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Manipulation of fermentation dynamics and its effect on silage production, rumen fermentation and animal performance

Kevin Timothy Leahy

To the Graduate Council:

I am submitting herewith a dissertation written by Kevin Timothy Leahy entitled "Manipulation of fermentation dynamics and its effect on silage production, rumen fermentation and animal performance." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Karl M. Barth, Major Professor

We have read this dissertation and recommend its acceptance:

R. A. McLean, J. B. McLaren, J. C. Waller

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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K R Cook

P Michael Davidson

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Vice Provost and
Dean of The Graduate School

MANIPULATION OF FERMENTATION DYNAMICS AND ITS EFFECT ON SILAGE PRODUCTION, RUMEN FERMENTATION AND ANIMAL PERFORMANCE

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Kevin Timothy Leahy

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ABSTRACT

Corn silage was either left untreated (control) or treated with α-amylase (.05% wet basis, a fermentation stimulant), sorbic acid (.10% wet basis, a fermentation inhibitor), or both in a 2 x 2 factorial arrangement. Experiments were conducted to ascertain treatment effects on: 1) fermentation and aerobic deterioration dynamics, 2) rumen microbial activity as estimated by in vitro gas production, and 3) the nutritive value of these silages by measuring performance and carcass characteristics of cattle.

During a 9-day fermentation study, silages treated with sorbic acid appeared to be better preserved since no yeast or mold growth was observed and because more ($P<.02$) water-soluble carbohydrates were retained as compared to the other silages (12.2 vs 9.0 % DMB). However, the concentrations of butyric ($P<.01$) and isobutyric acids ($P<.01$) were also elevated in sorbic acid-treated silages, indicating clostridial degradation.

Silages treated with sorbic acid were more aerobically stable as exhibited by lower temperatures ($P<.01$), fewer total aerobes ($P<.06$) and more retention of water-soluble carbohydrates ($P<.01$) as compared to the other silages. Dry matter loss during ensiling was not significantly affected by treatment. The average dry matter loss during ensiling was 9.7% and ranged from a low of 9.1% for the sorbic acid-treated silage to a high of 10.3% in the α-amylase-treated

silage. Spoilage losses were significantly decreased (P<.07) by sorbic acid treatment (7.5 vs 5.7%).

Rumen microbes from cattle fed α-amylase-treated silages produced more (14.5 vs 13.0 μl/g/min, P<.09) gas in vitro as did those from cattle fed sorbic acid-treated silages (14.4 vs 13.1, P>.16).

During an 85-day silage feeding trial with beef heifers, treatment with α-amylase resulted in no difference in DM intake (mean 5.7 kg/d), greater (P<.01) average daily gain (.84 vs .79 kg/d), and increased weight gain per feed intake (.146 vs .137, P<.01). This improved performance due to α-amylase treatment may have been caused partly by the increased microbial activity and partly by an increased (P<.09) nitrogen-free extract content (45.0 vs 43.2% DMB) of the silage.

Results from these studies indicate that sorbic acid treatment of silages resulted in improved preservation and protection from aerobic deterioration. Treatment with α-amylase resulted in improved animal performance. Therefore, better preservation may not result in improved animal performance.

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INTRODUCTION

The word "fermentation" as currently used has no precise meaning. There are many types of fermentations which occur due to microbial activity. Although it is an oversimplification, there do appear to be two major classes of fermentations.

The first class can be described as a production or synthesis fermentation. The purpose of this type of fermentation is to produce or synthesize a desired endproduct. Examples of synthesis fermentations are alcoholic fermentation, yielding products such as ethanol, beer or wine, fermentations which produce fuel such as methane and many others.

The second class of fermentation also produces a desired endproduct; however, rather than a direct requirement for the endproduct produced, the desire is for the endproduct to preserve a certain material. For this reason, this second type of fermentation can be referred to as a preservation fermentation. It should be noted that, generally, the fermentation process is quantitatively and qualitatively a destructive process; therefore, any product resulting from this process usually does not have a nutritive value greater than that of the original non-fermented material. It is, therefore, the purpose of this process to have a fermentation which will preserve the desired material while minimizing the nutrient loss. In addition, the fermented product must be

appealing in sight, taste, smell and texture. Examples of preservation fermentations are milk fermentation products, such as yogurt, and fermentation of vegetative materials, such as pickles, sauerkraut and various silages.

Most of the original studies of silage and the process by which it is produced were conducted in Europe in the early 1900s. The silage process is affected by both chemical and microbiological alterations and both these aspects are very much interrelated. There are many questions regarding where the effects of microorganisms end and those of substrates and/or endproducts begin.

Manipulation of the silage fermentation process can be accomplished by various means. Results of early studies, in addition to those from more recent research, have indicated the desirability of manipulation of the silage fermentation process in order to produce higher quality feed material. Types of manipulation of the silage fermentation process include ensiling at the proper moisture content and cutting of the crop at the proper stage of maturity and appropriate length.

Manipulation of the silage fermentation process has also been accomplished by the use of various types of additives. These manipulators fall into two categories, fermentation stimulants and inhibitors. Fermentation stimulants affect silage preservation by enhancing the rapid production of lactic acid. This is accomplished by several means, such as

inoculation of the silage with lactic acid-producing bacteria, addition of substrates used by the lactic acid-producing bacteria and addition of enzymes which convert complex carbohydrates of the green chop material to forms more easily available to lactic acid-producing bacteria. The action of fermentation stimulants results in a lowering of the pH to 4.00 or less more rapidly, which inhibits further destructive microbial activity. Addition of fermentation inhibitors to green chop material preserves the silage by direct inhibition of the activity of either selected or all microorganisms. Theoretically, the type of silage produced using fermentation stimulants could be different in composition from that produced by fermentation inhibitors.

Variations in the compositional makeup of silages due to varying fermentation processes have been shown to affect the aerobic stability of the silage. In addition, improvements in the nutritive quality of silages resulting from either retention of original substrates or production of more utilizable fermentation endproducts can result in increased activity of rumen microbes and possible improvement in animal performance. Finally, since additives to silage are designed to either stimulate or inhibit silage microorganisms, residual amounts of these original additives in the silage product could alter the microbial profile of the rumen which, in turn, could affect animal performance.

With these considerations, therefore, the objectives of this study were: 1) to determine the variations in the processes by which silages are preserved, as affected by manipulation of fermentation dynamics, 2) to quantify the effect of these silages on rumen microbial activity and 3) to ascertain the nutritive value of these silages by measuring performance of beef cattle under feedlot conditions.

CHAPTER I

LITERATURE REVIEW

Production or Synthesis Fermentations

Fuel Production

The ability of microorganisms to produce alcohol has been known for many years by those who manufactured beers and wines. Today several countries, including the United States, have begun programs using variations of this process to produce ethanol as a substitute for gasoline. In addition, the large-scale production of methane or biogas is also a reality. The production of ethanol from residues with high sugar content may someday become an economical means of offsetting the costs of more conventional fuel sources (NRC, 1979). The amount of alcohol yield is dependent on the amount of starch or fermentable sugars present in the substrate. Sugar cane, which is available in many parts of the world, would serve as an excellent raw material for this purpose (Hepner, 1977). The fermentation of molasses from sugar cane using yeast would be expected to produce a product which would be 95% ethanol. This product could then be converted to absolute ethanol (Faith et al., 1974). Corn can also be converted to 95% ethanol through a yeast fermentation process.

The microbial breakdown of organic materials to methane is a naturally occurring process. It should be noted that

this process may serve as an economical supplement to fossil fuel (NRC, 1979). Methane production occurs in many natural microbial ecosystems such as organic sediments of aquatic systems, marshes, soil and the rumens of herbivores. Anaerobic bacteria can convert up to 90% of the combustible energy of the degraded organic matter to methane and carbon dioxide (NRC, 1979).

The National Academy of Science (1977) suggests that anaerobic fermentation of organic solids is a three-stage process. Fats, cellulose and proteins are decomposed by appropriate microorganisms to soluble compounds. These soluble compounds are then converted to organic acids by acid-producing bacteria. Finally, methane and carbon dioxide are the endproducts of methane-producing bacteria. This process could theoretically convert 30-50% of the combustible material, such as cattle manure or human organic refuse, to methane. At least a part of this methane-producing fermentation process agrees with a definition of "fermentation" offered by Banwart (1979); he described the process of fermentation as the anaerobic breakdown of an organic substance by an enzyme system in which the final hydrogen acceptor is an organic compound.

Vitamin Production

Ono (1971) noted that there is an important byproduct which can be recovered from the liquid fraction of digested material produced by methane fermentation. Considerable

amounts of vitamin B_{12} are produced during the process and can be recovered directly from this digested liquid by chemical preparation and centrifugation. The production of vitamins, however, is attributed more often to other fermentation procedures.

The production of vitamins through fermentation processes on an industrial scale is a relatively recent development. Various organic substrates are required and acted upon by enzyme systems which are the result of the activity of specific microorganisms. Much data have been published by Japanese researchers regarding various techniques used to produce different vitamins. Fukui (1971) in a review discussed the production of vitamins B_2, B_6, B_{12} and others by fermentation processes. In addition to vitamins, proteins, amino acids, nucleotides and antibiotics are also produced by microbial enzyme systems (Kinoshita, 1971).

Waste Treatment

Another type of production or synthesis fermentation is that associated with several types of organic waste treatments. Here the purpose is to convert potentially harmful waste products to safer materials through microbial degradation. In microbiological terms, biological waste-treatment processes were described by Grant and Long (1981) as being large man-made microbial culture systems designed to transform large amounts of carbon-containing material into inoffensive products. Grant and Long (1981) went on to point

out that these processes range from thermophilic composting of materials with a relatively low water content to the transformation of matter dissolved or suspended in relatively large volumes of water. Unlike a "true fermentation" which is anaerobic, thermophilic composting is mainly the result of maintenance of aerobic conditions. Composting has been described as a process involving the acceleration of microbial decomposition through conditions favorable for microbial reproduction and metabolic activity (Golueke, 1972). These conditions include temperature, oxygen content, moisture and the type of substrates available. Interestingly, the conditions required for composting are similar to those associated with an undesirable fermentation, that is, composting requires constant inclusion of air within the composting material. This air inclusion results in increased temperatures due to the heat produced by the activity of aerobic microorganisms (NRC, 1979).

The treatment of sewage is perhaps a better example of the features associated with waste processing. The microbial decomposition of sewage is sometimes referred to as facultative ponding, that is, ponding involving both aerobic and anaerobic treatment. The process begins with the raw sewage being screened to remove large objects and the flow of the sewage is slowed. The material then enters the primary sedimentation tank which removes certain solids and floating fats and oils. The organic material which settles is known

as primary sludge. The supernatant or settled sewage can either be trickled through beds of small stones or aerated to produce what is called activated sludge. Both of these processes aerobically convert large amounts of the carbon-containing sewage material into carbon dioxide and water. These processes also produce a certain amount of microbial biomass which is known as secondary sludge. The primary and secondary sludge are then anaerobically digested, producing a product called digested sludge which is microbiologically less hazardous and suitable for use as a fertilizer. Also, methane is produced during this process and can be used to decrease energy costs (Hobson et al., 1974; Mah et al., 1977). This anaerobic digestion process of sewage has been likened to a technological intensification of the process which occurs naturally in the gastrointestinal tracts of ruminants (Grant and Long, 1981).

Production Fermentation in the Rumen

The ruminant animal has a four-part stomach. The fourth part, or abomasum, is similar to the stomach of other animals, that is, non-ruminants, in that it secretes digestive enzymes and stomach acid. The omasum, or third stomach, is where water absorption occurs; in addition, very little microbial activity occurs here. The first and second parts of the stomach, collectively named the reticulo-rumen, are the site of microbial fermentation of feed material to

volatile fatty acids. Microbial protein is also produced as microorganisms grow and multiply (Annison and Lewis, 1959).

Suto and Uemura (1971) regarded the rumen as a kind of fermentation vessel. Yet they felt its functioning was "far more ingenious and precise than that of the apparatus currently used in fermentation technology." The rumen was considered to be a "continuous fermentation vessel." In fact, Van Soest (1982) stated that man-made continuous fermentors have the objective of simulating rumen conditions. There are logistical difficulties in maintaining these continuous fermentors, however. In the rumen excess acids are removed by being absorbed across the rumen wall. This could not be accomplished in a glass vessel where removal is entirely dependent on washout (Van Soest, 1982). The rumen is also very selective in allowing only particles which are smaller than a certain size to flow into the omasum and abomasum. Fibrous materials therefore remain in the rumen longer, thus allowing for the additional time required by the microbes to break them down (Suto and Uemura, 1971). The rumen is obviously a unique environment for fermentation to occur. The removal of endproducts creates an environment where inhibition of microorganisms due to the formation of these endproducts is minimized. This allows for many types of fermentations to occur simultaneously and continuously in the rumen. In fact, the production of most of the previously

discussed products of fermentations, as well as others, occur in the rumen.

Methane is produced in the rumen through the action of Methano-bacterium ruminatium (Smith and Hungate, 1958). This bacterium has been shown to convert acetate, propionate and butyrate to methane in the rumen but not to any great extent (Opperman et al., 1961). More recently, however, it has been shown that a primary fermentation converts carbohydrates to propionate and butyrate, and higher fatty acids are converted to acetate and carbon dioxide (Van Soest, 1982). Methanogenic organisms can reduce carbon dioxide, but not acetate, to methane. Van Soest (1982) stated that this lack of conversion of acetate to methane in the rumen represents a significant preservation of energy. Acetate is not converted to methane because the generation time for acetate-utilizing methanogens is four days while the turnover rate for the slowest fractions, that is, fiber, is usually less than two days. Therefore, the methanogens are being washed out of the rumen before they are able to ferment acetate (Van Soest, 1982).

In addition to methane, ruminants, through the action of rumen microbes, are able to synthesize amino acids. The B complex and K vitamins are also synthesized by rumen microorganisms (Hungate, 1966; Van Soest, 1982).

As stated earlier, volatile fatty acids are produced as a result of rumen microbial fermentation. These VFAs are

able to be absorbed and utilized by the ruminant animal. These same VFAs also play a significant role in preservation fermentations.

Preservation Fermentations

Preservation Fermentations of Foodstuffs

The purpose of a successful preservation fermentation of foodstuffs is to have a fermentation which will preserve the desired material while minimizing the nutrient loss. In addition, the fermented product must be appealing in sight, taste, smell and texture.

The preservation fermentation process can be described as the use of microorganisms to create conditions which are detrimental to other microbes, while retaining in the foodstuff the nutrients desired (Desrosier, 1970). Generally, the production of substantial amounts of organic acids by desirable organisms create the unfavorable conditions for others. These organic acids are produced by microorganisms which degrade carbohydrates; in fact, Desrosier (1970) defines fermentation as a process of anaerobic or partially anaerobic oxidation of carbohydrates. Originally the term "fermentation" was used to describe the evolution of carbon dioxide gas in the production of alcoholic beverages. Pederson (1971), however, noted that there are diversities among fermented products. Besides those products produced from alcoholic fermentation by yeasts there are

fermented products which result from lactic acid bacterial fermentation, mold fermentation, acetic acid bacterial fermentation and many others through combinations of these fermentations. Banwart (1979) updated the definition of fermentation by describing it as the anaerobic breakdown of an organic substance by an enzyme system in which the final hydrogen acceptor is an organic compound. This definition includes types of fermentation processes other than alcoholic fermentation. This indicates the complexity of the fermentation process and specifically the relationship between microbial and enzymatic activity. In addition to the preceding definitions, the fermentation process has often been described by the conditions which allow for a desirable fermentation.

Factors Involved in a Desirable Fermentation

Though there are many types of fermentations, there are several factors which are required of microorganisms in all fermentations. According to Desrosier (1970), the microorganisms "should have rapid growth when in a suitable substrate and environment." The microorganisms must be able to maintain physiological consistency under these suitable conditions and yield the essential enzymes quickly and abundantly to insure that the desired chemical changes occur. Finally, the environmental conditions required for maximum growth and production should be relatively simple.

Fermented foodstuffs are the result of the activity of only a few of the thousands of species of bacteria, yeasts and molds. The lactic acid-producing bacteria, the acetic acid-producing bacteria and certain alcohol-producing yeasts are highly specialized and are extremely important in food fermentation processes. The lactic acid-producing bacterial group carries on essential metabolic biological processes without oxygen by means of a complex series of intramolecular oxidations and reductions (Pederson, 1971).

The raw materials which are used to produce fermented food products, such as plant and animal tissue, mammalian milk and cereal extracts, are excellent sources of microbial nutrients and thus are good sources for growth of the micro-organism (Rose, 1982). The ability to convert carbohydrates in these materials to lactic acid, acetic acid, alcohol and carbon dioxide with only minor changes in the other food components has made this group of bacteria extremely important in the preservation of edible and nutritious foodstuffs.

