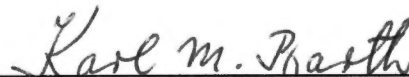


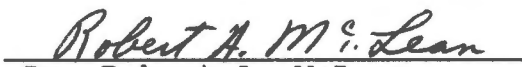
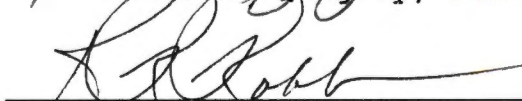
To the Graduate Council:

I am submitting herewith a dissertation written by Sylvia A. Nicklas-Bray entitled "Manipulation of Fermentation Dynamics of Corn Silage with Selected Additives and their Effects on Silage Quality, Digestibility and Animal Performance." I have examined the final 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.



Dr. Karl M. Barth,
Major Professor

We have read this dissertation
and recommend its acceptance:


Dr. P. Michael Davidson
Dr. Robert A. McLean
Dr. James B. Origley, III.
Dr. Kelly R. Robbins

Accepted for the Council:


Vice Provost and
Dean of The Graduate School

MANIPULATION OF FERMENTATION DYNAMICS OF CORN SILAGE
WITH SELECTED ADDITIVES AND THEIR EFFECTS ON
SILAGE QUALITY, DIGESTIBILITY AND
ANIMAL PERFORMANCE

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Sylvia A. Nicklas-Bray

August 1991

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THESIS

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DEDICATION

To my husband and best friend, James Michael Bray,
whose support, understanding, good sense of humor and most
of all love, were essential to successfully complete this
doctorate program.

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ABSTRACT

Study 1. A laboratory-scale experiment with 75-kg steel drums was conducted using sorbic acid (SA), an amylase-sterilant (AS) or both (SA+AS) as silage additives for whole-plant corn, to determine type of fermentation and change in nutrient composition over time due to treatments. Addition of SA, AS or SA+AS increased concentrations of water-soluble carbohydrates (WSC) by 60.3, 17.4 or 50.1%, respectively. Treatment with SA inhibited formation of butyric acid, and lowered ammonia-N and ethanol concentrations in silage by 6.4 and 34.2% respectively ($P < .02$). Use of AS reduced butyric acid concentration, but increased ammonia-N and ethanol content in silage by 6.8 and 33.1%, respectively ($P < .10$).

Study 2. Using the aforementioned silages, a digestion and nitrogen balance trial and a rumen fermentation experiment measuring in-vitro gas production and microbial protein synthesis were conducted. In-vitro gas production was increased twofold in sheep fed SA- or AS-treated silages ($P < .10$). Microbial protein synthesis with SA, AS or SA+AS treatment was increased by 125, 133 ($P < .10$) and 61%, respectively. Digestibility and nitrogen utilization were not affected by treatments.

Study 3. A fermentation time study with 8-kg plastic buckets using α -amylase, α - and β -amylases or α -, β -amylases and sulfur-salts (AS) as silage additives, and a growth

trial with beef heifers were conducted to identify the cause for increased animal performance with AS-treated silages. After 28 days of ensiling, all amylase-treated silages retained 41 to 60% higher concentrations of WSC ($P < .10$), and produced more lactic acid ($P < .10$) than untreated silage. Presence of fungi was reduced in two of the amylase-treated silages (1.10 to 1.17 log; $P < .10$). Dry matter recovery of amylase-treated corn silages was markedly increased (6.3 to 10.7%, $P < .10$). Beef heifers fed amylase-treated corn silages had 5.3 to 12.1% increased ADG. Results suggest that addition of SA and AS shows potential for improving silage quality, nutrient preservation and ultimately, animal productivity.

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INTRODUCTION

Silage-making has been a popular method of forage preservation in the U.S.A. since the early 1950s. It is less weather-dependent than hay-making, is well suited as part of total mixed rations for large-scale livestock production, and is adaptable to a variety of crops. The ensiling process is affected by many biological and technological factors. Since these elements are often interrelated, their individual contribution to a successful ensiling is difficult to predict.

