, 42.6%), responses to the inoculants were variable. When the grass was wilted quickly, inoculants gave a superior fermentation and a lower DM loss. However, if wilting was delayed by unfavorable weather and the WSC content was below 2% in the fresh crop, neither inoculants nor formic acid stabilized the preservation of the silages.

Grass suitable for inoculants contained a minimum of 1.5% WSC on a fresh basis and a DM content above 20-21%, but needed favorable wilting conditions to reach these values. *Lactobacillus*-based inoculants gave silages of fermentation quality equal to that of the positive control (formic acid), but with significantly more lactic acid and a 1.1 percentage unit higher DM recovery.

During the EUROBAC Conference held at Uppsala, Sweden in August, 1986, results of over 600 laboratory-scale experiments with silage inoculants were reported, and these were compiled by Spoelstra (1991). The data showed that inoculation of the majority of crops decreased pH values and ammonia-nitrogen levels and increased the lactic : acetic acid ratio by both increasing the lactic acid and decreasing the acetic acid contents of the silages. When averaged across all crops and ensiling conditions, *Lactobacillus*-based inoculants increased DM recovery by 2-3 percentage units.

In the EUROBAC results, the population of epiphytic LAB on the chopped forages was higher than expected, with counts below 10^3 cfu per g (fresh basis) being the exception (Pahlow, 1990). About 55% of the grasses were in the range of 10^4-10^5 LAB per g of crop. When this was compared to the average inoculation rate of 10^5 LAB per g provided by the bacterial products, about one-third of the forages had a LAB-flora count that was low enough to be increased by a factor of 10. For another 30% of the forages, the initial LAB population was doubled, but the remainder received significantly higher LAB populations only from products that provided 10^6 LAB per g of crop. Results showed that, in general, an inoculation factor (IF) of 2 was the minimum to achieve a positive effect of fermentation quality.

Bolsen et al. (1988) and Bolsen et al. (1990) determined the effect of bacterial inoculants on silage fermentation in a series of over 50 studies conducted from 1987-1989. The four principal crops used and their ranges in DM content were: alfalfa (32-54%), wheat (30-42%), corn (32-38%), and forage sorghum (28-34%). A summary of the results showed that over 90% of the nearly 300 inoculated silages had lower pH, higher lactic:acetic acid ratio, and lower ethanol and ammonia-nitrogen contents compared to control silages. The IF was not a good predictor of a crop's response to an inoculant, and applying more than 300,000 cfu of LAB per g of fresh forage did not provide additional benefit to fermentation quality. The data also suggest that strain selection for a particular bacterial inoculant was as important as the number of LAB it supplies per g of crop.

Pahlow (1991) indicated that a population of 10^6 LAB per g of fresh forage represents only a small fraction of the microflora that develop during the first 1-2 days of the ensiling process. Because few strains of epiphytic LAB have optimal properties for a silage environment, trying to establish a highly competitive strain or strains of silage-adapted LAB was still worthwhile. Pitt and Leibensperger (1987), in a modelling approach, found that the effectiveness of bacterial inoculants increased with the number of LAB supplied, and they concluded that an IF greater than 1 was necessary. However, this was based on the assumption that the epiphytic and inoculant LAB had equal maximum growth rates. They also reported that an increased acid tolerance of the inoculant strains was more important than a homofermentative fermentation.

As expected, the number of studies with bacterial inoculants that have measured animal performance (i.e., live weight gain, milk production, and feed efficiency) are much fewer than those that measured only fermentation and preservation criteria. In general, effects of inoculated silages on beef or dairy cattle performance appear to be small, but consistently positive. In a review of data collected from 1985-1992, Muck (1993) reported that DM intake, daily gain, and milk production were increased in about 25, 25, and 40%, respectively, of the studies with inoculated silages compared to control silages. Feed efficiency was improved in nearly 50% of the studies. When significant benefits from the bacterial inoculants were observed, DM intake, daily gain, milk production, and efficiency were increased by averages of 11, 11, 5, and 9%, respectively.

Harrison (1989) compiled data published primarily in North America between 1982 and 1989 on the effects of inoculants, enzymes, or their combinations on silage fermentation efficiency and dairy cattle performance. In 20 studies with predominantly alfalfa or grass silages, inoculants increased production of both actual and 4% fat-corrected milk by about 0.45 kg per cow per day. Cows fed inoculated silages also consumed more DM and had higher body weight gains. The greatest advantage for inoculated silages was obtained with wilted alflafa and inclusion of at least 60% silage in the total ration on a DM basis.