The majority of the yeasts grow on food surfaces and require oxygen for growth. Acetic acid bacteria require oxygen to oxidize alcohol to acetic acid. The major purpose of acetic acid production is to produce a preserved vinegar product. Lactic acid-producing bacteria are often associated with the preservation of foodstuffs. The lactic acid produced is effective in inhibiting the growth of other bacteria that are capable of decomposing the food. Lactic

acid-producing bacterial fermentations are used to preserve meats and milk products. In addition, lactic acid-producing bacteria are often involved in the preservation of vegetative materials, such as cucumbers (pickles) and cabbage (sauerkraut).

Materials Suitable for Ensiling

As is the case with the fermentation of various types of foods, the type and/or effectiveness of the fermentation which occurs with silages is dependent on the type of material ensiled. Silage can be made from a variety of crops. Many crops are specifically grown for animal consumption as silage; however, in addition silages have often been made from surplus materials and byproducts. In Europe, where much of the original studies on silage were conducted, grasses, grass mixtures or grass-legume mixtures were the materials ensiled most often. In the United States, these grass or hay-crop silages are used extensively in feeding dairy cows and heifers. These hay-crop silages are fed because they are usually higher in protein content than grain or seed silages. There are certain characteristics of hay crops which would make them less than an ideal crop for preserving as silage. According to McDonald (1981), the desired characteristics of a crop to be preserved as silage are: 1) an adequate level of fermentable substrate in the form of water-soluble carbohydrates; 2) a relatively low buffering capacity; 3) a dry matter content in the fresh crop

of greater than 20%; and 4) a physical structure which will allow it to compact readily in the silo after harvesting. Hay crops tend to have a low water-soluble carbohydrate content. An adequate supply of water-soluble carbohydrates is needed for proper microbial production of lactic acid which subsequently preserves the silage; therefore, a WSC source such as molasses has often been added to hay-crop silages (Carpintero et al., 1969; McCullough and Neville, 1960).

In the United States corn and to a lesser extent sorghum silage make up virtually all of the grain silages produced (USDA, 1980). Grain sorghum is usually grown in regions of the United States where the rainfall is not sufficient to produce corn. The production of corn over sorghum is preferred because corn yields more available energy per unit of land than sorghum when both are harvested as whole plant silage (Stoneberg et al., 1968). Sorghum has been ensiled with successful results (Ward et al., 1966) and has been used in the feeding programs of both beef cattle (Goodrich and Meiske, 1982) and dairy cattle (Ward et al., 1966).

Goodrich and Plegge (1984) noted that small grains are generally not harvested as silage because of their low dry matter yields and high harvesting costs per unit of dry matter as compared to corn and sorghum. According to the NRC (1982), however, small grain (barley, oats, wheat) silages contain more crude protein but less available energy than

corn silage. The stage of maturity at which small grains are harvested appear to be important to the nutritive quality of the silage produced and animal intake (Edwards et al., 1968; Bolsen and Berger, 1976). Corn silage is by far the most widely used grain silage. It is a highly palatable feed that can be used in many types of rations and feeding programs for both beef and dairy cattle. As far as ensiling characteristics are concerned, corn would be considered as being ideal. Corn is relatively high in dry matter content, it has a low buffering capacity and it contains adequate levels of WSC for a satisfactory fermentation, in terms of lactic acid production. In addition, corn silage may be harvested and successfully stored at 55% to 70% moisture, thereby extending the harvesting period (Goodrich and Plegge, 1984). Stoneberg et al. (1968) noted that corn silage can be stored for long periods and that it reduces the dustiness of rations containing dry grains, thereby improving ration palatability. A disadvantage of corn silage is that it contains less crude protein than most forages (NRC, 1982). For this reason urea or urea plus limestone has been added to corn green chop to improve the nitrogen content (Wise et al., 1944; Barth et al., 1974).

Other crops and crop byproducts are being utilized and stored as silages. In Germany, cooked potato silage has been produced for many years (Nash, 1978). Morrison in 1958 noted that beets, cabbage, pea vine and sunflowers were being used

to make silages. In fact, there has been some relatively recent interest in the use of sunflowers as a silage (Edwards et al., 1978). Crop byproducts from the production of liquor, such as brewers' grains and distillers' grains, and food and fruit processing residues, such as vegetable wastes and apple pomace, have been preserved as silage. Fontenot (1984) has noted that animal wastes in combination with other materials have been ensiled. The ensiling of these animal wastes appears to be an especially useful method of processing by making them more acceptable to livestock.

The Preservation Fermentation of Silages

The preservation fermentation process of silages is very similar to that of foodstuffs used for human consumption. The fermentation of vegetative material, such as cabbage, is particularly similar to that of the fermentation of silage. As with many fermentations of vegetative foodstuffs, the first essential objective in preserving crops by fermentation is the achievement of anaerobic conditions. Ohyama (1984), in his analysis of factors influencing the fermentation of silage, listed the moisture content and water-soluble carbohydrate content of the forage, in addition to attainment of anaerobic conditions, as main factors. He also listed several secondary factors which have effects under certain conditions. These factors were density, environmental temperature and level of inoculation with lactic acid bacteria. Stirling and Whittenbury (1963) in their

examination of lactic acid bacteria occurring on growing plants found that their numbers were usually very low. McDonald (1981) stated that, as a result of this low microbial population, the biochemical changes that occur immediately after cutting and during the early stages of ensiling are due primarily to the activities of the plant enzymes. These enzymes are responsible particularly for respiration and proteolysis. However, Stirling and Whittenbury (1963) found that 80% of the endogenous microorganisms found on growing plants were heterofermentative Leuconostocs. Heterofermentative microorganisms yield carbon dioxide in addition to lactic acid. Since this is the case, in an adequately sealed silo one might expect that the action of the Leuconostoc sp. might help to create anaerobic conditions which would be favorable for the activity of homofermentative lactic acid-producing bacteria, such as Lactobacilli. Theoretically, homofermentative bacteria should produce 100% lactic acid under anaerobic conditions (Edwards and McDonald, 1978). The action of these heterofermentative and homofermentative microorganisms could also be responsible for the biochemical changes which occur.

As previously stated, the moisture content of a material to be ensiled is extremely important to the success of the fermentative process. There are several reasons for this. When anaerobic conditions are achieved, there are microbes other than the lactic acid-producing bacteria which are

active. Of particular concern are the biochemical reactions of Clostridia. Clostridia break down sugars and lactic acid to the endproducts butyric acid, CO_2 and hydrogen (McDonald, 1981). Clostridia also degrade the nitrogen fraction of an ensiled crop to simple compounds such as amines. Edwards and McDonald (1978) stated that the resulting products of clostridial fermentation are likely to be unacceptable to animals; in addition, the nitrogen fraction is thought to be made less available to ruminants than the originally ensiled material. Clostridia are sensitive to water availability and require a very wet environment in order to grow actively. This is because the optimal pH for clostridial growth is approximately 7.0-7.4 (Pelczar and Reid, 1972). The critical pH at which Clostridia will not grow is directly related to the water activity of the plant material (Weissbach et al., 1974). Several researchers have also found that very wet silages may be an inadequate feedstuff because of low involuntary dry matter intakes (Gordon et al., 1961; Wilkens et al., 1971). Bolsen and Ilg (1981) stated that ensiling of wet crops with a moisture content above 74-76% can result in the production of large volumes of effluent which decrease the nutritive value of the silage and reduces intake by cattle. Hay-crop silages require the process of wilting of the crop prior to ensiling in order to have an acceptable moisture content while still maintaining its nutritive value. Corn and sorghum usually do not require pretreatment prior to

ensiling in order to have an acceptable moisture content and corresponding nutritive value.

As indicated previously, the water-soluble carbohydrate content of a forage is also an important factor in the preservation fermentation process of silages. McDonald and Edwards (1976) felt that an ideal fermentation, that is, minimum nutrient loss, would require a water-soluble carbohydrate content of 6-8%.

There are several factors which can affect the water-soluble carbohydrate content. McDonald (1981) discussed several of these factors for hay-crop silages. These factors were species, cultivar, stage of growth, daily variations, climate and fertilizer level. Many of these factors, if not all, would also be applicable to grain-crop silages. The water-soluble carbohydrates are important in the preservation fermentation of silages because they are used as precursors by the fermenting bacteria in the production of lactic acid which ultimately preserves the silage. As stated previously, hay-crop silages may require the supplementation of a carbohydate source. Grain-crop silages, such as corn, rarely require carbohydrate addition since they usually contain an adequate water-soluble carbohydrate content.

The degree of compaction of a crop during ensiling, or the density of the ensiled material, can also affect the quality of the silage produced. McDonald (1981), in summarizing an earlier work he and others conducted, found that

inadequate compaction of ryegrass resulted in a silage which was higher in butyric acid and ammonia, while being lower in lactic acid, crude protein and organic matter digestibilities as compared to a well-compacted ryegrass crop. In addition, approximately 35% of the original dry matter of the inadequately compacted silage was lost. Finally, in this same study there was a marked increase in the temperature of the silage which was not consolidated above those which were consolidated properly. Wood (1971) stated that adequate consolidation or compaction is important since silage quality and maximum temperature have been related to the extent of compaction. One method to improve the degree of compaction of an ensiled material is by applying pressure through the use of a silage press. The most common approach to improving compaction is simply by cutting the material before ensiling. In addition a finer cut of an ensiled material increases the density of packing in silos and subsequently the effectiveness of the fermentation process (McCullough, 1978). Several works have reported that lactic acid levels are higher, while butyric acid levels are lower, in chopped or cut silages as compared to uncut silages (Zimmer and Gordon, 1964; Wierenga, 1959).

The environmental temperature during the time of ensiling can affect silage quality. Aerobic deterioration can increase in both rate and magnitude due to the ambient temperature and the heat generated by the process itself.

Aerobic deterioration has been shown to be a problem at temperatures greater than 10 C (Ohyama and Hara, 1975); therefore, aerobic deterioration would be expected to be more of a problem in the summer than in the winter. A result of increased temperatures during ensiling is a decrease in the efficiency of the fermentation process and, subsequently, a decrease in the nutritive value of the silage. High temperature of ensiled materials, whether due to environmental temperature, respiration or both, can result in increased water-soluble carbohydrate loss by creating a favorable environment for clostridia and a less favorable environment for lactic acid-producing bacteria (Vetter and VonGlan, 1978).

As is the case with the preservations of several foodstuffs, silage can be preserved by acidification, under anaerobic conditions, due to lactic acid production by several lactic acid-producing bacteria. Orla-Jensen (1919) divided lactic acid-producing bacteria into two major categories. The first category he called the homofermentative lactic acid bacteria, or homolactics. These microorganisms ferment hexoses predominantly to two molecules of lactic acid. The second category, the heterofermentative lactic acid bacteria, or heterolactics, generally ferment hexoses to one molecule each of lactic acid, ethanol or acetic acid and one molecule of carbon dioxide.

The homolactics which are important to the fermentation of silages are several species of Lactobacilli, Pediococci and Streptococci. Several heterolactic species of Lactobacilli and Leuconostoc are the heterolactics associated with silage fermentation. If a crop has been properly ensiled and sealed, the oxygen trapped in the silo is usually rapidly removed. Initially Enterobacteriaceae are the predominant microorganisms found immediately after ensiling. Soon after ensiling, however, Streptococci and Leuconostoc replace the Enterobacteriaceae. These microorganisms are then dominated by the Lactobacilli which lower the silage pH to approximately 4.0 (Whittenbury, 1968). It is at this point that the silage is essentially preserved until opening.

Obviously, the preservation fermentation process of silages is dependent on many interrelated factors. Many of these factors can be controlled by the silage producer through proper management. Through proper management the probability of an effective fermentation can be greatly improved.

One of the more controversial methods of aiding or controlling the fermentation process of silages is through the use of silage additives. Since Virtanen first described the use of inorganic acids as a portion of his AIV method of preserving silage in 1938, the number and diversity among the types of silage additives has increased dramatically. The effectiveness of these many additives has been studied for

many years, often with conflicting results. Often it is difficult just to classify a silage additive in terms of its desired effect on the silage fermentation process.

Silage Additives

Classification of Silage Additives

As stated previously, most of the original studies of silage were conducted in Europe in the early 1900s, so it is not surprising that it was there that much of the original work studying additives to silage was also conducted. Since grass or hay crops were the material most often ensiled and since these crops are relatively low in water-soluble carbohydrate content, it was logical to add a source of carbohydrates to the silage. For this reason molasses were added as a fermentable carbohydrate source for use by the lactic acid-producing bacteria. This type of silage additive was considered to be an aid to the silage fermentation process. Molasses were used almost exclusively until A.I. Virtanen, in 1929, recommended the use of mineral acids to rapidly acidify the ensiled material to a pH of approximately 3.5. Virtanen believed that this type of silage additive would effectively inhibit the fermentation process by the inhibition of microbial and plant enzyme activities. So essentially in the early part of this century there already existed two classes of silage additives, fermentation

stimulants and fermentation inhibitors. Even today silage additives are generally divided into these two classes.

McCullough (1977) suggested that there were four basic methods used in manipulating silage fermentations. He thought that these methods could be classified using four categories: direct acidification, aids to acidification, preservatives and nutrient additions. Then in 1981 McDonald also suggested the use of the terms "fermentation stimulants" and "fermentation inhibitors" as the two major classes of silage additives. He also added two subclasses to each of these major classes. Fermentation stimulants included the subclasses bacterial cultures and carbohydrate or nutrient sources. Acids and other inhibitors or preservatives were the two subclasses of fermentation inhibitors. In addition McDonald (1981) suggested two other major classifications: aerobic deterioration inhibitors and nutrients. This "nutrient" classification was used to distinguish non-carbohydrate nutrients from carbohydrates which would also be considered nutrient sources. Leahy et al. (1988) made slight adaptations of McDonald's classifications (Table 1). The four main classifications used were fermentation stimulants, fermentation inhibitors, aerobic deterioration inhibitors and nitrogen-containing nutrients and minerals. Stimulants were subclassified as lactic acid bacteria, carbohydrate sources and enzymes.

Table 1. CLASSIFICATION OF FERMENTATION MANIPULATORS

Fermentation stimulants

Lactic acid bacteria	Carbohydrate sources
Lactobacillus	glucose
acidophilus	sucrose
casei	molasses
plantarum	grain
xylosus	whey
Leuconostoc	beet pulp
mesenteriodes	citrus pulp
Pediococcus	
acidilactici	Enzymes
cervisiae	
Streptococcus	amylases
cremoris	
faecalis	cellulases
lactis	

Fermentation inhibitors

Acids	Others
mineral acids	formaldehyde
formic acid	paraformaldehyde
acetic acid	sodium nitrate
propionic acid	sodium chloride
lactic acid	
acrylic acid	antibiotics
benzoic acid	bronopol
sorbic acid	carbon dioxide

Aerobic deterioration inhibitors

acetic acid	sorbic acid
butyric acid	caproic acid
propionic acid	pimaricin
acrylic acid	ammonia

Nitrogen-containing nutrients and minerals

ammonia	limestone
urea	other mineral sources
biuret	

Leahy et al., 1988; Adapted from McDonald, 1981

Fermentation Stimulants

As stated in the preceding section, Leahy et al. (1988) suggested three classes of fermentation stimulants: lactic acid bacteria, carbohydrate sources and enzymes.

Since lactic acid production during the ensiling process has been known to produce and preserve a high quality silage, the positive effect of addition of lactic acid-producing bacteria can be easily recognized. In fact, Oshima et al. (1979) has indicated that there might be a need to include these microorganisms in a silage due to low or inadequate numbers. Whittenbury (1961) suggested that a microorganism should meet the following criteria before being used with silage: 1) it must have rapid growth resulting in its dominance over other organisms in the silage; 2) it must produce lactic acid without other major byproducts, i.e., it must be homofermentative; 3) it must be acid tolerant and lower the silage pH to 4.0 rapidly; 4) it must be able to ferment glucose, fructose, sucrose and preferably fructosans and pentosans; and 5) it should have no action on organic acids. There have been many different preparations containing lactic acid-producing bacteria, not all of which would meet Whittenbury's criteria for an adequate bacterial fermentation stimulant. Among the species of Lactobacilli used as fermentation stimulants have been Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus brevis and Lactobacillus plantarum. Many of these species of

Lactobacilli are sold as commercial preparations, either individually or as a mixture, under various trade names. While many of these and other microorganisms are sold as fermentation stimulants, McCullough (1977) has discussed his concern that only a small number of these preparations has been subjected to controlled research.

The second class of fermentation stimulants includes carbohydrate sources, such as grains and molasses. Carbohydrate sources are often added to silage in order to serve as a substrate for either the endogenous or added microorganisms.