Silage of good quality is produced by inhibiting activities of plant enzymes and undesirable microorganisms, and encouraging the dominance of lactic-acid producing bacteria. Immediately after ensiling, respiratory activities of plant enzymes result in energy-wasteful reactions by oxidizing glucose and maltose (WSC) which would otherwise be available for fermentation to lactic acid, to CO_2 , H_2O and heat. Growth of undesirable microorganisms, such as yeasts, molds, clostridia and coliforms, should be inhibited, because they compete with lactic-acid producing bacteria for substrate and most of their fermentative end products have no preservative action.

Rapid growth of lactic-acid producing bacteria results in a drop in pH to 4.0 in the ensiled crop which inhibits other microorganisms. While silage inoculants containing

lactic acid bacteria do not greatly improve fermentation of whole-plant corn due to the substantial number of indigenous lactic acid bacteria in the fresh crop, fermentation efficiency may be enhanced by increasing the availability of substrate to these bacteria. The main carbohydrate source in chopped whole-plant corn is starch which cannot be utilized by lactic acid bacteria without being hydrolyzed to smaller molecules, such as glucose and maltose.

The addition of amylases to the ensiled corn crop could increase the rate of starch hydrolysis, thereby increasing the amount of substrate available to lactic acid bacteria. These, in turn, could produce high quantities of lactic acid which lead to a low pH and reduced nutrient losses. Anti-microbial agents, such as sorbic acid may make substrate more available to lactic acid bacteria by reducing competing microorganisms. They also may inhibit growth of microorganisms which utilize lactic acid as energy source.

Silage additives may induce changes in the type of fermentation and extent of nutrient transformation in the silo. These changes may improve the nutritive quality of the silage, enhance the fermentation activity and metabolic pathways in the rumen and ultimately, affect animal productivity.

Thus, the objectives of the three studies were:

- 1) to determine the magnitude and type of nutrient transformation during ensiling caused by fermentation

manipulation, 2) to study the effects of selected silage additives on digestibility, nitrogen utilization, rumen microbial growth and protein synthesis which could explain improved animal performance and 3) to identify the causative agent responsible for increased growth performance with an amylase product. Effectiveness of selected amylases in improving fermentation and nutrient recovery of ensiled whole-plant corn was tested. Furthermore, the same silage additives were used in a feedlot trial with beef heifers to compare extent of improvement in weight gains.

I. LITERATURE REVIEW

1. PRESERVATION FERMENTATION OF FORAGES

Factors Affecting the Ensiling Process

Preservation fermentation can be described as a metabolic process in which feed components are converted to desired organic compounds through enzymatic action of specific microorganisms (Bjurstrom, 1987; Leahy, 1988). However, no matter how successful the preservation of the ensiled forage may be, nutrient losses in silage are inevitable (Watson and Smith, 1956). Factors which influence the fermentation of silage either relate to the nature of the raw plant material or to the ensiling conditions imposed by the silage producer. Crop characteristics such as presence of epiphytic microflora, amount of fermentable carbohydrates, dry matter content and buffering capacity at harvest play an important role in the course of fermentation. Through proper management and technological 'know-how' the same crop may become more suitable for ensiling by using silage additives and by improving storage conditions in the silo (Zimmer, 1974; Woolford, 1984). Knowledge of the primary factors that control microbial metabolism in the silo is necessary to regulate the fermentation process and to minimize nutrient losses.

Oxygen inclusion. Rapid achievement of anaerobiosis is essential for the successful ensiling of forage (Woolford, 1984). This ensures a lactic-acid fermentation which will reduce the pH of the plant mass to a level low enough to terminate any undesired action of plant enzymes. Infiltration of small amounts of air is thought to delay plant cell autolysis, and therefore delay the release of plant juices, which is a prerequisite for growth of lactic-acid bacteria during early stages of ensiling (Ruxton et al., 1975). Inclusion of air also promotes growth of coliforms (acetic-acid producers), proteolytic bacteria, and yeasts which compete with lactic acid bacteria for substrate. This causes the silage pH to rise making conditions in the ensiled material favorable for growth of proteolytic clostridia (Langston et al., 1962). Clostridial fermentation of glucose and lactic acid to butyric acid, is the primary cause for spoilage and loss of nutrients during ensiling. Thus, insufficient exclusion of air can result in silage of lower quality which when fed to ruminants affects animal performance (Miller et al., 1961).