Bolsen et al. (1992a) summarized results from 26 studies conducted over a 14-year period at Kansas State University comparing fermentation efficiency, DM

recovery, and beef cattle performance for inoculated or NPN-treated corn and forage sorghum silages. Treatment means for untreated, control silages and treated silages are shown in table 3. The 19 inoculated corn silages had a 1.3 percentage unit higher DM recovery compared to untreated silages, and the inoculated silages supported a 1.8% more efficient gain and a 1.8 kg increase in gain per tonne of crop ensiled. When the 10 untreated and inoculated sorghum silages were compared, inoculants increased DM recovery, improved feed conversion, and produced 2.3 kg more gain per tonne of crop ensiled. In both crops, inoculants significantly reduced the acetic acid content of the silages and tended to decrease the ethanol content and increase the lactic:acetic acid ratio.

Overall, the magnitudes of animal performance responses to inoculated silages are higher than what might be expected from the shifts in fermentation products and the impact of increased DM recovery. One rather surprising finding from the recent reviews of Muck and Bolsen (1991), Spoelstra (1991), and Muck (1993) was that bacterial inoculants significantly increased both DM digestibility (in over 60% of the studies) and fiber digestibility (in over 35% of the studies). Why this should occur is not completely understood, because LAB are not known to degrade the cell wall or other forage components that are believed to limit digestibility in beef and dairy cattle. Muck (1993) speculated that the lower pH of inoculated silages causes additional acid hydrolysis of hemicellulose, which opens the cell wall fraction for more rapid and extensive digestion by rumen microorganisms.

Muck (1993) reported that animal performance benefits were linked closely to increases in digestibility in the 31 studies reviewed. Animal performance was improved in nearly 60% of the studies (9 of 16) in which bacterial inoculants improved DM digestibility, but when digestibility was not affected by the inoculants, improved animal performance was observed in only 13% of the studies (2 of 15).

TABLE 3. SUMMARY OF TREATMENT MEANS FOR SILAGE FERMENTATION, DM RECOVERY, AND CATTLE PERFORMANCE FROM BACTERIAL INOCULANT AND NPN ADDITIONS TO CORN AND FORAGE SORGHUM SILAGES

Crop and silage treatment	No. of silages	DM recovery[1]	Avg. daily gain (kg)	Daily DM intake (kg)	DM/kg of gain (kg)	Gain/tonne of crop ensiled (kg)	pH	Lactic acid	Acetic acid	Ethanol[2]
Corn :							– % of the silage DM –			
Control	15	90.2	1.09	7.73	7.10	49.5	3.82	5.3	2.5	0.8
Inoculant	19	91.5	1.12	7.76	6.97	51.3	3.82	5.5	2.3	0.6
Probability level	–	0.01	NS	NS	0.11	0.01	NS	0.12	0.03	NS
Control	3	91.5	1.04	7.80	7.52	48.1	3.81	4.7	2.0	–
Anhydrous NH_3	3	89.4	1.01	7.96	7.84	45.0	4.19	6.1	2.5	–
Probability level	–	NS	0.16	NS	NS	0.07	0.01	0.01	0.12	–
Forage sorghum :										
Control	10	83.1	0.75	5.96	8.32	35.3	3.94	5.1	2.6	1.4
Inoculant	10	85.2	0.76	5.85	7.98	37.6	3.93	5.2	2.1	1.2
Probability level	–	0.01	NS	0.20	0.04	0.01	NS	NS	0.02	NS
Control	3	87.7	0.61	5.41	9.52	37.3	3.91	5.1	2.0	–
Anhydrous NH_3 or urea[3]	3	82.6	0.49	5.13	10.58	30.3	4.63	6.1	3.6	–
Probability level	–	0.09	NS	NS	NS	0.24	0.10	NS	0.08	–

Source: Bolsen et al. (1992a).

[1] As a percent of the crop DM ensiled.

[2] Ethanol was not measured in studies conducted prior to 1984.

[3] One study with anhydrous NH_3 and two studies with urea.