Many types of grains in many forms have been added to silages as carbohydrate sources. Andrews and Stob (1958) were able to decrease pH in second cut alfalfa by addition of 90.7 kg of corn/ton. Gordon et al. (1957) increased dry matter and had no change in milk production when a silage consisting of orchard grass and ladino clover had 90.7 kg of corn meal per ton added. Clifton et al. (1963) added 50.0 kg of corn per ton of pre-boot sudan grass and found it to result in milk production equal to that of cows fed corn silage.

Murdock et al. (1955) found that an alfalfa-timothy silage treated by the addition of 45.4 kg of barley meal/ton adequately preserved the material. However, the ensiled crop was no better preserved when compared to wilting the crop or adding of molasses. In laboratory silos, Anderson (1970)

added ground barley to perennial ryegrass-white clover at the rates of 6, 10 and 19%. The addition of the ground barley increased dry matter and decreased pH and dry matter losses, while increasing dry matter digestibility and metabolizable energy content of the silage.

The use of molasses as a carbohydrate source for hay-crop silages has occurred for many years. In addition, there have been many experiments conducted to determine the effects of molasses addition on factors affecting fermentation and the quality of silage. Murdock et al. (1955) found that molasses served as a good carbohydrate source for lactic acid-producing bacteria. When molasses was added to a hay-crop silage, the silage pH was reduced (McCarrick, 1969). The addition of molasses has also been shown to decrease silage dry matter loss (Colovos et al., 1957; Lanigan, 1961).

Whittenbury (1968) stated that plant leaves contain enzymes which hydrolyze their own non-structural carbohydrates to the monomers glucose and fructose, which are the main substrates used by silage microorganisms. Gates and Simpson (1968) found that many plant species showed α-amylase activity; therefore, the addition of α-amylase could theoretically assist in producing a more effective fermentation by converting starch to a form more easily utilized by lactic acid-producing bacteria. In fact, Bolsen and Ilg (1980a,b) found that addition of a commercial product containing

α-amylase did improve the dry matter recovery of sorghum and corn silages as compared to their respective controls.

Though many different types of nutrients have been added to silages to stimulate the fermentation process, improve the nutritive value of a silage, or both, several scientists realized that the quality of a silage could be improved by inhibiting the fermentation process.

Fermentation Inhibitors

The first class of fermentation inhibitors includes organic and inorganic acids used for direct acidification of the silage. In much of his early studies of silage, A.I. Virtanen discovered that a silage required a pH of approximately 4.0 in order to restrict respiration, proteolysis and butyric acid fermentation. This early work led Virtanen (1938) to the development of one of the first programs to use an acid as a fermentation inhibitor. The AIV method, as it became known, consisted of the addition of enough of a sulfuric and hydrochloric acid mixture in a dilution of approximately 2N to decrease the pH of a desired forage to the required 4.0 (Virtanen, 1938). Even though the AIV method caused burns, destroyed clothing and corroded metal equipment and silos (Owen, 1971), it was not until recently that its use in Europe was virtually discontinued. Since the 1950s the relatively less caustic organic acids are the ones which have been used in both Europe and the United States.

Sulfur dioxide has been used in several forms as a fermentation inhibitor in the food industry. Sulfur dioxide has been added as a gas, in a solution with water as sulfurous acid, or as a constituent of the sodium salts sulfite and metasulfite. In work conducted by Skaggs and Knodt (1952), silages that had been treated with sulfur dioxide were shown to have lower levels of volatile fatty acids, lactic acid and ammonia nitrogen levels than untreated silages. Knodt et al. (1952) found that sulfur dioxide treatment of a timothy clover silage has lower dry matter losses than that of a control. Bratzler et al. (1956) found that addition of sodium metabisulfate to alfalfa silage reduced dry matter loss. In contrast, Ramsey et al. (1959) found no difference in dry matter recovery among a sodium metabisulfate-treated and untreated grass silage. Lanigan (1961) indicated that there was a problem with the use of sodium metabisulfate. He found that even though the additive inhibited undesirable fermentation, its effect was dependent on silage temperature. The sodium metabisulfate acted well as a preservative at 23 C but was considerably less active at 30 C.

Formic acid was one of the first organic acids recommended for use as a fermentation inhibitor and, even though it is one of the strongest of the fatty acids, it is still weaker than the mineral acids used by Virtanen (McDonald, 1981). Addition of formic acid to cut ryegrass resulted in a

drop in pH from approximately 5.5 to 4.6 (Henderson and McDonald, 1976). Yahara and Nishibe (1975) found that alfalfa ensiled with 0.5% of 85% formic acid resulted in lower acid and ammonia production and higher dry matter recovery than other organic acids that were tested. This effect of formic acid has been attributed to an antimicrobial action resulting in a hydrogen ion concentration effect and selective bactericidal effect of the undissociated acid (McDonald, 1981). Formic acid has been shown to inhibit the growth and the spore formation of Clostridia (Richard, 1946), while Lactobacilli were not completely inhibited (Mann, 1975). The addition of formic acid to hay-crop silages has been effective in preserving silage by improving water-soluble carbohydrate and protein nitrogen concentrations when compared with other additives (Barry et al., 1978). Other acids such as acetic and propionic acids have also been added to silage, resulting in restriction of the fermentation process (Mann and McDonald, 1976); however, it was found that silages treated with acetic acid had a lower water-soluble carbohydrate content than those treated with formic and propionic acid.

Propionic acid has been shown to be effective in controlling the fermentation of silage by inhibiting fungal growth (Britt et al., 1975) and reducing ensiling temperatures (Yu and Thomas, 1975; Thomas, 1976). In their studies of the possible use of other longer chain fatty acids, Wilson

et al. (1979) concluded that most could not be recommended as fermentation inhibitors. Initial work by Woolford (1975) and more recently Bolsen and Hinds (1984) have indicated that acrylic acid may serve as an effective silage additive. In addition, benzoic acid as sodium benzoate had antimicrobial effects by being selective for heterofermentative lactic acid bacteria (Woolford, 1975).

Sorbic acid has been safely used for decades as a mold inhibitor in foods (Deuel et al., 1954). Potassium sorbate has been shown to have an inhibitory effect on spore-bearing bacteria, yeasts and molds at pH 5 in silage (Woolford, 1975). When sorbic acid was added to corn silage, the results were a decrease in the populations of yeasts and molds and in dry matter loss, with increased levels of water-soluble carbohydrates (Alli et al., 1985).

The second class of fermentation inhibitors, classified as "others," included formaldehyde, sodium chloride and many other substances. Formaldehyde or the commercial substance formalin was reported as being a successful bacteriostat in silage production by Kuchler and Von Wachter in 1931 (Watson and Nash, 1960). More recently Brown and Valentine (1972) were able to inhibit silage fermentation and protect protein degradation by formaldehyde addition; however, dry matter intake and digestibility by sheep offered the silages were drastically reduced. There have been several concerns expressed with the addition of formaldehyde and/or formalin

to silage. While Brown and Valentine (1972) found that formaldehyde treatment of alfalfa decreased animal dry matter consumption, others (Barry, 1975; Valentine and Radcliffe, 1975) obtained increased consumption of forages treated with formaldehyde above untreated controls. The work of Waldo et al. (1975) indicated that paraformaldehyde, though it was not as effective as formic acid in inhibiting undesirable fermentations, produced comparable body weight gains.

Many of the substances used to preserve foods have also been used as silage preservatives. Sodium chloride, for example, has been used for either slowing down the rate of fermentation of vegetables or to stop fermentation completely (Ayers et al., 1980). Watson and Nash (1960), in a summary of early studies where sodium chloride was used as a silage preservative, concluded that salt has little if any effect on bacterial action. In a more recent study, Goering and Gordon (1973) found that sodium chloride did not have an inhibitory effect on mold growth.

The use of antibiotics as food preservatives has been limited by the Food and Drug Administration to use with poultry; however, after surface and deep tissue residues were still detectable in the meat after 13 days, antibiotic use was again prohibited (Ayers et al., 1980). McDonald (1981) notes that antibiotic use as a feed additive for livestock is also very strictly controlled worldwide. The effects of terramycin, neomycin, penicillin, aureomycin and zinc

bacitracin as silage preservatives for alfalfa were tested by Dexter (1957). He found that of all these antibiotics only zinc bacitracin produced a silage that would have been considered of good quality. Other antibiotic treatments resulted in silages with off odors and discoloration. Woolford (1975) tested the effects of the antibiotics tylosin and pimaricin as fermentation inhibitors. He felt that tylosin, though it was effective in preventing bacterial growth, would not make a good silage additive since the required level of application would make it cost prohibitive. Pimaricin, however, because of its ability to prevent aerobic deterioration, would have potential as a silage additive if used in combination with an antibacterial agent. Woolford (1975) also tested a synthetic antimicrobial agent, bronopol, and concluded that it had promise for preventing the activity of spore-forming bacteria. Of all the antibiotics used as fermentation inhibitors, zinc bacitracin has been the most widely tested. Zinc bacitracin treatment of silages has resulted in increased silage intake (Rusoff et al., 1959a), dry matter digestibility (Alexander et al., 1961) and an improved fermentation quality (McCarrick, 1969). Other studies, however, have found the treatment of silage with zinc bacitracin has lowered consumption of silage fed to cows as compared to a control (Rusoff et al., 1959b). McCarrick (1969) determined that zinc bacitracin had no effect on improving fermentation quality.

Aerobic Deterioration Inhibitors

Several researchers have noted that higher organic acids, such as butyric, valeric and caproic acid, have successfully inhibited microorganisms involved in the aerobic deterioration of silage (Ohyama and McDonald, 1975; Ohyama et al., 1975; Woolford and Cook, 1978). When silage was treated with formic acid alone it deteriorated, while that treated with formic acid in combination with either isovaleric or caproic remained stable for seven days under aerobic conditions (Ohyama and McDonald, 1975). Propionic acid was added to Italian ryegrass producing a silage which was more stable than similar silages which were untreated (Ohyama et al., 1975). Propionic acid has been shown not only to improve the quality of a silage produced by quickly lowering pH but also to inhibit the growth of fungi (Britt et al., 1975). Caproic acid addition to either Italian ryegrass or orchardgrass was also found to be successful in preventing aerobic deterioration as determined by increases in temperature, pH and yeast and mold counts (Ohyama et al., 1977). Corn silage treated with sorbic acid was more aerobically stable when compared to an untreated control (Alli et al., 1985).

Woolford and Cook (1978) noted that aerobic deterioration is becoming more of a problem due to the use of additives to silage which restrict fermentation. This presents serious practical considerations, in that by manipulation of

the silage fermentation process to produce a silage of improved nutritive quality, the stability of that silage may be decreased when in the presence of oxygen. Barry et al. (1980) found that treatment of silage with formaldehyde-containing additives increased the amount of heating and deterioration during prolonged exposure to air as compared to an untreated control. Conversely, when butyric acid, which is often considered an indication of an undesirable silage fermentation, was added to aerobically unstable silages, they became more stable (Ohyama and Hara, 1975; Henderson et al., 1979).

Nitrogen-Containing Nutrients and Minerals

Sources of nitrogen, such as ammonia and urea, are usually added to crops that are nitrogen (protein) deficient, such as corn. The addition of these nitrogenous compounds to the silage may decrease the amount of supplemental protein which would be required in a ruminant's diet.

Urea has been the most widely used source of nitrogen added to silage to increase the nitrogen content of a silage. As early as 1944 urea was being added to protein-deficient silages with resulting increases in crude protein content (Wise et al., 1944; Davis et al., 1944). Urea has usually been added at the rate of 0.5%, but has been added at the rate of 0.75%, with resulting improvement in animal performance (Huber and Thomas, 1971).

In addition, corn silages tend to be low in minerals such as calcium; therefore, limestone has also been added to silages in order to eliminate the need for later supplementation. Besides increasing the calcium content of silages, limestone addition has helped to prolong the fermentation process and increase organic acid production (Ely, 1978). Addition of limestone resulted in a slight elevation in pH and an increase in lactic acid production (Condon et al., 1969). Barth et al. (1974) found that when urea and limestone were each added at 0.5% there was an additive effect which resulted in a significant improvement of nitrogen utilization by cattle over that of urea (0.05%) added alone.

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CHAPTER II

EFFICIENCY OF SILAGE PRODUCTION AS AFFECTED BY THE MANIPULATION OF FERMENTATION DYNAMICS

Abstract

Corn silage was either left untreated (control) or treated with α-amylase, sorbic acid or both in a 2 x 2 factorial arrangement. The effects of treatments on fermentation and on aerobic deterioration were studied using 208-liter steel drums. Dry matter losses of the treatments were determined using 9-ton capacity silos.

After one day of fermentation it was apparent that sorbic acid treatment resulted in significantly more ($P<.03$) water-soluble carbohydrates being retained from the originally ensiled material. Alpha-amylase treatment of corn silage had no effect ($P>.43$) on the water-soluble carbohydrate content at any time during fermentation. There were no effects on lactic ($P>.10$) or acetic acid ($P>.21$) concentrations at any time during the fermentation study. Butyric acid concentrations of silages treated with sorbic acid were nearly twice ($P<.07$) those of the other two treatments. Both butyric acid ($r=.951$) and isobutyric acid ($r=.988$) concentrations were significantly correlated ($P<.05$) with retained water-soluble carbohydrate content.

During the aerobic deterioration study, sorbic acid-treated silages began with significantly more ($P<.03$)

retained water-soluble carbohydrates and this increase was sustained through day 3 ($P<.02$) Lactic acid concentrations in corn silages were not significantly affected by α-amylase treatment or sorbic acid treatment. Temperatures increased less rapidly ($P<.03$) during anaerobic deterioration due to sorbic acid treatment and it resulted in significant decreases in the log counts of all aerobic microorganisms at all sampling times (day 0, $P<.01$; day 2, $P<.06$; day 3, $P<.01$). Alpha-amylase treatment had no significant effect on total aerobe numbers at day 0 or day 2 of deterioration.

Dry matter lost due to spoilage was significantly decreased ($P<.07$) when a silage was treated with sorbic acid (7.5 vs 5.7%). Treatment of silages with α-amylase had no significant effect ($P>.28$) on spoilage dry matter loss.

Introduction

Virtanen (1938) was one of the earliest researchers to realize the possibilities of manipulation of the silage fermentation process when he added inorganic acids to green chop material. Since that time, hundreds of materials have been suggested and utilized as additives to silage. The effects of various silage additives can be better elucidated by studying the chemical changes which occur at various intervals during the fermentation process. Preservation of the nutrients in the originally ensiled material occurs as a result of inhibition of microbial activity. In the case of

fermentation stimulants, lactic acid-producing bacteria are stimulated so that yeasts, molds and Clostridia are inhibited by the lower pH resulting from the lactic acid produced. It is, therefore, essential that lactic acid be produced as early and rapidly in the ensiling process as possible. Fermentation inhibitors are added to silages to inhibit all or selected fermentations (microorganisms). The resulting silage produced using these two types of fermentation manipulators could be very different. In this study, small-scale silos (208 liter drums) such as those suggested by Ohyama (1984) were used as models to closely monitor the chemical changes which occur during the early stages of the fermentation due to the manipulation of the fermentation process using the fermentation stimulant, α-amylase, and the inhibitor, sorbic acid.

Alpha-amylase stimulates the fermentation process by converting starch to a form which can be readily utilized by lactic acid-producing bacteria. Treatment of corn green chop with an α-amylase-containing commercial product resulted in increased dry matter recovery in both sorghum and corn silage (Bolsen and Ilg, 1980a,b). Sorbic acid is a selective inhibitor of yeasts and molds and has resulted in reduced surface spoilage when added to corn green chop (Alli et al., 1985; Salunke, 1956). Addition of these two fermentation manipulators to corn green chop together could, theoretically, have an additive effect in preserving the

material during ensiling. Corn silage produced after treatment with sorbic acid at ensiling was found to be more aerobically stable upon exposure to air (Alli et al., 1985).

There has also been interest in the effect of silage additives regarding their ability to decrease secondary aerobic deterioration. Most of this type of loss has been attributed to yeasts and molds; however, Woolford (1984) has indicated that bacteria may also be involved. In addition, silages which preserve materials well during the fermentation phase of ensiling are known to be very unstable and susceptible to aerobic deterioration when opened. Conversely, poorly preserved silages (those where butyric acid is produced) tend to be more unstable when exposed to air. Finally, attempts have been made to determine if there is a secondary effect of addition of silage fermentation manipulators at the time of ensiling into this phase of aerobic deterioration. For this reason, a second experiment, using the same manipulators, was conducted to better define the dynamics of aerobic deterioration and to determine the fermentation manipulators' secondary effects.

Since small-scale silos, such as 208-liter drums, are not considered good models to measure dry matter loss (Ohyama, 1984), corn silage dry matter loss data were obtained using 9-ton capacity silos. Dry matter lost due to ensiling and spoilage were determined.