The main nutrients affected by the presence of O_2 are water-soluble carbohydrates (mono- and disaccharides). During aerobic ensiling conditions (respiration), mono- and disaccharides are completely oxidized to CO_2 and H_2O with a substantial loss of energy in the form of heat. These detrimental effects of O_2 appear to diminish when

carbohydrate reserves in the crop are adequately high to sustain greater losses of sugars without affecting fermentation (Ohyama et al., 1975). Fast exclusion of air, however, inhibits activity of respiratory enzymes and growth of yeasts and some aerobic bacteria and favors proliferation of lactic acid bacteria.

Ensiling temperature. In silage making, ensiling temperature has always been regarded as a major factor influencing the course of fermentation (McDonald et al., 1966). Generally, heating should be avoided and ensiling temperatures should not exceed 30°C (Woolford, 1984). Rate of microbial metabolism is increased by a rise in temperature (Woolford, 1984). However, microorganisms represented in the ensiled plant material differ in their temperature optima. Most lactic-acid bacteria are unable to grow at temperatures above 40°C, whereas clostridia thrive at 35° to 45°C (Zimmer, 1966; McDonald, 1981). Thus, any beneficial effects of increased temperature on rate of fermentation are counteracted by favoring clostridial growth which may result in formation of butyric acid and ammonia. Presence of large amounts of butyric acid causes pH to rise allowing putrefying clostridia to proliferate which causes spoilage and results in reduced nutritive value of the silage (Woolford, 1984).

The energy released by aerobic breakdown of carbohydrates in plant respiration causes a rise in temperature of the ensiled crop (Wieringa et al., 1961). Quantity of air present, insulation properties of the silo structure, and specific heat of the plant mass will determine the changes in temperature within the silo (McDonald, 1981).

Overheating of silages may cause Maillard reactions to occur, resulting in reduced protein digestibility. The formation of heat-damaged proteins by the browning reaction is a consequence of condensation of carbonyl and amine groups, resulting in the irreversible binding of nitrogen compounds (Bergen, 1984). Browning and polymerization reactions generate highly insoluble, biologically unavailable, brown nitrogenous polymers and copolymers, called 'melanoidins'. Heat, O₂ and pH greater than 6.0 enhance the rate and extent of these processes. Lower DM in silages may prevent the heat developed in the interior of the silo from being transferred to the exterior because higher water content in the harvested crop allows the plant mass to be compacted more densely (Bergen, 1984). Damaged carbohydrates and indigestible protein formed through Maillard reaction are quantitatively recovered in artifact lignin and acid detergent fiber nitrogen (ADFN) (Van Soest, 1987; Yu and Thomas, 1976). Thus, heat damage of silage is extensive when large amounts of lignin and ADFN are found.

Detrimental effects of high temperature on silage may be offset to some degree by ensiling forage with a high content of sugar or by the use of sugar-containing additives, because the energy-yielding substrate is not limiting to the lactic acid bacteria (Ohyama and Masaki, 1974).

Importance of Silage Additives in Fermentation of Forage

Ensiling of forage has been, for the most part, an unpredictable venture. Potential direction of the fermentation process and therefore, quality of the ensiled feed, may be improved by the use of silage additives.

Methods for altering natural fermentation of forage feeds have been recognized and studied for nearly a century. The primary purpose for adding various fermentation aids to ensiled feeds has been to improve preservation of the crop and minimize changes in its nutritive value. This in turn will affect intake, utilization of the ensiled feed and finally, productivity of livestock (Zimmer, 1976).

More than fifty years ago, Virtanen (1933) developed the so-called 'A.I.V. process', where he applied a diluted mixture of mineral acids to rapidly lower the pH of the ensiled plant material at onset of fermentation and thus induce qualitative microbial changes. Lactic acid bacteria survived and proliferated at this low pH because of their acid tolerance, whereas the growth of coliforms, fungi and clostridia was inhibited. This method employed highly

corrosive acids which caused feeding problems such as reduced palatability by livestock and in some cases even acidosis (Holzschuh, 1964) and hypomagnesemia (Breirem et al., 1954). Chamberlain and Quig (1987) also noted that undesirable microorganisms were not always successfully inhibited when inorganic acids were used. Recently there has been an increased interest in promoting 'natural' fermentation using 'biological' additives. This trend has been caused by technological progress in the production of bacterial inoculants and enzymes, and greater awareness of the danger of using potentially corrosive chemical additives. The purpose of using biological additives is to stimulate fermentation, so higher concentrations of lactic acid can be expected (Chamberlain, 1987). Such changes in chemical composition can affect the feeding value of silage.