The data reviewed indicate that bacterial inoculants are not always effective. The IF has been used to predict when an inoculant would be expected to give a significant improvement in animal performance for a particular silage crop. In studies at the U.S. Dairy Forage Research Center, increases in milk production from wilted alfalfa silages occured only when the inoculant supplied at least 10 times more LAB than the epiphytic, acid-tolerant LAB population on the forage (Satter et al., 1991). Grass silage studies in Europe also confirm that an inoculant must provide a 10-fold increase in LAB to produce significant effects on animal performance (Spoelstra, 1991). Corn usually has 10^5-10^6 epiphytic LAB per g, and commercial inoculants provide an IF of only 1 or less (Pahlow, 1990). However, data from farm-scale, inoculant studies often show increases in animal performance, and these benefits are not always explained by differences in fermentation efficiency (Bolsen et al., 1992b). Gordon (1989) reported that DM intake and milk production were higher for cows fed inoculant-treated ryegrass silage (10^6-cfu of *L. plantarum* per g of fresh crop) than for cows fed formic acid-treated (85% w/w applied at 2.7 liters per tonne of fresh crop) or untreated silages. These improvements occurred despite similar fermentation characteristics for the three silages. Kung (1992) suggested that as yet unidentified constituents in the inoculated silages might be responsible for the nutritive value benefits.

Nonprotein nitrogen

Earlier research with urea and anhydrous ammonia additions to corn and sorghum silages was reviewed by Ely (1978). Urea-treated silages gave small but consistent improvements in daily gain, milk production, and feed efficiency compared to untreated silages that were supplemented with a similar amount of urea at feeding. Ammonia-treated silages provided benefits less frequently and had negative effects in several studies. The review did not include silage preservation results; however, retention of added nitrogen was 95% or higher for urea-treated silages but only 50-75% for ammonia-treated.

The addition of ammonia immediately raises the pH of the crop to 8-9, and the combined effect of the ammonia and high pH reduces the yeast and mold populations and usually increases aerobic stability of the silage. Ammonia also decreases the number of LAB, and this delays the start of the fermentation phase. However, the amount of fermentation products (i.e., lactic and acetic acids) increases because of the much higher initial forage pH. Ammonia breaks some of the linkages between hemicellulose and other cell wall components, which should increase both rate and extent of digestion. The high initial pH also inactivates plant proteases, and this reduces the extent of protein degradation in the ensiled crop.

In a series of six studies conducted in farm-scale silos, Bolsen et al. (1992a) observed that anhydrous ammonia applied at 3.5-4.0 kg per tonne or urea at 5.0 kg per tonne (fresh basis) increased the pH value, lactic and acetic acid contents, and DM loss in both corn and forage sorghum silages (table 2). Performance of growing cattle was not improved by the NPN-treatments, and gain per tonne of crop ensiled was reduced by 3.1 and 7.0 kg in the corn and sorghum silages, respectively.

In a review of 39 studies reported since 1985, Muck (1993) found that NPN additives increased fermentation acids in approximately 60% of the silages, and clostridial activity was a problem in low-DM crops (less than 30%). In 12 of 21 studies, DM recovery in the NPN-treated silages was decreased and it increased in only three studies. Aerobic stability was improved consistently in the NPN silages. Digestibility of the NPN silages (i.e., DM, NDF or ADF) was increased in 16 of 19 studies, and most treated silages had higher true protein content. However, these apparent improvements in nutritive value did not increase daily gain, milk production, or feed efficiency in most studies, especially in recent research with grass and legume silages. The author noted that the apparent paradoxes of improved bunk life with reduced DM recovery and improved digestibility with no benefit in animal performance need further clarification.

Anhydrous ammonia is used widely in a few regions of North America; however, it is unlikely to become a popular additive in the future unless the problems of handling, application, preservation, reduced DM intake, and the increased risk of clostridial fermentation are overcome. When economic analysis includes the increased silage DM loss and the cost of replacing the volatile nitrogen loss, ammonia can be an expensive source of supplemental protein for beef and dairy cattle (Bolsen, 1993). Urea is easier and safer to handle than ammonia, but unless future studies show significantly increased nutritive value for the traditional silage crops (i.e., corn and grasses) it will not become a commonly used additive.