Experimental Procedure

Whole corn plants in the early dent stage, with a dry matter content of approximately 34%, were harvested and chopped into lengths of approximately 6.4 to 12.7 mm. Chopped plant material was transported in a wagon for less than one km and quickly unloaded into 208-liter steel drums lined with 4-ply plastic bags and temporarily placed in a cooler at 4.4 C to minimize fermentation activity prior to addition of fermentation manipulators. The green chop was removed from the cooler and placed in a Marion Mixer (Model #2030) with a speed of 60 rpm and mixed for approximately two minutes. When necessary α-amylase (0.05% fresh weight basis), sorbic acid (0.10% fresh weight basis) or both α-amylase and sorbic acid were added and mixed with the green chop. Therefore, there were four experimental silage treatments: 1) an untreated control (which was also mixed for two minutes); 2) α-amylase-treated corn green chop; 3) sorbic acid-treated corn green chop, and 4) green chop treated with both fermentation manipulators. The experiment was arranged as a 2 x 2 factorial with two levels of α-amylase (- or + α-amylase) and two levels of sorbic acid (- or + sorbic acid) being used.

Fermentation Study

Changes in pH, water-soluble carbohydrates, volatile fatty acids and lactic acid were determined at various times

during the fermentation process. To accomplish this, 15 kg of the respective green chop was placed into a four-ply polyethylene bag. Air was expelled by hand and the bag was then sealed and placed into a 208-liter steel drum. Each drum contained five of these 15-kg bags. Each treatment consisted of two drums, Which allowed for ten sampling times, namely one 15-kg bag was removed from the drums at days .25, .50, 1, 2, 3, 6, 9, 18, 27 and 36 of ensiling. This system allowed for sampling of the fermenting green chop at various times without interruption of the remaining fermenting samples. Representative samples were immediately frozen at -20 C for later analyses.

Frozen silage samples were prepared for analysis by immersing them in liquid nitrogen placing them into an industrial-type Waring blender (Model #1120) and macerating them at a speed of 21,000 rpm (Parker, 1978). The resulting material was a coarse powder which was homogenous in appearance. All subsequent analyses were performed using this powdered form of the silage sample. Ten grams of silage was mixed with 50 ml of distilled water, shaken for five minutes and pH was determined. The procedure described by El Hag et al. (1982) was used to prepare a corn silage water extract for volatile fatty acid and lactic acid analyses. Volatile fatty acid analysis was conducted according to the procedure of El Hag et al. (1982) using a Hewlett-Packard HP5890A gas chromatograph fitted with a 10.0 m x 0.53 mm fused silica

capillary column (Model #HP-FFAP). Lactic acid content was determined according to the procedure of Barker and Summerson (1941) and water-soluble carbohydrate content was determined using a water extract preparation and procedure developed by Dubois et al. (1956).

Silage pH was determined on samples obtained on day 0 through day 36 of fermentation, while all other analyses were determined on samples obtained from 0 through 9 days of fermentation.

Aerobic Deterioration Study

The previously described corn green chop, treated in a similar manner, was placed into one large polyethylene bag which lined a 208-liter drum and allowed to ferment for 480 days prior to opening. After opening, the top 15 kg of silage was discarded to remove any yeasts, molds or other contaminants that might alter aerobic deterioration characteristics. Of the remaining 60 kg of silage, two 2-kg samples were removed, aerated by hand to stimulate unloading, and placed into open replicate polystyrene containers with walls 2.54 cm thick and an inside volume of 30.5 cm^3 to simulate in-silo conditions. The polystyrene containers were placed in a room where simulated summer temperatures of 30-38 C and a relative humidity between 30 and 40% were maintained in order to obtain conditions suitable for microbial activity.

Heat would have been rapidly dissipated, had the silage material not been placed in the polystyrene containers which have excellent insulation properties. Therefore, accurate measurements of temperature increases were able to be obtained. Temperatures were measured by placing a thermocouple in the silage at the geometric center of the mass. Temperature readings were taken at 4-hour intervals for the first 60 hours and a one time measurement was obtained at 72 hours of aerobic deterioration.

Silage samples were taken at 24-hour intervals for determinations of water-soluble carbohydrate, volatile fatty acid and lactic acid according to the previously described procedures. Silage samples were also taken at 0, 2 and 3 days for enumeration of total aerobic microorganisms, and for yeasts and molds. Total numbers of viable organisms were determined, according to procedures as outlined by Hausler (1977), by placing a 25-g silage samples into a stomacher bag and aseptically adding 225 ml of peptone water. The silage and peptone water were blended in a stomacher for two minutes and dilutions to 10^{-8} were made. Selected dilutions were made by the pour plate method using standard methods agar for enumeration of total viable aerobic organisms and Rose Bengal agar for enumerating yeasts and molds. The standard methods and Rose Bengal agars were sterilized at 121 C for 15 minutes. In addition, just prior to plating, .2

ml per 100 ml of molten agar of Rose Bengal dye and chlortetracycline were added to prepare the Rose Bengal agar.

Dry Matter Losses

Eight upright brick silos measuring 1.8 x 9.1 m and holding an average of nine tons were used during each of the three consecutive trials to measure dry matter recovery. Two silos were assigned to each of the four experimental treatments for 1985 and 1986 and one silo was used in 1987. Pioneer Hybrid 3147 corn green chop was harvested in the early dent stage. The four experimental treatments were: 1) an untreated control; 2) α-amylase-treated (.05% on a wet basis) corn green chop; 3) sorbic acid-treated (.10% on a wet basis) corn green chop; and 4) green chop treated with both fermentation manipulators. The green chop was allowed to ferment for at least 117 days. Silage samples were taken during each of the trials when a silo was first opened and at approximately 30-day intervals thereafter.

Green chop and silage samples were dried at 60 C for 72 hours in a forced air oven, then allowed to air equilibrate for 72 hours, air-equilibrated dry matter ground through a 1 mm mesh screen of a Wiley Mill, redried at 100 C for 16 hours and total dry matter content was calculated.

Dry matter losses and total dry matter recovery were determined for each silo of each treatment for the three years. First, dry matter loss during the ensiling process was calculated; second, loss of inedible silage due to

spoilage, as a proportion of dry matter ensiled, was calculated with the use of spoiled silage dry matter; and finally, the two losses were combined in the calculation of total dry matter lost as a proportion of dry matter ensiled, and of total dry matter recovery.

Amount of dry matter ensiled was calculated as the product of the amount of wet green chop ensiled and the average dry matter for all the green chop samples. Similarly, dry matter loss after ensiling was the product of the silage removed from the silo and its average dry matter percentage for all the silages. Dry matter loss from the ensiling process was the quotient of the amount of dry matter lost after ensiling and the amount of dry matter ensiled.

Dry matter lost due to spoilage was obtained from the amount of spoiled silage determined after the silo was first opened and throughout the emptying of the silo and from the dry matter percentage of the spoiled silage.

Statistical Analysis

Significance levels associated with calculated F-statistics were calculated using contrasts which analyzed the data as a 2 x 2 factorial. Test statistics were determined for the main effects and interaction of α-amylase and sorbic acid (SAS, 1985). In discussing the results, the probability level of $P<.10$ was chosen to delineate between significance and non-significance.

Results and Discussion

Fermentation Study

The water-soluble carbohydrate content, on a dry matter basis, at various times during the fermentation process is included in Table 1. At the beginning of the study, it averaged 20.4% for the four treatments. Water-soluble carbohydrate content of the treatments declined until it stabilized on day 6 of fermentation at an average of 4.7%. From the start, it was apparent that the fermentation inhibitor, sorbic acid, resulted in higher retention of water-soluble carbohydrates from the originally ensiled material. Higher retention became significant ($P<.03$) one day after ensiling, and remained higher ($P<.01$ to .06) for the remainder of the trial. On the sixth day of fermentation, when stabilization occurred, sorbic acid-treated silages retained approximately 2.3 percentage units more water-soluble carbohydrates than silages not treated with sorbic acid (5.8 vs 3.5%). Alli et al. (1985) also reported that sorbic acid treatment resulted in higher water-soluble carbohydrate content. This effect was probably due to the known inhibiting action of sorbic acid on fermentation by yeasts and molds (Salunke, 1956), which compete for the same substrates (water-soluble carbohydrates) that are required by lactic acid-producing bacteria for growth. In our study, yeast and mold growth was absent in sorbic acid-treated silages but was observed in the silages that had not been

Table 1. WATER-SOLUBLE CARBOHYDRATES IN CORN SILAGE AT VARIOUS TIMES DURING THE FERMENTATION PROCESS AS AFFECTED BY FERMENTATION MANIPULATORS

Constituent	Time of fermentation, d	Corn silage					Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
Water-soluble carbohydrates, % DMB[b]	0	18.8	19.1	23.3	20.5	1.23	.67	.33	.60
	.25	16.1	14.5	17.9	20.7	1.48	.86	.28	.53
	.50	14.2	13.1	18.8	15.4	1.28	.43	.25	.69
	1	8.6	8.5	13.3	13.0	1.00	.87	.03	.96
	2	4.6	4.1	8.0	8.3	.75	.81	.01	.52
	3	4.1	4.3	6.4	6.2	.43	.95	.01	.54
	6	3.6	3.5	6.4	5.3	.57	.56	.06	.59
	9	3.5	3.9	5.9	5.8	.44	.70	.01	.51
	Mean	9.2	8.9	12.5	11.9	2.35[c]	.64	.02	.97

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]DMB = Dry matter basis.

[c]Standard error of the overall treatment mean is based on 16 observations per treatment.

treated with sorbic acid. Alpha-amylase treatment of corn silage had no significant effect (P>.43) on the water-soluble carbohydrate content at any time during the fermentation process. Alli and Baker (1982), however, reported that corn silage treated with an amylase product (diastase) resulted in water-soluble carbohydrates higher than those of the control.

Table 2 contains the pH of the silages. Initially, sorbic acid treatment of corn silage significantly decreased (P<.01) pH from an average of 5.55 in silages not treated with sorbic acid to 5.25 in silages treated with sorbic acid. This indicates that, at least initially, sorbic acid's fermentation inhibitory action may be pH related. After two days of fermentation, pH values of all four treatments had dropped below 4.00, which is considered to be the required pH for proper preservation. Throughout the fermentation, values of the silages treated with sorbic acid were lower than those not treated with sorbic acid. This lowered pH approached significance (P>.12) on days 3 and 6 of fermentation and was significant on days 27 (P<.01) and 36 (P<.03).

Table 3 contains the lactic acid concentrations through day 9 of fermentation. At 0 time, lactic acid was not detectable in any of the silages. After six hours of fermentation it averaged .71% on a dry matter basis and continued to increase in each of the silages throughout the nine days of fermentation. Overall α-amylase treatment resulted in lower (P<.07) lactic acid concentrations; five of

Table 2. pH IN CORN SILAGE AT VARIOUS TIMES DURING THE FERMENTATION PROCESS AS AFFECTED BY FERMENTATION MANIPULATORS

	Time of fermentation, d	Corn silage					Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
pH	0	5.58	5.53	5.25	5.25	.062	.63	.01	.63
	.25	4.90	4.93	4.81	4.86	.043	.72	.46	.93
	.50	4.54	4.56	4.50	4.55	.021	.54	.66	.80
	1	4.20	4.16	4.15	4.16	.012	.65	.34	.34
	2	3.89	3.87	3.84	3.87	.015	.83	.26	.62
	3	3.86	3.81	3.77	3.75	.022	.45	.12	.76
	6	3.81	3.75	3.69	3.69	.024	.46	.12	.59
	9	3.74	3.68	3.68	3.66	.015	.22	.18	.44
	18	3.70	3.71	3.67	3.66	.011	.92	.21	.77
	27	3.76	3.75	3.66	3.65	.020	.61	.01	.97
	36	3.75	3.75	3.66	3.65	.021	.80	.03	.80
	Mean	4.16	4.14	4.06	4.06	.060[b]	.48	.01	.34

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Standard error of the overall treatment mean is based on 22 observations per treatment.

Table 3. LACTIC ACID IN CORN SILAGE AT VARIOUS TIMES DURING THE FERMENTATION PROCESS AS AFFECTED BY FERMENTATION MANIPULATORS

	Time of fermentation, d	Corn silage					Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
Lactic acid, % DMB[b]	0	ND[c]	ND	ND	ND				
	.25	.84	.67	.73	.61	.080	.49	.70	.92
	.50	1.17	1.03	.97	.83	.061	.22	.11	.99
	1	2.29	2.30	1.88	1.91	.139	.95	.26	.98
	2	5.16	4.66	4.47	4.72	.123	.56	.19	.13
	3	5.39	5.01	5.33	5.19	.197	.64	.91	.82
	6	4.46	4.38	5.02	4.94	.284	.91	.47	.99
	9	8.52	6.98	8.36	5.88	.577	.14	.59	.69
	Mean	3.44	3.09	3.30	2.97	.011[d]	.07	.48	.97

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]DMB = Dry matter basis.

[c]ND = Not detectable.

[d]Standard error of the overall treatment mean is based on 16 observations per treatment.

the eight measurement times the control silage had numerically greater lactic acid concentrations than the treated silages. This increase in lactic acid production is probably due to the increased fermentation activity occurring in the control silage. Also, the increased lactic acid produced was at the expense of water-soluble carbohydrates, originally present in the ensiled material, from which it is produced.

Acetic and butyric acid concentration changes during fermentation are presented in Table 4. Propionic acid could not be detected during the fermentation study. Acetic acid was present immediately in each of the four treatments, indicating that some aerobic deterioration of the green chop material had occurred between the time the plants were cut and when they were ensiled. In general, acetic acid concentrations increased at each successive sampling time until day 6. There were no significant effects due to treatment.

Butyric acid was detectable in all four experimental silages during day 1 of fermentation. After two days of fermentation and for the remaining sampling times, no butyric acid was detected in any of the silages. Butyric acid concentrations steadily decreased throughout the first day of fermentation in each treatment; however, the rate of decline was slower in silages treated with sorbic acid. At one day of fermentation the concentration of butyric acid due to

Table 4. ACETIC ACID AND BUTYRIC ACID IN CORN SILAGE AT VARIOUS TIMES DURING THE FERMENTATION PROCESS AS AFFECTED BY FERMENTATION MANIPULATORS

	Time of fermentation, d	Corn silage				SE	Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid		Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
Acetic acid, % DMB[b]	0	.15	.14	.13	.13	.013	.89	.70	.78
	.25	.55	.47	.50	.44	.045	.55	.77	.92
	.50	.49	.46	.46	.42	.018	.46	.46	.91
	1	.58	.55	.56	.58	.022	.94	.92	.70
	2	.69	.63	.64	.66	.014	.37	.76	.21
	3	.69	.71	.66	.65	.023	.92	.51	.77
	6[c]	.82	.79	.87	.85				
	9	.91	.78	.79	.75	.037	.38	.39	.60
	Mean	.61	.57	.58	.56	.028[d]	.17	.41	.49
Butyric acid, % DMB	0	.05	.05	.05	.06	.004	.67	.51	.60
	.25	.03	.04	.04	.05	.004	.68	.18	.91
	.50	.03	.03	.03	.02	.003	.54	.63	.20
	1	.01	.01	.03	.03	.003	.69	.04	.71
	2	ND[e]	ND	ND	ND				
	3	ND	ND	ND	ND				
	6[c]	ND	ND	ND	ND				
	9	ND	ND	ND	ND				
	Mean	.01	.01	.02	.02	.002[d]	.85	.01	.44

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]DMB = Dry matter basis.

[c]Replicate sample for day 6 of fermentation was lost.

[d]Standard error of the overall treatment mean is based on 16 observations per treatment.

[e]ND = Not detectable.

sorbic acid treatment was significantly higher at P<.04 (.03 vs .01%).

The concentrations of isobutyric acid in the experimental silages are included in Table 5. Isobutyric acid concentrations of the four experimental silages generally declined throughout the nine days of fermentation. Beginning with day 2, the rate of this decline was significantly (P<.01) slower in the sorbic acid-treated silages until, on day 9, (P<.07), concentrations were nearly twice those of silages not treated with sorbic acid (.11 vs .06%). Table 12 presents the correlation coefficients obtained using treatment means of selected parameters over the 9-day measurement period from each of the three studies. Within the fermentation study, several parameters were significantly correlated. As pH values of a silage decreased the amount of water-soluble carbohydrates maintained in the silage increased (r=-.971, P<.05). Both butyric acid (r=.951) and isobutyric acid concentrations (r=.988) were significantly (P<.05) and positively correlated with retained water-soluble carbohydrate content. This occurrence is probably explained by the concurrent decreases in water-soluble carbohydrate and butyric acid percentages in the silages while the percentage of lactic acid increased. In fact, the water-soluble carbohydrate content of the silages did decrease as lactic acid of the silages increased and therefore they were

Table 5. ISOBUTYRIC ACID IN CORN SILAGE AT VARIOUS TIMES DURING THE FERMENTATION PROCESS AS AFFECTED BY FERMENTATION MANIPULATORS

	Time of fermentation, d	Corn silage					Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
Isobutyric acid, % DMB[b]	0	.46	.45	.47	.51	.018	.68	.44	.61
	.25	.33	.36	.43	.48	.030	.56	.14	.91
	.50	.28	.27	.34	.30	.018	.44	.31	.72
	1	.16	.15	.24	.27	.020	.69	.01	.53
	2	.06	.07	.16	.17	.019	.74	.01	.78
	3	.05	.06	.09	.10	.008	.58	.01	.90
	6[c]	.04	.04	.11	.07				
	9	.07	.05	.13	.10	.015	.38	.07	.81
	Mean	.18	.18	.25	.25	.020[d]	.99	.01	.92

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]DMB = Dry matter basis.