Recent attempts to classify the multitude of available silage additives based on their mode of action have been made by McDonald (1981), Woolford (1984) and Leahy et al. (1988). Researchers have tested inorganic and organic acids, carbohydrate sources, enzymes and combinations thereof, for their potential stimulatory effect on the desired lactic-acid fermentation or their inhibitory action on undesired clostridial growth in ensiled feed.

Some organic acids elicit selective antimicrobial activities against fungi and certain aerobic bacteria. Prevention of fungal growth in the ensiled plant material

has been recommended by McDonald (1981) and Woolford (1984). Yeasts and molds contribute to nutrient losses in the ensiled plant material during fermentation when air is still trapped in the plant mass. More importantly, fungi may cause the ensiled forage to undergo secondary fermentation when the silo is opened. Nutrient losses due to spoilage by generating molds can also occur when silage is fed from the silo over a long period of time or when it is exposed to air in the feedbunk (Beck and Gross, 1964; Leahy and al., 1988).

In contrast to the aforementioned group of inhibitors, fermentation stimulants promote the growth of lactic acid bacteria. Fermentation stimulants include direct energy substrates (molasses, dextrose), enzymes (amylases, cellulases) which degrade forage plant components rich in potentially fermentable substrate and/or microbial cultures (some Lactobacillus species, Streptococcus, Pediococcus) that establish a dominance of homofermentative lactic acid bacteria (Woolford, 1984). Under many ensiling conditions, the number of lactic acid bacteria is not preventing a good fermentation to occur, but the availability of substrate, such as glucose, can be limiting for lactic acid bacteria to utilize (Gordon, 1989).

2. FERMENTATION MANIPULATION

Industrial Applications of Enzymes

Enzymes are widely used in the food industry. Many traditional fermentation processes, such as production of beer, wine, bread and cheese, rely on hydrolytic reactions catalyzed by indigenous or deliberately added enzymes. Enzymes are often added to accelerate or supplement the effect of natural enzymes during fermentations (Kulp, 1975). The majority of industrial enzymes are derived from specific microorganisms, although some are still extracted from animals and higher plants (Godfrey and Reichelt, 1983).

Generally, microorganisms secrete enzymes which catalyze the hydrolysis of polymers. The resulting molecular fragments are then transported into microbial cells and become substrates for their metabolism (Godfrey, 1985). A limiting factor for greater exploitation of enzymes in the food industry is their costly production. However, application of recombinant DNA techniques to production of enzymes may soon reduce the cost and contribute to new product development.

Biotechnological tools such as genetic engineering, molecular cloning or mutagenesis, will have a significant impact on microbial enzyme production in the future. Possible applications of this rather new technology to food fermentations appear unlimited.

Polymeric carbohydrates, such as starch and cellulose, are present in most agricultural raw materials. In processing these materials into various food products, both polymers are either removed as waste, hydrolyzed to smaller compounds, such as glucose, or used as source of dietary fiber (Meade et al., 1987).

Malted cereals have been used to produce carbohydrases of which α - and β -amylases are the most common (Godfrey, 1985). Alpha-amylases also have been commercially obtained from Bacillus species by applying rDNA technology to increase enzyme yield from the original production organism (Meade et al., 1987). These enzymes have many uses in the food industry. For example, α - and β -amylases derived from malting barley are used in the brewing industry to convert carbohydrates to fermentable sugars, which in turn are metabolized to alcohol by yeasts. Amylases are added to supplement, or replace acid hydrolysis of starch to produce syrup (highly concentrated sugar solution) or remove starch from fruit juices (Shellenberger, 1978). In the baking industry, addition of amylases to doughs provides the sugar required for yeast fermentation (Kulp, 1975).

Biological mechanism of starch-degrading enzymes.