Fermentable substrates

Molasses is the most widely used source of sugars and is particularly effective in improving the fermentation quality in low-DM grasses and low-WSC legumes and tropical forages. Castle and Watson (1985) compared molasses and formic acid additions to low-DM ryegrass silages and concluded that at 20-30 liters per tonne, molasses was as effective as the acid. The application of moderate to high levels (i.e., 40-60 kg per tonne of fresh

crop) of cereal grains, wheat or rice brans, or citrus or beet pulps to low-DM forages has improved the fermentation characteristics of the silages, and in some studies, the amount of effluent was reduced (Jones et al., 1990; Bolsen et al., 1995). Thorough mixing of the substrates with the crop is particularly important because, if the absorbents are added in layers in silos fitted with internal drains, effluent production can be increased (Wilkinson, 1990).

Acids and acid salts

In Europe and in many other areas where low-DM grasses are important silage crops, formic acid is the standard against which most other silage additives have been tested. Formic acid not only restricts the growth of bacteria through its acidifying (hydrogen ion) effect, it also has a selective antibacterial action, as does the weaker propionic acid (Woolford, 1975). In contrast, the mineral acids, sulfuric and hydrochloric, act solely by reducing pH and have no specific antimicrobial properties. Yeasts have been shown to be particularly tolerant of formic acid (McDonald et al., 1991), and the aerobic stability of silages made with formic acid is often poor, partly because of the likely elevated yeast counts in the silage, and also because of the restricted fermentation, which leads to relatively higher contents of residual WSC in the silage.

Effects of formic acid on animal performance have been reviewed extensively (Thomas and Thomas, 1985; McDonald et al., 1991). The magnitude of the improvement in animal performance depends on the preservation quality of the untreated forage, with large benefits recorded when the untreated silage is badly preserved.

Salts of acids are used widely in Europe as safer alternatives to the acids themselves and their efficacy is similar, if the same rates of active ingredients are applied to the crop. Very recently, the combined addition of bacterial inoculants and acid salts has been evaluated with encouraging results (Kalzendorf and Weissbach, 1993). In 10 studies with a wide range of crops, a concentrated solution of sodium formate, in which freeze-dried LAB was dispersed, gave reduced fermentation losses, especially with forages that were difficult to ensile. Aerobic stability also was improved compared to silage with inoculant alone. The salt has little damaging effect on the LAB and develops its antibacterial action as the pH of the silage decreases. Because the LAB are relatively more acid-tolerant than the undesirable epiphytic microorganisms, they dominate the ensiling process.

Literature Cited

Anonymous. 1992. Silage additives. In: 1993. Direct-fed Microbial, Enzyme, and Forage Additive Compendium. Miller Publ. Co., Minnetonka, Minnesota. pp. 217-261.

Aimutis, W. R. and K. K. Bolsen. 1988. Production of biological silage additives. In: Biological Silage Additives. Chalcombe Publ., Church Lane, Kingston, Canterbury, Kent, UK. pp. 45-72.

Bolsen, K. K. 1985. New technology in forage conservation-feeding systems. In: Proc. XV Int. Grassl. Congr. Kyoto, Japan. pp. 82-88.

Bolsen, K. K. 1993. Manejo del ensilaje (part II). In: Mexico Holstein. 24(3):21-24.

Bolsen, K. K. and J. L. Heidker. 1985. Silage Additives USA. Chalcombe Publ., Church Lane, Kingston, Canterbury, Kent, UK.

Bolsen, K. K., A. Laytimi, R. Hart, L. Nuzbach, F. Niroomand and L. Leipold. 1988. Effect of commercial inoculants on fermentation of 1987 silage crops. In: Kansas Agric. Exp. Sta. Rpt. of Prog. 539. Kansas State University, Manhattan. pp. 137-153.

Bolsen, K. K., J. L. Curtis, C. J. Lin and J. T. Dickerson. 1990. Silage inoculants and indigenous microflora: With emphasis on alfalfa. In: T. P. Lyons (ed). Biotechnology in the Feed Industry. Alltech Tech. Publ., Nicholasville, Kentucky. pp. 257-269.

Bolsen, K. K., R. N. Sonon, B. Dalke, R. Pope, J. G. Riley and A. Laytimi. 1992a. Evaluation of inoculant and NPN silage additives: A summary of 26 trials and 65 farm-scale silages. In: Kansas Agric. Exp. Sta. Rpt. of Prog. 651. Kansas State University, Manhattan. pp. 101-102.