[c]Replicate sample for day 6 of fermentation was lost.

[d]Standard error of the overall treatment mean is based on 16 observations per treatment.

negatively correlated (r=-.218) though this was not significant (P>.10).

The expected negative correlation of lactic acid production and pH (r=.410, P>.10) was not observed, indicating that products other than lactic acid were influencing the pH of the silages.

Aerobic Deterioration Study

All variables measured in the fermentation study were also measured in the aerobic deterioration study. Similar values at the end of the fermentation study and at the beginning of the deterioration study might be expected. If differences occurred, they could be due to the originally ensiled materials, method of ensiling (5 vs 1 polyethylene bag) and time the material was allowed to ferment.

Water-soluble carbohydrates, pH and lactic acid concentrations in corn silages at various times during aerobic deterioration are presented in Table 6. Results of measurements at various times during the aerobic deterioration study indicate that sorbic acid-treatment of corn green chop at ensiling resulted in significantly higher (P<.03) retention of water-soluble carbohydrates (mean of 19.2 vs 6.8%). Though water-soluble carbohydrate contents were somewhat variable over the 3-day study, there was no decrease of its concentration as deterioration progressed. Addition of α-amylase did not significantly affect (P>.59)

Table 6. WATER-SOLUBLE CARBOHYDRATES, pH AND LACTIC ACID IN CORN SILAGE AT VARIOUS TIMES DURING AEROBIC DETERIORATION AS AFFECTED BY FERMENTATION MANIPULATORS

		Corn silage					Significance level for contrasts[a]		
	Time of deterioration, d	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
Water-soluble carbohydrates, % DMB[b]	0	6.6	6.6	16.0	16.2	2.10	.97	.03	.98
	1	6.8	5.6	14.9	19.7	2.48	.59	.02	.39
	2	8.0	7.8	23.6	25.3	3.34	.81	.01	.76
	3	6.9	5.5	19.8	18.7	2.85	.75	.02	.97
	Mean	7.1	6.4	18.5	20.0	.81[c]	.83	.01	.52
pH	0	4.19	4.16	3.99	4.19	.070	.62	.64	.53
	1	4.22	4.13	4.09	4.08	.057	.78	.57	.75
	2	4.21	4.03	3.98	4.05	.075	.78	.60	.52
	3	4.13	4.05	3.94	4.05	.060	.94	.54	.54
	Mean	4.19	4.09	4.00	4.10	.032[c]	.98	.26	.24
Lactic acid, % DMB	0	4.09	3.00	5.86	3.87	.652	.33	.39	.76
	1	2.43	2.64	3.07	3.39	.289	.71	.35	.94
	2	2.39	3.70	4.86	3.99	.562	.86	.31	.41
	3	2.47	3.32	5.59	4.21	.626	.83	.17	.40
	Mean	2.82	3.17	4.84	3.87	.276[c]	.57	.03	.27

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]DMB = Dry matter basis.

[c]Standard error of the overall treatment mean is based on 8 observations per treatment.

water-soluble carbohydrate content at any time during the aerobic deterioration process.

During the aerobic deterioration study there were no significant treatment effects on pH. However, treated silages had numerically lower pH values at all sampling times. Lactic acid concentrations during aerobic deterioration were also not significantly affected by treatments. However, the values in the control silage were numerically lower at all sampling times.

In Table 7 the acetic, propionic and butyric acid percentages during the aerobic deterioration study are presented. Acetic acid percentages were not significantly affected by treatment or length of deterioration. However, the silage treated with α-amylase alone had numerically higher, and both silages treated with sorbic acid had numerically lower, acetic acid concentrations throughout the study.

Minimal concentrations of propionic acid were detectable in three of the four treatments throughout the study. Sorbic acid treatments contained significantly lower propionic acid percentages on day 0 ($P<.06$) and day 1 ($P>.10$) of deterioration and contained numerically lower, but not significant concentrations on day 2 ($P>.20$) and day 3 ($P>.12$) of deterioration.

The concentrations of butyric acid in the silages were not significantly affected at any time during the

Table 7. ACETIC ACID, PROPIONIC ACID AND BUTYRIC ACID IN CORN SILAGE AT VARIOUS TIMES DURING AEROBIC DETERIORATION AS AFFECTED BY FERMENTATION MANIPULATORS

	Time of deterioration, d	Corn silage					Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
Acetic acid, % DMB[b]	0	1.67	2.03	1.79	1.44	.135	.98	.44	.28
	1	1.70	1.94	1.63	1.33	.142	.93	.32	.42
	2	1.97	2.08	1.84	1.51	.166	.78	.41	.59
	3	1.77	1.95	1.61	1.24	.138	.72	.16	.35
	Mean	1.78	2.00	1.72	1.38	.071[d]	.71	.04	.09
Propionic acid, % DMB	0	.091	.080	ND[c]	.080	.0166	.59	.06	.36
	1	.097	.076	ND	.061	.0169	.49	.10	.19
	2	.108	.076	.021	.045	.0193	.91	.20	.51
	3	.093	.080	ND	.047	.0183	.63	.12	.40
	Mean	.098	.078	.005	.048	.008[d]	.47	.01	.06
Butyric acid, % DMB	0	.39	.12	.09	.66	.143	.66	.71	.23
	1	.64	.13	.07	.72	.129	.74	.96	.03
	2	.59	.36	.27	.71	.136	.75	.96	.34
	3	.47	.16	.18	.61	.134	.85	.82	.28
	Mean	.53	.19	.15	.67	.065[d]	.51	.70	.01

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]DMB = Dry matter basis.

[c]ND = Not detectable.

[d]Standard error of the overall treatment mean is based on 8 observations per treatment.

deterioration process due to an α-amylase effect (P>.66) or a sorbic acid effect (P>.71). However, they were numerically higher in the control silage and the silage treated with both manipulators at all times during the study. In addition, on day 1 of deterioration there was a significantly higher (P<.03) butyric acid concentration due to an interaction effect than in the single manipulator-treated silages (.68 vs .10%).

As during the fermentation study, isobutyric acid concentrations of silages treated with sorbic acid were significantly higher on each day of the study (Table 8). Similarly, Ohyama et al. (1975) demonstrated that no aerobic deterioration occurred in silages which contained more than .5% of butyric and isobutyric acid combined. In the same study, however, they noted that losses of water-soluble carbohydrates and organic acids during aerobic deterioration were higher in the silages with higher water-soluble carbohydrate contents. In the present study, silages that had been treated with sorbic acid at ensiling had higher water-soluble carbohydrate and butyric acid contents. Treatment of corn green chop with α-amylase during ensiling did not affect (P>.18) isobutyric acid concentration at any time during the study.

Temperature (C) changes of the corn silages at 12-hour intervals during the deterioration study are presented in Table 9. Ohyama et al. (1977) considered an increase in

Table 8. ISOBUTYRIC ACID IN CORN SILAGE AT VARIOUS TIMES DURING AEROBIC DETERIORATION AS AFFECTED BY FERMENTATION MANIPULATORS

	Time of deterioration, d	Corn silage					Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
Isobutyric acid, % DMB[b]	0	.21	.13	.31	.26	.029	.18	.05	.78
	1	.19	.16	.30	.37	.033	.48	.01	.11
	2	.20	.22	.47	.40	.043	.26	.01	.05
	3	.23	.21	.43	.38	.039	.51	.02	.80
	Mean	.21	.18	.38	.35	.019[c]	.16	.01	.93

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]DMB = Dry matter basis.

[c]Standard error of the overall treatment mean is based on 8 observations per treatment.

Table 9. TEMPERATURE (C) CHANGES OF CORN SILAGE DURING AEROBIC DETERIORATION AS AFFECTED BY FERMENTATION MANIPULATORS

Time of deterioration, h	Ambient temperature	Corn silage				SE[b]	Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid		Amylase effect	Sorbic acid effect	Interaction
0	26.9	22.6	22.5	22.7	22.3	.08	.34	.93	.25
12	32.1	25.9	25.6	25.6	25.8	.18	.79	.67	.21
24	29.9	27.9	27.3	27.2	27.2	.26	.24	.15	.28
36	32.8	28.3	27.8	27.7	27.6	.27	.44	.23	.48
48	29.6	28.2	28.0	27.7	27.2	.30	.48	.23	.81
60	31.4	27.2	27.2	26.8	26.5	.27	.83	.26	.70
72	28.8	27.0	27.8	26.9	26.2	.32	.95	.24	.31
Mean	30.2	26.8	26.6	26.4	26.1	.26[c]	.22	.01	.51

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Standard error of the treatment mean is based on two observations per treatment.

[c]Standard error of the overall treatment mean is based on 14 observations per treatment.

temperature to be a good determinant of aerobic deterioration. Temperature readings of the four experimental silages throughout the entire study remained below the ambient temperatures at each of the reading times. The mean temperature of the silages treated with sorbic acid, over the 72-hour study, was .45 C lower than that of the silages not treated with sorbic acid (26.25 vs 26.70 C). Ohyama and Hara (1975) reported that the process of aerobic deterioration of silage was due mainly to the action of lactic acid-assimilating yeasts and molds, which results in production of heat. Woolford (1984) has indicated that bacteria may be involved in the aerobic deterioration of silage, also producing heat. Results of the present study indicate that treatment with sorbic acid resulted in decreased metabolism of lactic acid and therefore less heat was produced.

Table 10 contains the counts of colony forming units, expressed as log counts per gram of wet silage, of the total aerobes and yeasts and molds associated with the silages at various times during the progression of the aerobic deterioration process. Log counts of both total aerobic microorganisms, and of yeasts and molds clearly indicate that sorbic acid treatment of silages was effective in inhibiting aerobic microbial degradation. Sorbic acid treatment of corn silages resulted in significant decreases in the log counts of all aerobic microorganisms at each time counts were taken (day 0, $P<.01$; day 2, $P<.06$; day 3, $P<.01$). Alli et

Table 10. AEROBIC MICROORGANISMS ASSOCIATED WITH CORN SILAGE DURING AEROBIC DETERIORATION AS AFFECTED BY FERMENTATION MANIPULATORS

Microorganisms	Time of deterioration, d	Corn silage				SE	Significance level for contrasts[a]		
		Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid		Amylase effect	Sorbic acid effect	Interaction
No. observations/ treatment		2	2	2	2				
Total aerobes, log counts/g wet silage	0	7.05	5.87	3.80	3.72	.573	.38	.01	.32
	2	7.80	8.52	5.90	5.59	.581	.83	.06	.60
	3	7.44	7.35	6.06	3.89	.584	.10	.01	.12
Yeasts and molds, log counts/g wet silage[b]	0	3.50	<2.00	<2.00	<2.00				
	2	6.00	7.00	<3.00	<3.00				
	3	<3.00	7.28	<3.00	<3.00				

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Statistical analyses were not conducted on yeast and mold counts since most values were estimated.

al. (1985) also showed that treatment of corn silage with sorbic acid reduced the population of yeasts and molds during a 48-hour period of exposure to air. Alpha-amylase treatment of corn silages did not exhibit an effect on total aerobe numbers at day 0 ($P>.38$) or day 2 ($P>.83$) of deterioration. By the third day of the deterioration study, however, the effect of α-amylase treatment of corn silages was significant ($P<.10$) in lowering the counts of aerobic microorganisms. This was primarily due to the very low log count of the α-amylase/sorbic acid-treated silage (3.89).

Statistical analyses were not conducted on yeast and mold counts since most values were estimated; however, some inferences can be made from the actual and estimated counts. The log counts of yeasts and molds of all three treated silages were lower than those of the control (<2.00 vs 3.50) on day 0. On day 2 of the study, yeast and mold log counts increased to 6.00 in the control silage and 7.00 in the silage treated only with α-amylase. Log counts of yeasts and molds on day 2 remained relatively stable in silages treated with sorbic acid (<3.00).

By day 3 of the study the two silages that had been treated with sorbic acid at ensiling had the same estimated log counts as they had on day 2 (<3.00). The log counts of the α-amylase-treated silage had increased to 7.28, while the control count appears to have decreased from 6.00 to <3.00, though this seems unlikely.

The higher initial water-soluble carbohydrate content of the silages treated with sorbic acid should have left them more susceptible to aerobic deterioration (Ohyama et al., 1975). This was not the situation in this study. Still unclear is whether the silages treated with sorbic acid were more stable because of the increased butyric and isobutyric acids they contained or because of continued sorbic acid inhibition of aerobic microorganisms during aerobic deterioration.

Table 12 contains simple correlation coefficients between parameters of the fermentation and aerobic deterioration studies. Of interest was the positive correlation between the butyric acid ($r=.919$, $P<.10$) and isobutyric acid ($r=.997$, $P<.05$) contents of a silage during the fermentation study and the water-soluble carbohydrate content of the aerobically deteriorating silage, indicating once again butyric acid's ability to stabilize a silage during aerobic deterioration (Ohyama and Hara, 1975; Henderson et al., 1979).

The content of butyric acid present in silages during the fermentation study was positively correlated ($r=.970$, $P<.10$) with the amount of lactic acid present in a silage during the aerobic deterioration study.

Dry Matter Losses

The effect of corn silage fermentation manipulators on dry matter losses from silos with a 9-ton capacity are

presented in Table 11. Dry matter lost during ensiling was not affected by α-amylase addition (P>.51) or sorbic acid addition (P>.95). The average dry matter loss during ensiling was 9.7% and ranged from a low of 9.1% for the sorbic acid-treated silage to a high of 10.3% in the α-amylase-treated silage.

Dry matter lost due to spoilage was significantly decreased (P<.07) when a silage was treated with sorbic acid as compared to the other two treatments (5.7 vs 7.5%). Treatment of silages with α-amylase had no effect (P>.28) on spoilage dry matter loss.

Total dry matter lost was obtained by the addition of the two previous loss figures. Total dry matter recovery was improved (P>.23) by addition of sorbic acid (84.8 vs 82.4%). Silage treated with α-amylase resulted in only slight improvement in dry matter recovery (P>.79). Bolsen and Ilg (1980) were able to improve the dry matter recovery of corn silage by treatment with α-amylase (87.4 vs 93.7%).

When the correlations between total dry matter recovery and the parameters of the other two studies were determined (Table 12), there was an inexplicable positive correlation between the pH of both the fermentation study (r=.917, P<.10) and aerobic deterioration study (r=.918, P<.10) with dry matter recovery. Better understood is the negative correlation between the butyric acid content of a silage during the fermentation study and dry matter recovery (r=-.942, P<.10).

Table 11. EFFECT OF CORN SILAGE FERMENTATION MANIPULATORS ON DRY MATTER RECOVERY, % (3-TRIAL MEANS)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE[b]	Amylase effect	Sorbic acid effect	Interaction
Dry matter lost during ensiling	9.8	10.3	9.1	9.8	.58	.51	.95	.71
Dry matter lost due to spoilage	8.9	6.2	5.7	5.7	.61	.28	.07	.27
Total dry matter lost	18.7	16.5	14.8	15.5	.95	.79	.23	.33
Total dry matter recovery	81.3	83.5	85.2	84.5	.95	.79	.23	.33

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Standard error of the treatment mean is based on five observations per treatment.

Table 12. CORRELATION COEFFICIENTS OF SELECTED FERMENTION STUDY, AEROBIC DETERIORATION STUDY AND DRY MATTER RECOVERY PARAMETERS

	Fermentation study					Aerobic deterioration study					
	Water-soluble carbohyd.	pH	Lactic acid	Butyric acid	Iso-butyric acid	Water-soluble carbohyd.	pH	Lactic acid	Butyric acid	Iso-butyric acid	Dry matter recovery
Fermentation study											
Water-soluble carbohyd.	1.000	-.971*	-.218	.951*	.988*	.977*	-.693	.920*	.049	.998*	-.804
pH	-.971*	1.000	.410	-.987*	-.980*	-.966*	.787	-.928*	.034	-.962*	.917†
Lactic acid	-.218	.410	1.000	-.347	-.356	-.380	.259	-.131	-.213	-.212	.560
Butyric acid	.951*	-.987*	-.347	1.000	.944†	.919†	-.870	.970†	-.189	.935†	-.942†
Isobutyric acid	.988*	.980*	-.356	.944†	1.000	.997*	-.657	.875	.138	.988*	-.824
Aerobic deterioration study											
Water-soluble carbohyd.	.977*	-.966*	-.380	.919†	.997*	1.000	-.605	.837	.210	.981†	-.795
pH	-.693	.787	.259	-.870	-.657	-.605	1.000	-.905†	.638	-.654	.918†
Lactic acid	.920†	-.928†	-.131	.970*	.875	.837	-.905†	1.000	-.346	.899	-.883
Butyric acid	.049	.034	-.213	-.189	.138	.210	.638	-.346	1.000	.099	.313
Isobutyric acid	.998*	-.962*	-.212	.935†	.988*	.981*	-.654	.899	.099	1.000	-.777
Dry matter recovery	-.804	.917†	.560	-.942†	-.824	-.795	.918†	-.883	.313	-.777	1.000

*Significant P<.05.

†Significant P<.10.

Butyric acid production is considered an indication of an undesirable fermentation.

The results obtained from these experiments indicate that sorbic acid treatment of corn silage at ensiling improves the retention of water-soluble carbohydrates during both fermentation and aerobic deterioration. In addition, silages treated with sorbic acid appear to be more stable in the presence of oxygen as evidenced by decreased temperature, decreased total aerobic microorganisms and yeast and mold counts as determinants.

These data also indicate that generalizations cannot be made in determining the aerobic stability or instability of a silage based on a single factor obtained during either fermentation or aerobic deterioration. The relationship between fermentation and aerobic deterioration may depend more on the interaction of several factors other than just chemical factors.

Bolsen and Hinds (1984) noted that ruminants fed silages which had a lower loss of nutrients do not necessarily perform better. This indicates that evaluation of a silage is more complex than simple measurement of several constituents. The interaction between amount of nutrients retained in a silage and animal performance when fed that silage is also important.

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CHAPTER III

EFFECT OF MANIPULATION OF SILAGE FERMENTATION DYNAMICS ON RUMEN MICROBIAL ACTIVITY AND THE RELATION BETWEEN MICROBIAL ACTIVITY AND CATTLE PERFORMANCE

Abstract

Beef cattle were fed corn silages produced by altered fermentations using α-amylase, sorbic acid or both in a 2 x 2 factorial arrangement. The effect of these corn silages on rumen microbial activity was estimated by measuring in vitro gas production and the relationship between microbial activity and cattle performance was determined. Microbes from cattle fed α-amylase-treated silages produced significantly more (P<.09) gas in vitro compared to those from cattle fed control and sorbic acid-treated silages (14.6 vs 13.0 μl/g/min). Sorbic acid addition to corn silage increased (P>.16) gas production from 13.2 to 14.4 μl/g/min. Interaction between the two manipulators was not significant (P>.46). Therefore, the two effects were additive and gas production increased to 15.5 μl/g/min.

Heifers fed silages treated with α-amylase gained .09 kg per day more (P<.03) than heifers fed silages which were not treated with α-amylase. Sorbic acid treatment of corn silages resulted in no significant effect (P>.59) on heifers' average daily gains. Carcass data of heifers fed α-amylase-treated silages showed increased fat deposition. Gas

production was positively correlated (P>.42) with average daily gain of heifers during the silage-feeding phase. In addition, in vitro gas production was significantly positively correlated with marbling score (P<.01), carcass grade (P<.04) and yield grade (P<.10).

Introduction

The nutritional benefits to ruminants of products from rumen microbial fermentations have been known for several decades. Volatile fatty acids produced by rumen microorganisms serve as energy sources for the ruminant animal. In addition, as these microorganisms grow and multiply, microbial protein is being synthesized, which later is digested and absorbed post-ruminally and serves as an important energy source for the ruminant animal. Therefore, anything which changes rumen microbial activity could affect the production of nutritionally beneficial endproducts either positively or negatively, depending on the microorganisms affected.

The silage fermentation process can be manipulated by fermentation stimulants, such as enzymes (Henderson and McDonald, 1977; LaBarbera et al., 1980; Bolsen et al., 1980) and microbial inoculants (O'Leary and Bull, 1977; McCullough, 1975; Leahy et al. 1981) or fermentation inhibitors, such as organic acids (Waldo et al., 1971; Britt et al. 1975; Yu and Thomas, 1975) and inorganic acids (Virtanen, 1938) with the expectation of stimulating desirable and/or inhibiting

undesirable microbial fermentations and thus improving the quality and/or quantity of the silage produced. The resulting endproducts of these altered fermentations, as well as residual amounts of the original additive, could affect the activity of microbes by altering their growth and reproduction.

Among the methods that measure or estimate microbial growth is the one proposed by el-Shazly and Hungate (1965) which measures in vitro gas production and uses this measurement to calculate rumen microbial growth. Using a slightly modified method, el-Din and el-Shazly (1969a) found that there was significant positive correlation between gas production and colony counts of viable rumen bacteria. Thus, gas production was considered to be a good predictor of total microbial activity.

The primary objective of this study was to estimate the effect of feeding silages produced by altered fermentations on rumen microbial activity of heifers. Microbial activity was estimated by measuring in vitro gas production using rumen fluid obtained from heifers fed the altered silages. The present research differed markedly from others in that gas production of a large number of samples, rather than just a few, was measured in rapid succession. In addition, in vitro gas production was related to growth and body composition parameters of the heifers fed these altered silages.

Experimental Procedure

Cattle Feedlot Trial

The experiment was carried out using 40 Angus beef heifers which were part of a feedlot trial. Ten heifers were allotted to four experimental silage treatments with two replicate lots (five heifers per lot). The feedlot trial consisted of a silage-feeding phase which was 82 days in length. This was followed by the finishing phase (105 days) after which the animals were slaughtered and carcass data were obtained.

The four experimental silages produced were: 1) a control corn silage which received no treatment; 2) corn silage treated with α-amylase, an enzyme which acts as a fermentation stimulant at the time of ensiling; 3) corn silage treated with sorbic acid, a selective fermentation inhibitor, specifically of yeasts and molds; and 4) corn silage treated with both α-amylase and sorbic acid. The green chop materials were ensiled for a minimum of 173 days before they were fed. The treatments were arranged as a 2 x 2 factorial using two factors (α-amylase and sorbic acid) added at two levels each. Alpha-amylase was added at the levels of 0 and .05% on a wet weight basis and sorbic acid was added at 0 and .10% on a wet weight basis. The heifers had been fed their respective experimental silages for a period of 78 days when in vitro gas production was deter-

mined. Rumen fluid samples were collected between 1.5 and 5.0 hours after the morning feeding.

Sampling Procedure

Two rumen fluid samples were collected from each of the experimental animals. They were obtained using a stainless steel strainer connected to tygon tubing which was similar to that developed by Raun and Burroughs (1962). The strainer and tube were placed into a heifer's rumen per os. A 50-ml rumen fluid sample was obtained and divided into two replicate 20-ml subsamples for in vitro gas production determination.

Gas Production Apparatus

In vitro gas production was measured using an adaptation of the manometric method developed by Hungate (1956) and modified by el-Din and el-Shazly (1969a). Figure 1 is a sketch of the gas production apparatus which consisted of a 40 C water bath, a 250-ml glass jar, a rubber stopper with a hypodermic needle pierced through it, tygon tubing and a 10-cc glass syringe.

The 250-ml glass jars had openings which could be completely sealed by the rubber stoppers. The hypodermic needle pierced through the stoppers allowed for the release of gas from the jar into the tygon tubing which delivered the gas to the graduated 10-cc glass syringe where it could be quantified. Glass syringes with individually fitted plungers

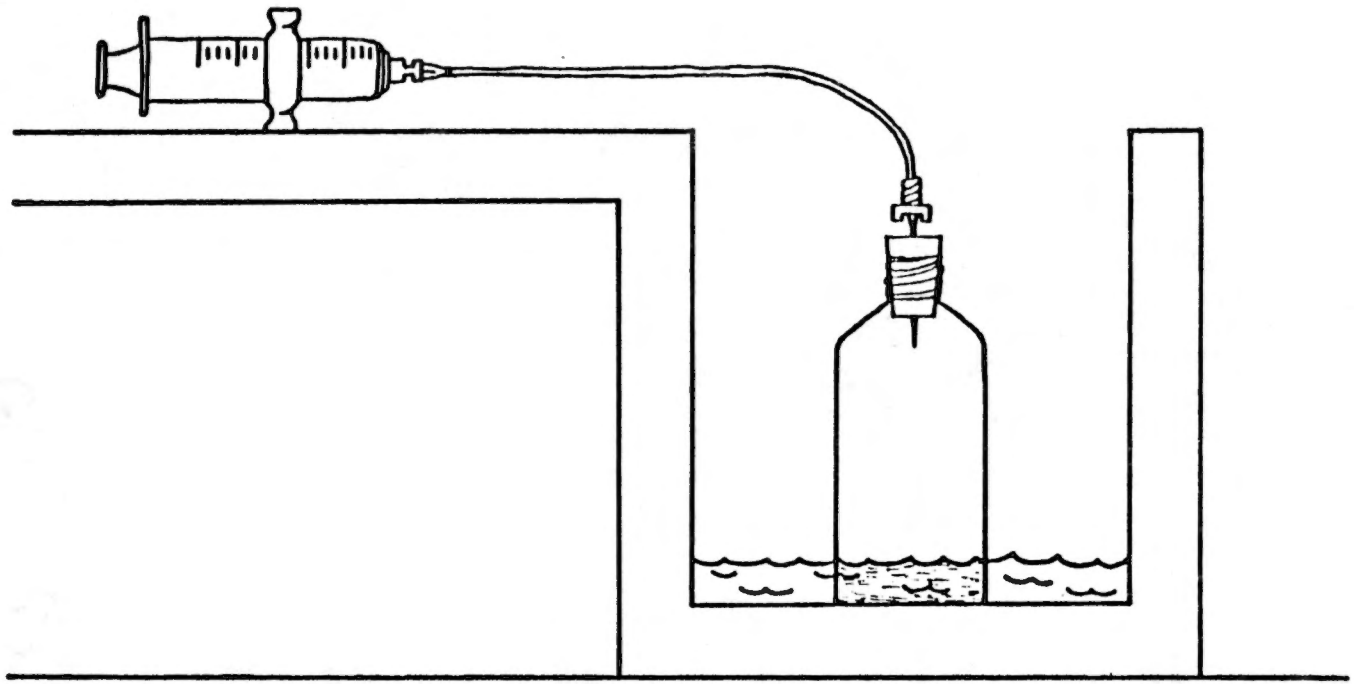

Figure 1. Gas production apparatus.

were used because of their low resistance of plunger movement. Just before a syringe and plunger were attached to the system, both were placed into a tray of soapy water because the glass plunger moved more freely within the syringe when lubricated in this manner; also, leaks between the syringe and plunger would cause bubbling, thereby alerting the operator.

Each glass jar contained .5 grams of ground alfalfa hay as substrate for the microbes in the rumen fluid samples. Just prior to collection of a rumen fluid sample each jar containing the alfalfa hay substrate was pre-warmed in the 40 C water bath; 20-ml rumen fluid sample was added; and carbon dioxide was blown into the jar to force out the air to create an anaerobic environment. The jar was immediately sealed with the rubber stopper and the unattached end of the tygon tubing was quickly attached to the 10-cc glass syringe and a closed system was obtained. The entire process, beginning with the collection of the rumen fluid and ending with the creation of the closed in vitro system, required approximately five minutes.

As soon as the system was closed, the contents of the jars were gently shaken to mix the alfalfa hay substrate with the rumen fluid and designated as "time 0." It was observed that immediately on attaching the end of the tygon tubing to the glass syringe gas rapidly entered the syringe. This gas was considered not produced as a result of instantaneous

metabolism by the microorganisms but more likely, it was a result of the increased pressure built up in the glass jar due to the immediate sealing of the jar after carbon dioxide gas had been blown into it. Therefore, this "time 0" measurement was deducted from the subsequent 30-minute measurement to determine the actual amount of gas produced as a result of microbial activity after the 30-minute incubation period.

Statistical Analysis

El-Din and el-Shazly (1969b) reported that length of time between feeding of the animals and rumen fluid sample collection influenced in vitro gas production. Therefore, time after feeding as a covariate was included in the statistical model. Since time was found not to have a significant ($P>.54$) effect, it was not included in the final model.

Differences in gas production due to treatment were determined by an analysis of variance. Heifer was the experimental unit. The α-amylase main effect, sorbic acid main effect and interaction effect of the 2 x 2 factorial arrangement were tested using contrast statements (SAS, 1985).

Average daily gains and carcass data of heifers were also analyzed to determine differences due to treatment by an analysis of variance. A lot (pen) of five heifers was the experimental unit. Main effects and the effect due to the

interaction of the two manipulators were tested using contrast statements (SAS, 1985).

Simple correlation coefficients (r), and their probability levels (P>|r|), between in vitro gas production and average daily gain and carcass data were also calculated. Probability levels of P<.10 were considered to be significant while levels of P>.10 were considered not to be significant.

Results and Discussion

In vitro gas production using rumen fluid obtained from the heifers fed the four corn silage treatments are presented in Table 1. The data analyzed as a 2 x 2 factorial indicate that in vitro gas production from rumen fluid of heifers fed silages treated with α-amylase was significantly higher (P<.09) than that of heifers fed silages without α-amylase (14.6 vs 13.0 μl/g/min). Sorbic acid addition to corn silage increased in vitro gas production and approached significance (P>.16). The effect of the interaction between α-amylase and sorbic acid was not significant (P>.46); therefore, there was an additive effect between the two as observed by the increase in gas production to 15.5 μl/g/min.

Hungate (1956) considered increased in vitro gas production to be a beneficial effect because it indicates increased microbial activity which results in increased production of microbial protein and volatile fatty acids. Therefore, using gas production as an indicator of microbial

Table 1. IN VITRO GAS PRODUCTION USING RUMEN FLUID OBTAINED FROM HEIFERS FED CORN SILAGE OF ALTERED FERMENTATIONS

Corn silage	Number of observations	Gas Production µl/g/min	Significance level for contrasts[a]
Control	10	12.7	
+α-Amylase	10	13.6	.09
+Sorbic acid	8	13.3	.16
+α-Amylase & sorbic acid	8	15.5	.46
SE[b]		.41	

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase effect, sorbic acid effect and interaction of α-amylase and sorbic acid are presented.

[b]Standard error of the mean.

activity, it appears that the addition of either manipulator was beneficial.

Table 2 contains the average daily gain and carcass data of the cattle. All three of the treated silages resulted in at least a numerical improvement in average daily gain during the silage-feeding phase when compared to the control.

Heifers fed the silages treated with α-amylase gained significantly more during the silage-feeding phase ($P<.03$) and when both phases were combined ($P<.08$) compared to heifers fed the silages not treated with α-amylase. During the full-feeding phase there was no improvement in average daily gain due to the treatments.

Heifers fed silages treated with α-amylase had significantly greater ($P<.01$) hot carcass weights which most probably was due to increased fat deposition in these carcasses.

The treatment with α-amylase either significantly or numerically improved several carcass parameters related to the degree of fat deposition. For example, backfat thicknesses on these heifers averaged 11.4 mm, which was greater ($P>.15$) than those of heifers fed silages not treated with α-amylase 10.5 mm). Marbling scores were improved ($P>.19$) in heifers fed the α-amylase-treated silages and kidney fat was significantly improved ($P<.03$) in the cattle.

The carcass grade of heifers fed α-amylase was improved ($P<.07$) by more than half a grade on the average, and yield

Table 2. AVERAGE DAILY GAIN AND CARCASS DATA OF HEIFERS FED CORN SILAGES OF ALTERED FERMENTATIONS

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. lots/treatment	2	2	2	2				
Average daily gain, kg								
Silage-feeding phase	.75	.85	.78	.85	.020	.03	.59	.65
Full-feeding phase	.80	.84	.71	.78	.025	.31	.21	.78
Combined phases	.78	.84	.75	.82	.018	.08	.46	.88
Carcass data								
Hot carcass weight, kg	212	223	209	222	2.4	.01	.48	.83
Backfat thickness, mm	10.4	11.4	10.6	11.4	.011	.15	.99	.68
Marbling score[b]	4.7	5.0	4.8	5.2	.10	.19	.44	.91
Kidney fat, %	2.0	2.4	2.0	2.3	.08	.03	.55	.84
Loineye area, cm^2	27.6	28.5	27.6	26.9	.33	.86	.46	.76
Carcass grade[c]	11.5	12.5	11.7	12.0	.13	.07	.91	.35
Yield grade	2.1	2.4	2.2	2.5	.08	.07	.46	.76

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase effect, sorbic acid effect and interaction of α-amylase and sorbic acid are presented.

[b]Slight=4.0; small=5.0.

[c]High good=11.0; low choice=12.0.

grade, which accounts for the market value of a carcass was significantly improved ($P<.07$) from 2.15 to 2.45.