Bolsen, K. K., D. G. Tiemann, R. N. Sonon, R. A. Hart, B. Dalke, J. T. Dickerson and C. Lin. 1992b. Evaluation of inoculant-treated corn silages. In: Kansas Agric. Exp. Sta. Rpt. of Prog. 651. Kansas State University, Manhattan. pp. 103-106.

Bolsen, K. K., J. T. Dickerson, B. E. Brent, R. N. Sonon, Jr., B. S. Dalke, C. J. Lin and J. E. Boyer, Jr. 1993. Rate and extent of top spoilage in horizontal silos. J. Dairy Sci. 76:2940-2962.

Bolsen, K. K., P. S. Faylon, U. M. Lustria, M. Loresco, N. F. Bolsen and B. Beltran. 1995. Unpublished data. Dairy Training and Research Institute. University of the Philippines, Los Banos, Laguna, Philippines.

Castle, M. E. 1990. Conclusions and future prospects. In: S. Lindgren and K. L. Pettersson (eds). Proc. of the EUROBAC Conf. Swedish University of Agric. Sciences, Uppsala. pp. 184-188.

Castle, M. E. and J. N. Watson. 1985. Silage and milk production: studies with molasses and formic acid as additives for grass silage. Grass and Forage Sci. 40: 85-92.

Dickerson, J. T., G. Ashbell, K. K. Bolsen, B. E. Brent, L. Pfaff and Y. Niwa. 1992. Losses from top spoilage in horizontal silos in western Kansas. In: Kansas Agric. Exp. Sta. Rpt. of Prog. 651. Kansas State University, Manhattan. pp. 131-134.

Donald, S., D. R. Fenlon and B. Seddon. 1993. The influence of oxygen tension on the growth of *Listeria monocytogenes* in grass silage. In: Proc. 10th Silage Res. Conf. Dublin City University, Dublin, Ireland. pp. 18-19.

Ely, L. O. 1978. The use of added feedstuffs in silage production. In: M. E. McCullough (ed). Fermentation of Silage - A review. Nat. Feed Ingred. Assoc., West Des Moines, Iowa. pp. 233-280.

Fenton, M. P. 1987. An investigation into the sources of lactic acid bacteria in grass silage. J. of Applied Bacteriology. 62:181-188.

Gordon, F. J. 1989. An evaluation through lactating dairy cattle of a bacterial inoculant as an additive for grass silage. Grass and Forage Sci. 44:169-179.

Harrison, J. H. 1989. Use of silage additives and their effect on animal productivity. In. Proc. of the Pacific Northwest Animal Nutr. Conf. Boise, Idaho. pp. 27-35.

Jones, D. I. H., R. Jones and G. Moseley. 1990. Effect of incorporating rolled barley in autumn cut ryegrass silage on effluent production, silage fermentation and cattle performance. J. Agric. Sci. Camb. 115:399-408.

Kalzendorf, C. and F. Weissbach. 1993. Studies on the effect of a combined application of inoculants and sodium formate. In: Proc. 10th Silage Res. Conf. Dublin City University, Dublin, Ireland. pp. 89-90.

Kung, L., Jr. 1992. Use of additives in silage fermentation. In: 1993 Direct-fed Microbial, Enzyme and Forage Additive Compendium. Miller publ. Co., Minnetonka, Minnesota. pp. 31-35.

Lin, C., K. K. Bolsen, B. E. Brent, R. A. Hart, J. T. Dickerson, A. M. Feyerherm and W. R. Aimutis. 1992. Epiphytic microflora on alfalfa and whole-plant corn. J. Dairy Sci. 75:2484-2493.

Lindgren, S. 1984. Silage inoculation. In: F. J. Gordon and E. F. Unsworth (eds). Proc. 7th Silage Conf. Summary of Papers. The Queen's University, Belfast, Northern Ireland. pp. 3-4.

McDonald, P. 1980. Silage fermentation. In: Occ. Symp. No. 11. Brit. Grassl. Soc., Brighton, UK. pp. 161-174.

McDonald, P., A. R. Henderson and S. J. E. Heron. 1991. The Biochemistry of Silage (2nd ed.). Chalcombe Publ., Church Lane, Kingston, Canterbury, Kent, UK.

Muck, R. E. 1989. Initial bacterial numbers on lucerne prior to ensiling. Grass and Forage Sci. 44:19-25.