Simple correlation coefficients (r) between in vitro gas production and average daily gain and carcass parameters are included in Table 3. All growth parameters and measures, including degree of carcass fatness (backfat, marbling, kidney fat, carcass grade and yield grade), were positively correlated with in vitro gas production, and loineye area was negatively correlated. Though a positive correlation existed between gas production and average daily gain for each phase separately and when both phases were combined, these correlations were not significant ($P>.42$). Hungate (1956) obtained comparable results. He observed that when one ration had a greater rate of fermentation (gas production) than another there was an associated increase in weight gain in steers. Several of these parameters related to the fat content were significant (marbling score, $P<.01$; carcass grade, $P<.04$; and yield grade, $P<.10$). In addition, kidney fat correlation with gas production approached significance ($P>.12$).

These data indicate that the type of fermentation which occurs during the ensiling of corn green chop can result in changes in rumen microbial activity when these silages are fed to heifers. The treatment of corn green chop with the fermentation stimulant, α-amylase, and/or the fermentation inhibitor, sorbic acid, resulted in silages of different composition. It may be hypothesized that these altered

Table 3. CORRELATION (r) BETWEEN IN VITRO GAS PRODUCTION AND AVERAGE DAILY GAIN AND CARCASS CHARACTERISTICS OF HEIFERS

Gas production vs.	Correlation	P>|r|
Average daily gain		
Silage-feeding phase	.33	.42
Full-feeding phase	.06	.89
Combined phases	.25	.55
Hot carcass weight	.39	.33
Backfat thickness	.21	.62
Marbling score	.91	.01
Kidney fat	.59	.12
Loineye area	-.45	.26
Carcass grade	.72	.04
Yield grade	.62	.10

silages, when fed to beef cattle, resulted in changes in the rumen microbial population (numbers, genera and profile). These changes resulted in an increase in VFAs and microbial protein which ultimately was responsible for improved animal growth and increased fat deposition. The positive correlation between in vitro gas production and animal performance and carcass fat parameters suggests the use of the measurement of gas production as a means of predicting animal and carcass effects in response to corn silage feeding.

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CHAPTER IV

EFFECT OF TREATING CORN SILAGE WITH ALPHA-AMYLASE AND/OR SORBIC ACID ON BEEF CATTLE GROWTH AND CARCASS CHARACTERISTICS

Abstract

The effects of feeding silages produced by altered fermentations on beef cattle performance and carcass characteristics were determined. Alpha-amylase was added at 0 or .05% (wet basis) and sorbic acid was added at 0 or .10% (wet basis) to corn green chop before ensiling in a 2 x 2 factorial arrangement.

Three feedlot trials were conducted using Angus heifers of the Select grade which had mean initial body weights of approximately 213, 232 and 228kg for trials 1, 2 and 3, respectively. The trials consisted of two phases each, a silage-feeding phase averaging 85 days and a subsequent finishing phase averaging 111 days in length. When heifers attained an average estimated backfat thickness over the 13th rib of 12 mm, they were slaughtered and carcass data were obtained. Data from the finishing phase and carcass data from the first two trials only are presented.

Silages treated with α-amylase had a slightly lower percentage of crude fiber ($P<.08$) and a slightly higher percentage of nitrogen-free extract ($P<.09$) than silages that had not been treated with α-amylase. Silages treated with

sorbic acid had significantly lower (P<.03) percentages of acid-detergent fiber nitrogen.

During the silage-feeding phase, heifers fed silages treated with α-amylase gained significantly more (P<.01) than heifers fed the other two treatments (71 vs 67 kg). In addition, they were significantly more efficient (P<.01) in gaining weight per unit of feed consumed (.146 vs .137). During the finishing phase, heifers that had previously been fed the α-amylase-treated silage continued to gain well, as exhibited by their higher (P<.01) final weights; however, though gains of these heifers were greater, they were not significantly different (P>.16) from those not previously fed α-amylase-treated silage.

For the entire trial, the α-amylase treatment resulted in a significantly greater (P<.03) average daily gain among heifers of .06 kg per day. The percentage of kidney fat on the carcass of heifers from the α-amylase treatments was significantly increased (P<.01). There were no clear-cut results due to main effects regarding lean or fat deposition. The results of this study indicate that fermentation manipulation of corn silage with the enzyme fermentation stimulant α-amylase did improve gain and feed efficiency of beef heifers fed the treated silage.

Introduction

McDonald (1981) suggested the use of the terms "fermentation stimulants" and "fermentation inhibitors" as the two major classes of silage fermentation manipulators. Fermentation stimulants such as bacterial inoculants, nutrients and enzymes are added to silage in order to increase microbial production of lactic acid which preserves the silage. Fermentation inhibitors, including organic and inorganic acids, have been added to silage to quickly lower the pH, creating an environment which inhibits most microbial activity. The ultimate goal of the silage-making process is the production of a product with a minimum amount of nutrient loss and, when fed, improvement in animal performance. A summary of feedlot trials, in which cattle were fed corn silages produced using fermentation stimulants, showed minimal treatment effects on gains or feed efficiencies (Bolsen and Hinds, 1984). While addition of fermentation inhibitors to hay-crop silages have generally resulted in improved average daily gains in growing cattle, inhibitors added to corn silage have yielded variable results. Huber and Soejono (1976) suggested that the use of propionic acid would be preferable to formic or acetic acid as a silage fermentation inhibitor in that it improved nutrient recovery and animal performance. In contrast, Leahy et al. (1988) reported that the fermentation inhibitor, sodium diacetate,

did not improve average daily gain or feed efficiency of cattle fed corn silage.

McDonald and Whittenbury (1973) suggested that addition of a fermentation stimulant like α-amylase could result in a more effective fermentation since starch was the primary carbohydrate in cereal grains, and since lactic acid bacteria are unable to utilize starch. Bolsen and Ilg (1980a,b) tested a commercial product which contained α-amylase and found that it resulted in improved fermentation in both sorghum and corn silages. Addition of α-amylase to corn silage resulted in slightly higher average daily gains when fed to cattle (Bolsen and Ilg, 1980b).

When sorbic acid, a selective inhibitor of yeasts and molds, was added to corn green chop, it resulted in reduced surface spoilage (Salunkhe, 1956; Alli et al., 1985). Alli et al. (1985) noted that sorbic acid-treated corn silage was also more aerobically stable. A palatability test using yearling heifers indicated that hay-crop silages treated with sorbic acid were not as well accepted as an untreated silage (Shearer and Cordukes, 1962).

Theoretically the addition of the fermentation stimulant, α-amylase, along with the selective inhibitor, sorbic acid, could have an additive effect in preserving a silage during fermentation, and protecting it from aerobic deterioration when exposed to oxygen. Thus, the nutritive value of the resulting silage to cattle could be improved. In the

present experiments, feedlot trials were conducted to study the effect of feeding silages produced by altered fermentations on animal growth and carcass parameters. Fermentations were manipulated by adding α-amylase, sorbic acid or both together to fresh corn green chop prior to ensiling.

Experimental Procedure

Three feedlot trials were conducted in consecutive years. Trials 1 and 2 consisted of two phases, a silage-feeding phase and a finishing phase. Trial 3 consisted of only the silage-feeding phase.

During the silage-feeding phase, heifers were fed one of four experimental silages ad libitum. They were: 1) an untreated corn silage; 2) corn green chop treated with .05% α-amylase on a wet basis before ensiling; 3) corn green chop treated with .10% sorbic acid on a wet basis; and 4) corn silage treated with both α-amylase and sorbic acid at the aforementioned rates. In addition, heifers received .85 kg of grass hay and .54 kg of (44% crude protein) soybean meal which met NRC protein requirements for growth. The silage-feeding phase lasted for 72, 82 and 100 days, respectively.

The finishing phase began with a transition period of about 10 days during which the proportion of silage decreased while the proportion of concentrate in the ration increased. The heifers were finished on corn-and-cob meal ad libitum, .90 kg of hay daily and .48 kg of soybean meal daily which

met NRC (1984) requirements. The trials ended when heifers reached an average thickness over the 13th rib of 12 mm, which was estimated using a Scanco Scanprobe II somascope (Model # 7310). The finishing period lasted for 118 and 105 days, respectively. Animals were then slaughtered and carcass data were obtained.

Experimental Silages

Pioneer Hybrid 3147 variety of corn was seeded at the rate of 54,000 kernels per hectare. The corn plants were produced on various silt loams with slopes of 2-5%. The fields were fertilized in the fall with 4,485 kg of lime per hectare. The field the corn was grown in for Trial 1 required 729 kg of 0-20-20 and 493 kg of 34-0-0 fertilizer per hectare. The same field was used to grow corn silage for Trials 2 and 3 and required 779 kg and 902 kg of 8-24-24 fertilizer per hectare before planting, respectively. In addition, 336 kg of 34-0-0 per hectare was side dressed on the field for both trials. The precipitation during the three growing periods was 9.55, 6.62 and 9.46 cm, respectively. In each of the trials, the corn green chop was harvested in the early dent stage.

Eight upright brick silos measuring 1.8 x 9.1 m and holding an average of nine metric tons were used. Two silos were assigned to each of the four experimental treatments. The green chop was allowed to ferment for at least 117, 141 and 124 days respectively, until the first silos were opened.

The green chop samples and composited silage samples of the three trials were analyzed for total dry matter, Proximate Analyses constituents, acid-detergent fiber and acid-detergent fiber-nitrogen (AOAC, 1980).

Experimental Animals

Prior to the beginning of each trial, 45 Angus heifers were purchased at fall graded feeder calf sales. All heifers were of the Select grade and had mean initial body weights of approximately 213, 232 and 228 kg, respectively. Each heifer was administered 25 mg of the abortifacient prostaglandin $F_{2\alpha}$ approximately 30 days prior to the beginning of each trial so that no heifer would be pregnant during the trial. Animals were placed on permanent pastures for about two months prior to the beginning of each trial. In early December, just before the trial began, body weight, frame and muscling were measured and used to allot heifers to eight groups of five animals each. Two groups of heifers were then randomly allotted to each silage treatment.

Prior to the beginning of the silage-feeding phase of each group of heifers was placed in a 6.1 x 7.6 m lot located on both sides of an open-sided feeding barn and fed their respective experimental corn silage ad libitum once daily. At approximately 30-day intervals and at the end of both phases, individual body weights were taken.

Hot carcass weights were obtained at the slaughterhouse. After the carcasses had been chilled for 48 hours, loineye

area, adjusted backfat thickness, percent kidney fat, yield grade and carcass grade were determined by a USDA grader.

Statistical Analyses

Data from the three trials were collected and differences due to treatment were determined by an analysis of variance. The effect due to trial was accounted for when considering treatment differences. Alpha-amylase at 0 or .05% addition of wet weight corn silage and sorbic acid at 0 or .10% addition were used in a 2 x 2 factorial arrangement. Contrasts were made to test the main effects and interaction of α-amylase and sorbic acid (SAS, 1985). In discussing the results, the probability level of $P<.10$ was chosen to delineate between significance and non-significance.

Results and Discussion

Silage Nutrient Composition

Table 1 contains the nutrient composition of each of the four experimental silages as 3-trial means. The average dry matter content of the four treatments was nearly identical, ranging from 31.2% dry matter in α-amylase-treated corn silage to 31.9% in the silage treated with both manipulators.

Nitrogen-free extract percentages of the silages treated with α-amylase were significantly higher ($P<.09$) than silages which were not treated with α-amylase (45.1 vs 43.2%). The percent of crude fiber in silages treated with α-amylase was

Table 1. NUTRIENT COMPOSITION OF EXPERIMENTAL SILAGES, %
(3-TRIAL MEANS)

Component	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE[b]	Amylase effect	Sorbic acid effect	Interaction
Dry matter	31.8	31.2	31.5	31.9	.99	.55	.34	.79
Crude protein[c]	7.8	7.0	7.6	7.6	.16	.18	.55	.21
Crude fiber[c]	41.5	41.2	42.2	39.8	.87	.08	.63	.20
Ether extract[c]	2.4	2.4	2.4	2.5	.07	.36	.70	.71
Ash[c]	5.1	5.0	4.5	4.3	.37	.83	.26	.90
Nitrogen-free extract[c]	43.2	44.4	43.3	45.6	1.16	.09	.42	.47
Acid-detergent fiber[c]	30.6	31.8	30.2	28.5	.86	.82	.11	.19
Acid-detergent fiber nitrogen[c]	7.8	8.3	6.9	7.4	.29	.16	.03	.96

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Standard error of treatment mean is based on 6 observations per treatment.

[c]DMB = Dry matter basis.

less ($P<.08$) than that found in silages not treated with α-amylase, most probably as a function of the higher nitrogen-free extract content. The percent of acid-detergent fiber nitrogen (ADFN) was significantly ($P<.03$) less in silages treated with sorbic acid. ADFN content of a silage is thought to be increased as a result of heating of the ensiled material during storage (Van Soest, 1982). Heat is produced as a result of the activity of yeasts and molds (McDonald, 1981). Since sorbic acid inhibits yeast and mold activity, a decrease in the temperature of the ensiled material probably occurred and thereby resulted in a decrease in ADFN.

Silage-Feeding Phase

Three year means of feed consumption and animal performance of the heifers during the silage-feeding phase are included in Table 2. Silage-feeding phase data of the individual trials are included separately in Appendix Tables A1 through A3.

The average length of the silage-feeding phase was 85 days. The heifers in these three trials consumed an average of 4.34 kg of corn silage daily. Phipps and Wilkinson (1985) estimated that beef cattle weighing approximately 225 kg should consume about 4.50 kg of corn silage daily on a dry matter basis. Significance levels for the corn silage dry matter intake and the total dry matter intake are the same, since hay and soybean meal intake were held at constant levels across all treatments, The addition of the

Table 2. ANIMAL FEED INTAKE, GAIN AND FEED EFFICIENCY DURING THE SILAGE-FEEDING PHASE AS EFFECTED BY FERMENTATION MANIPULATION (3-TRIAL MEANS)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	30	30	30	30				
Days on feed	85	85	85	85				
Average daily feed intake/ heifer, kg								
Corn silage-as fed	13.87	13.98	13.95	13.82	.288	.85	.59	.15
Corn silage-dry matter	4.36	4.31	4.33	4.35	.036	.49	.82	.16
Hay[b]	.85	.85	.85	.85				
Soybean meal[b]	.54	.54	.54	.54				
Total dry matter[c]	5.75	5.70	5.72	5.74	.036	.49	.82	.16
Average weight, gain and feed efficiency								
Initial wt., kg[d]	225	223	224	224	1.8			
Final wt., kg	291	294	292	295	3.1	.13	.65	.94
Total wt. gain, kg	66	71	68	71	1.7	.01	.47	.20
Average daily gain, kg	.78	.84	.80	.84	.010	.01	.47	.20
Gain/feed-dry matter	.134	.147	.140	.146	.002	.01	.45	.11

[a]Significance levels associated with calculate F-statistics are presented from contasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Hay and soybean meal were fed at constant levels across all treatments.

[c]Significance levels for total dry matter intake are the same as those of the corn silage intake on a dry matter basis since hay and soybean meal intakes were held constant.

[d]Initial weights of heifers were used in allotting them to treatment.

fermentation stimulant, α-amylase, did not alter dry matter intake (P>.49). Jaster and Moore (1988) also found no effect on the dry matter intake of heifers fed an alfalfa silage treated with an amylase-cellulase preparation. In some cases, the use of other fermentation stimulants, such as microbial inoculants, in treating corn silage has resulted in improved dry matter intake (Wohlt, 1987; Kennedy et al., 1987).

Addition of sorbic acid, a selective fermentation inhibitor, to corn silage also did not significantly affect dry matter intake. Shearer and Cordukes (1962) noted that free choice palatability tests of a sorbic acid-treated hay-crop silage with an untreated control resulted in a slight preference for the untreated control by yearling heifers. Many researchers have reported that other organic acids, such as formic acid, resulted in increased dry matter intake when used to treat corn silage (Castle and Watson, 1970a,b; Derbyshire et al., 1971; Waldo et al., 1971). In contrast, Hinks et al. (1976) found no effect of formic acid treatment of silage on dry matter intake. Propionic acid has also been used as a fermentation inhibitor in corn silage. Huber and Soejono (1976) reported that propionic acid addition improved corn silage intake above a high dry matter control and dry matter silage with added water. Yu and Thomas (1975), however, found no difference in dry matter consumption between propionic-treated and control silages.

The total and average daily weight gain of heifers during the silage-feeding phase indicate a strong positive effect of α-amylase. Significance levels for total weight gain and ADG are identical, since days on feed were the same in all treatments. Alpha-amylase stimulation of fermentation processes significantly improved ($P<.01$) ADG an average of .05 kg. Similar results were obtained by Bolsen and Ilg (1980b) who reported that average daily gain of cattle fed corn silage treated with α-amylase increased from 1.13 to 1.21 kg above cattle fed the control. Sorbic acid inhibition of fermentation processes resulted in a .01 kg increase ($P>.47$).