Muck, R. E. 1993. The role of silage additives in making high quality silage. In: Proc. Nat. Silage Prod. Conf. NRAES-67, Ithaca, New York. pp. 106-116.

Muck, R. E. and K. K. Bolsen. 1991. Silage preservation and silage additives. In: K. K. Bolsen, J. E. Baylor and M. E. McCullough (eds). Hay and Silage Management in North America. Nat. Feed Ingred. Assoc., West Des Moines, Iowa. pp. 105-125.

Muck, R. E. and R. E. Pitt. 1993. Progression of aerobic deterioration relative to the silo face. In: Proc. 10th Silage Res. Conf. Dublin City University, Dublin, Ireland. pp. 38-39.

Pahlow, G. 1990. Microbiology of inoculants, crops, and silages. In: S. Lindgren and K. L. Pettersson (eds). Proc. of the EUROBAC Conf. Swedish University of Agric. Sciences, Uppsala. pp. 13-22.

Pahlow, G. 1991. Role of microflora in forage conservation. In: G. Pahlow and H. Honig (eds). Forage Conservation towards 2000. Inst. Grassl. Forage Res., Braunschweig, Germany. pp. 26-36.

Pahlow, G. and Th. Muller. 1990. Determination of epiphytic microorganisms on grass as influenced by harvesting and sample preparation. In: Proc. 9th Silage Conf. Newcastle-upon-Tyne, UK. pp. 23-24.

Pitt, R. E. 1990. Silage and hay preservation. Cornell University Coop. Ext. Bul. No. NRAES-5, Ithaca, NY.

Pitt, R. E. and R. Y. Leibensperger. 1987. The effectiveness of silage inoculants: A systems approach. Agric. Syst. 25:27-49.

Risley, C. 1992. An overview of microbiology. In: 1993 Direct-fed Microbial, Enzyme and Forage Additive Compendium. Miller Publ. Co., Minnetonka, Minnesota. pp. 11-13.

Satter, L. D., R. E. Muck, B. A. Jones, T. R. Ohiman, J. A. Woodford and C. M. Wacek. 1991. Efficacy of bacterial inoculants for alfalfa silage. In: G. Pahlow and H. Honig (eds). Forage Conservation towards 2000. Inst. Grassl. Forage Res., Braunschweig, Germany. pp. 342-343.

Spoelstra, S. F. 1991. Chemical and biological additives in forage conservation. In: G. Pahlow and H. Honig (eds). Forage Conservation towards 2000. Inst. Grassl. Forage Res., Braunschweigh, Germany. pp. 48-70.

Spoelstra, S. F. and V. A. Hindle. 1989. Influence of wilting on chemical and microbial parameters of grass relevant to ensiling. Netherlands. J. Agric. Sci. 37:

355-364.

Thomas, C. and P. C. Thomas. 1985. Factors affecting the nutritive value of grass silages. In: W. Haresign and D. J. A. Cole (eds). Recent Advances in Animal Nutrition. Butterworths, London. pp. 223-256.

Watson, S. J. and M. J. Nash. 1960. The Conservation of Grass and Forage Crops. Oliver and Boyd, Edinburgh, Scotland.

Whittenbury, R. 1961. An investigation of lactic acid bacteria. Ph.D. dissertation. University of Edinburgh, Edinburgh, Scotland.

Wilkinson, J. M. 1990. Silage UK (6th ed.). Chalcombe Publ., Church Lane, Kingston, Canterbury, Kent, UK.

Wilkinson, J. M. and B. A. Stark. 1992. Silage in Western Europe (2nd ed.). Chalcombe Publ., Church Lane, Kingston, Canterbury, Kent, UK.

Woolford, M. K. 1975. Microbiological screening of the straight chain fatty acids (C_2-C_{12}) as potential silage additives. J. Sci. Food and Agric. 26:219-228.

Woolford, M. K. 1984. The Silage Fermentation. Marcel Dekker, Inc., New York. NY.

Woolford, M. K. and M. K. Sawczyc. 1984. An investigation into the effect of cultures of lactic acid bacteria on fermentation in silage. 1. Strain selection. Grass and Forage Sci. 39:139-148.

Zimmer, E. 1990. Evaluation of fermentation parameters from the silage experiments. In: S. Lindgren and K. L. Pettersson (eds). Proc of the EUROBAC Conf. Swedish University of Agric. Sciences, Uppsala. pp. 19-44.