Feed efficiency of the heifers is presented as the ratio of gain per unit of dry feed intake. The effect of α-amylase is again evident since feed efficiency improved from an average of .137 to .147 ($P<.01$). Bolsen and Ilg (1982) were also able to improve the gain-to-feed ratio of cattle from .139 to .148 by feeding a corn silage treated with an enzyme additive similar to α-amylase. When an α-amylase preparation was used during the ensiling of forage sorghum and later fed to calves, they were 4.2% more efficient in converting feed to gain than calves fed the control (Bolsen and Hinds, 1984). Heifers fed silages treated with sorbic acid were no more efficient in weight increase per unit of feed intake than those consuming silage which had not been treated with sorbic acid ($P>.45$).

Finishing Phase

Data contained in Table 3 include 2-trial mean feed consumption and animal performance figures of the heifers during the finishing phase. Data of the individual trials are included in Appendix Tables A4 and A5. The average length of time required for the heifers to reach an average estimated backfat thickness of 12 mm was 111 days at which time they were slaughtered. The amounts of corn silage fed during the short change-over period and the amount of hay fed were held constant across all treatments and corn-and-cob meal was fed free choice. Voluntary dry matter intakes were similar among heifers fed the four silage treatments.

Final weights of the heifers indicate that animals which began the finishing phase with higher initial weights, that is, animals fed the α-amylase-treated silage, also ended the phase with higher ($P<.01$) final weights.

The heifers previously fed corn silages treated with α-amylase during the silage-feeding phase continued to have higher ($P>.16$) average daily gains during the finishing phase, also. The gain-to-feed ratio was also improved ($P>.14$) in heifers previously fed α-amylase-treated corn silage.

During the finishing phase, heifers that had been previously fed silages treated with sorbic acid showed no effect on final weight gain, total weight gain, average daily gain or feed efficiency. It was observed, however,

Table 3. ANIMAL FEED INTAKE AND PERFORMANCE DURING THE THE FINISHING PHASE AS EFFECTED BY FERMENTATION MANIPULATION (2-TRIAL MEANS)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	20	20	20	20				
Days on feed	111	111	111	111				
Average daily feed intake/ heifer, kg								
Corn silage - as fed[b]	.89	.89	.89	.90				
Corn silage - dry matter[b]	.25	.25	.25	.25				
Hay[b]	1.23	1.23	1.23	1.23				
Corn grain	5.81	5.78	5.83	5.81	.051	.31	.33	.92
Soybean meal	.49	.47	.49	.49	.007	.21	.25	.25
Total dry matter	7.78	7.73	7.80	7.78	.031	.22	.20	.59
Average weight, gain and feed efficiency								
Initial wt., kg	283	288	284	289	3.5	.08	.72	.89
Final wt., kg	373	381	369	383	2.1	.01	.71	.33
Total wt. gain, kg	90	93	85	94	2.7	.16	.56	.45
Average daily gain, kg	.81	.84	.77	.85	.017	.16	.56	.45
Gain/feed-dry matter	.104	.109	.098	.109	.002	.14	.50	.48

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Corn silage and hay were fed at constant levels across all treatments.

that heifers that had previously been fed silage treated only with sorbic acid had decreases (P>.56) in their total weight gain and average daily gain.

Combined Phases and Carcass Data

The total number of days on experiment, the ADG for the entire experiment (combined silage and finishing phases) and the carcass data of heifers are presented as two-trial means in Table 4. Data of Trials 1 and 2 are presented individually in Appendix Tables A6 and A7. The total number of days on feed of the two trials averaged 188 days.

When data of both phases were combined, heifers fed the α-amylase treated silage gained .06 kg/day more (P<.03) than heifers fed silages not treated with α-amylase.

As expected, the animals with the higher final live weights, those on the α-amylase treatment, had higher (P>.11) hot carcass weights. The percentage of kidney fat on these carcasses was significantly increased (P<.01) .3 percentage units from 1.8 to 2.1 %. The percentage of kidney fat was not related to other measurements of fat in the carcasses.

The results of this study indicate that fermentation manipulation of corn silage can result in changes in animal performance. In part, these differences are probably related to changes in the nutritive quality of the silages produced.

Table 4. CARCASS DATA OF ANIMALS FED SILAGES PRODUCED BY FERMENTATION MANIPULATION (2-TRIAL MEANS)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	20	20	20	20				
Days on silage	77	77	77	77				
Days on full-feed	111	111	111	111				
Total days on experiment	188	188	188	188				
Average daily gain[b]	.79	.84	.78	.85	.013	.03	.96	.88
Carcass data								
Hot carcass weight, kg	211	219	208	214	2.2	.11	.40	.82
Backfat thickness, mm	11.4	11.4	10.7	11.2	.30	.55	.42	.76
Marbling score[c]	4.7	4.9	4.9	5.0	.07	.19	.22	.74
Kidney fat, %	1.9	2.2	1.8	2.1	.05	.01	.19	.87
Loineye area, cm^2	26.0	27.1	26.3	25.7	.32	.65	.37	.13
Carcass grade[d]	11.6	12.0	11.8	11.7	.12	.41	.83	.31
Yield grade	2.5	2.4	2.3	2.5	.05	.27	.57	.12

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Refers to average daily gain for entire trial.

[c]Slight=4.0; small=5.0.

[d]High good=11.0; low choice=12.0.

Addition of the fermentation stimulant α-amylase appeared to result in silages of a somewhat higher nutritive quality, i.e., they had less crude fiber and more nitrogen-free extract than silages which were not treated with α-amylase. Other researchers have suggested that improvement in cattle performance from the use of fermentation stimulants such as α-amylase may be due to their effect of preserving more of the nitrogenous and other constituents of the originally ensiled material (Alli and Baker, 1982). Bolsen and Hinds (1984), however, have noted that, in theory, silage fermentations which result in decreased losses during ensiling would be expected to have a higher nutritional value than a silage with higher losses, because the dry matter lost probably could have been completely digested by the animal. However, in practice they found that in most of the experiments reviewed, silages which had lower ensiling losses due to addition of fermentation stimulants were less digestible.

In a companion study to this experiment, rumen microbial activity, as estimated by in vitro gas production, was increased ($P<.09$) among heifers fed α-amylase-treated silages. It may be hypothesized that silages produced by α-amylase addition resulted in beneficial changes in the rumen microbial population (numbers, genera and profile) which yielded increases in VFA and microbial protein production and which were ultimately responsible for improved animal growth and feed efficiency.

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APPENDIX

Table A1. ANIMAL FEED INTAKE, GAIN AND FEED EFFICIENCY DURING THE SILAGE-FEEDING PHASE AS EFFECTED BY FERMENTATION MANIPULATION (TRIAL 1)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	10	10	10	10				
Days on feed	72	72	72	72				
Average daily feed intake/ heifer, kg								
Corn silage-as fed	14.73	15.11	15.01	14.63	.111	.98	.67	.16
Corn silage-dry matter	4.09	4.16	4.19	4.10	.028	.93	.77	.28
Hay[b]	.85	.85	.85	.85				
Soybean meal[b]	.54	.54	.54	.54				
Total dry matter[c]	5.49	5.55	5.58	5.49	.028	.93	.77	.28
Average weight, gain and feed efficiency								
Initial wt., kg[d]	218	212	211	210	1.3			
Final wt., kg	274	277	272	271	1.4	.74	.22	.50
Total wt gain, kg	56	65	61	61	1.4	.13	.74	.10
Average daily gain, kg	.77	.90	.85	.85	.020	.13	.74	.10
Gain/feed-dry matter	.140	.162	.152	.155	.003	.08	.76	.11

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Hay and soybean meal were fed at constant levels across all treatments.

[c]Significance levels for total dry matter intake are the same as those of the corn silage intake on a dry matter basis since hay and soybean meal intakes were held constant.

[d]Initial weights of heifers were used in allotting them to treatment.

Table A2. ANIMAL FEED INTAKE, GAIN AND FEED EFFICIENCY DURING THE SILAGE-FEEDING PHASE AS EFFECTED BY FERMENTATION MANIPULATION (TRIAL 2)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	10	10	10	10				
Days on feed	82	82	82	82				
Average daily feed intake/ heifer, kg								
Corn silage-as fed	14.92	14.87	14.88	14.87	.010	.14	.23	.21
Corn silage-dry matter	4.38	4.41	4.30	4.30	.018	.05	.01	.02
Hay[b]	.85	.85	.85	.85				
Soybean meal[b]	.54	.54	.54	.54				
Total dry matter[c]	5.77	5.80	5.69	5.69	.018	.05	.01	.02
Average weight, gain and feed efficiency								
Initial wt., kg[d]	230	230	232	236	1.6			
Final wt., kg	292	300	296	307	2.5	.08	.21	.76
Total wt. gain, kg	62	70	64	71	1.6	.03	.59	.65
Average daily gain, kg	.75	.85	.78	.85	.020	.03	.59	.65
Gain/feed-dry matter	.130	.147	.137	.150	.003	.04	.34	.72

[a]Significance levels associated with calculated F-statistics are presented from contrasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Hay and soybean meal were fed at constant levels across all treatments.

[c]Significance levels for total dry matter intake are the same as those of the corn silage intake on a dry matter basis since hay and soybean meal intakes were held constant.

[d]Initial weights of heifers were used in allotting them to treatment.

Table A3. ANIMAL FEED INTAKE, GAIN AND FEED EFFICIENCY DURING THE SILAGE-FEEDING PHASE AS EFFECTED BY FERMENTATION MANIPULATION (TRIAL 3)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	10	10	10	10				
Days on feed	100	100	100	100				
Average daily feed intake/ heifer, kg								
Corn silage-as fed	11.97	11.96	11.96	11.97	.002	.01	.01	.01
Corn silage-dry matter	4.60	4.36	4.50	4.64	.040	.01	.01	.01
Hay[b]	.85	.85	.85	.85				
Soybean meal[b]	.54	.54	.54	.54				
Total dry matter[c]	5.99	5.75	5.89	6.03	.040	.01	.01	.01
Average weight, gain and feed efficiency								
Initial wt., kg[d]	228	227	229	227	1.6			
Final wt., kg	307	305	307	307	.8	.53	.67	.53
Total wt. gain, kg	79	78	78	80	.8	.99	.73	.44
Average daily gain, kg	.79	.78	.78	.80	.009	.99	.73	.44
Gain/feed-dry matter	.132	.136	.132	.133	.001	.72	.80	.65

[a]Significance levels associated with calculate F-statistics are presented from contasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Hay and soybean meal were fed at constant levels across all treatments.

[c]Significance levels for total dry matter intake are the same as those of the corn silage intake on a dry matter basis since hay and soybean meal intakes were held constant.

[d]Initial weights of heifers were used in allotting them to treatment.

Table A4. ANIMAL FEED INTAKE AND PERFORMANCE DURING THE THE FINISHING PHASE AS EFFECTED BY FERMENTATION MANIPULATION (TRIAL 1)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	10	10	10	10				
Days on feed	118	118	118	118				
Average daily feed intake/ heifer, kg								
Corn silage-as fed[b]	1.14	1.15	1.15	1.16				
Corn silage-dry matter[b]	.32	.32	.32	.32				
Hay[b]	1.23	1.23	1.23	1.23				
Corn grain	5.65	5.54	5.61	5.66	.021	.38	.35	.07
Soybean meal	.52	.47	.52	.52	.010	.25	.25	.18
Total dry matter	7.72	7.56	7.68	7.73	.029	.25	.18	.05
Average weight, gain and feed efficiency								
Initial wt., kg	274	277	272	271	1.4	.74	.22	.56
Final wt., kg	370	374	367	376	2.1	.21	.92	.55
Total wt. gain, kg	96	97	95	105	2.6	.37	.56	.45
Average daily gain, kg	.81	.82	.80	.89	.022	.37	.56	.45
Gain/feed-dry matter	.105	.108	.104	.115	.003	.33	.66	.60

[a]Significance levels associated with calculate F-statistics are presented from contasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Corn silage and hay were fed at constant levels across all treatments.

Table A5. ANIMAL FEED INTAKE AND PERFORMANCE DURING THE THE FINISHING PHASE AS EFFECTED BY FERMENTATION MANIPULATION (TRIAL 2)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	10	10	10	10				
Days on feed	105	105	105	105				
Average daily feed intake/ heifer, kg								
Corn silage-as fed[b]	.63	.63	.63	.64				
Corn silage-dry matter[b]	.19	.19	.18	.19				
Hay[b]	1.23	1.23	1.23	1.23				
Corn	5.96	6.02	6.05	5.96	.019	.62	.71	.07
Soybean meal	.46	.46	.46	.46	.001	.67	.67	.09
Total dry matter	7.84	7.90	7.92	7.84	.020	.65	.80	.08
Average weight, gain and feed efficiency								
Initial wt., kg	292	300	296	307	2.5	.08	.20	.76
Final wt., kg	376	388	371	389	3.1	.01	.64	.43
Total wt. gain, kg	84	88	75	82	2.6	.31	.21	.78
Average daily gain, kg	.80	.84	.71	.78	.025	.31	.21	.78
Gain/feed-dry matter	.102	.106	.090	.099	.002	.29	.20	.66

[a]Significance levels associated with calculate F-statistics are presented from contasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Corn silage and hay were fed at constant levels across all treatments.

Table A6. CARCASS DATA OF ANIMALS FED SILAGES PRODUCED BY FERMENTATION MANIPULATION (TRIAL 1)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	10	10	10	10				
Days on silage	72	72	72	72				
Days on full-feed	118	118	118	118				
Total days on experiment	190	190	190	190				
Average daily gain[b]	.80	.85	.82	.88	.019	.21	.52	.95
Carcass data								
Hot carcass weight, kg	209	214	206	206	3.0	.65	.39	.65
Backfat thickness, mm	11.9	11.2	10.4	10.7	.33	.77	.12	.46
Marbling score[c]	4.7	4.9	5.0	4.9	.09	.76	.36	.56
Kidney fat, %	1.9	2.0	1.7	1.8	.06	.48	.19	.94
Loineye area, cm^2	24.4	25.8	25.1	24.6	.43	.63	.77	.27
Carcass grade[d]	11.7	11.9	11.9	11.5	.17	.77	.79	.41
Yield grade	2.7	2.5	2.4	2.5	.07	.95	.34	.25

[a]Significance levels associated with calculate F-statistics are presented from contasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Refers to average daily gain for entire trial.

[c]Slight=4.0; small=5.0.

[d]High good=11.0; low choice=12.0.

Table A7. CARCASS DATA OF ANIMALS FED SILAGES PRODUCED BY FERMENTATION MANIPULATION (TRIAL 2)

	Corn silage					Significance level for contrasts[a]		
	Control	+Amylase	+Sorbic acid	+Amylase & sorbic acid	SE	Amylase effect	Sorbic acid effect	Interaction
No. animals/treatment	10	10	10	10				
Days on silage	82	82	82	82				
Days on full-feed	105	105	105	105				
Total days on experiment	187	187	187	187				
Average daily gain[b]	.78	.84	.75	.82	.018	.08	.46	.88
Carcass data								
Hot carcass weight, kg	212	223	209	222	3.1	.07	.74	.89
Backfat thickness, mm	10.4	11.4	10.7	11.4	.51	.37	.96	.85
Marbling score[c]	4.6	4.9	4.8	5.1	.10	.14	.42	.93
Kidney fat, %	2.0	2.4	1.9	2.3	.07	.01	.59	.88
Loineye area, cm^2	27.6	28.5	27.6	26.9	.36	.89	.31	.29
Carcass grade[d]	11.5	12.2	11.7	12.0	.17	.15	.97	.54
Yield grade	2.3	2.3	2.2	2.5	.06	.07	.78	.30

[a]Significance levels associated with calculate F-statistics are presented from contasts which analyzed the data as a 2 x 2 factorial. Test statistics for the α-amylase main effect, sorbic acid main effect and effect of interaction of the main effects are presented.

[b]Refers to average daily gain for entire trial.

[c]Slight=4.0; small=5.0.

[d]High good=11.0; low choice=12.0.

VITA

Kevin Timothy Leahy was born in Jersey City, New Jersey, on June 18, 1956. Soon after his birth his family relocated to Montville, New Jersey, where he attended primary and secondary schools, the latter being Montville Township High School. He entered Berry College, located in Mount Berry, Georgia, in September 1974 and graduated in the fall of 1978 with a Bachelor of Science degree in both Animal Science and Chemistry. In September 1979, he began graduate studies as a graduate research assistant at The University of Tennessee in Knoxville in the Department of Animal Science. In December 1981, he received a Master of Science degree in Animal Science. Immediately after graduation he was employed with Wayne Feeds Company, Memphis, Tennessee, until he reenrolled at The University of Tennessee in June 1984. In August 1988, he was awarded a Doctor of Philosophy degree in Animal Science with a concentration in Nutrition.