Starch may appear in two forms, amylose and amylopectin, and their molecular sizes are variable. For example, the number of glucose units in amylose ranges between 300 and 400 (Whitaker, 1972). Because of its hydrated nature, starch

can be broken down by amylases at high rates. The enzymes involved in its degradation comprise multicomponent enzyme systems. The various forms of starch-degrading enzymes differ in amino acid composition, extent of glycosylation, substrate specificity and activity (Fogarty and Kelley, 1979). Binding of amylases to the active site appears to be very substrate specific (Kilara and Benhura, 1990).

Degradation of polymers, such as amylose, may require a large active site compared to the hydrolysis of maltose to glucose. The groups composing the active binding site are not sequentially proximal to one another. Therefore, the majority of amino acid residues in the enzyme are involved in keeping these few necessary groups in close spatial proximity. If spatial orientation is distorted by factors, such as heat or pH, the enzyme's activity is diminished or even lost (Kilara and Benhura, 1990).

Four enzymes can act synergistically to degrade starch to glucose, maltose and longer glucose-containing fragments. These enzymes are α -amylase, β -amylase, α -glucosidase and glucoamylase. Only α -amylase and glucoamylase seem to be necessary to completely hydrolyze starch (Maede et al., 1987).

Alpha-amylase (1,4- α -D-glucan glucanohydrolase, EC 3.2.1.1) is an endosplitting enzyme, which hydrolyzes internal α -1,4-glycosidic bonds of starch in a random fashion, yielding maltose, maltotriose and dextrin. It

shows stability at high temperatures (Meade et al., 1987). As a metalloenzyme α -amylase requires calcium ion for its activity and stability (Dasinger et al., 1985).

Beta-amylase (1,4- α -D-glucan maltohydrolase, EC 3.2.1.2) is an exosplitting enzyme, which cleaves successive maltose units from nonreducing ends of the polysaccharide chains (Whitaker, 1972). In contrast to the endosplitting amylase, it has lower temperature stability (Meade et al., 1987).

Alpha-glucosidase (maltase, 1,4- α -D-glucoside glucohydrolase, EC 3.2.1.20) hydrolyzes maltose units to glucose. Glucoamylase (amyloglucosidase, 1,4- α -D-glucan glucohydrolase, EC 3.2.1.3) can produce glucose directly from the nonreducing end of starch. Some glucoamylases even act on both α -1,4 and α -1,6- glycosidic linkages (Meade et al., 1987).

Use of amylases in silage fermentation. Forage plants have a large reserve of carbohydrates, which in their natural state occur as polysaccharide. The predominant polysaccharide in whole-plant corn is starch, which cannot be metabolized as such by most lactic-acid producing bacteria (McDonald and Whittenbury, 1973). Amylase activity from the epiphytic microflora appears to be limited, particularly at the time of ensiling. Therefore, it has been suggested that corn silage may be more efficiently fermented, if amylases were added prior to ensiling

(Woolford, 1984). After a series of hydrolyzing reactions by amylolytic enzymes, starch can be degraded to glucose and some maltose which provide substrate for lactic acid bacteria. This may increase the concentration of preserving acids, namely lactic and acetic acids, early in fermentation.

As indicated previously, the fate of glucose during the ensiling process depends primarily on the microflora of the plant material, since each group of microorganisms ferments its substrate to different metabolic end products. Homofermentative lactic-acid bacteria utilize glucose to generate two moles of lactic acid, whereas heterolactics convert glucose to equal moles of lactic acid, acetic acid or ethanol and CO_2 (McDonald, 1981). Two moles of ethanol are formed during the fermentation of glucose by yeasts. Saccharolytic clostridia ferment glucose and lactic acid to butyric acid.

Barancic and Sevcic (1966) and Bolsen and Ilg (1980) reported increased generation of lactic acid and reduced production of acetic and butyric acid after adding a commercial amylase preparation to alfalfa and whole-plant corn prior to ensiling. Forest (1977) also found that adding an amylase-containing product to whole-plant corn increased the amount of soluble carbohydrates and propionic acid, while reducing concentrations of butyric acid compared to untreated silage. Propionic acid is generated by

proteolytic clostridia from the deamination of three moles of alanine, leading to a badly fermented silage with lower quality and nutritive value (McDonald, 1981).

The Food Additive Sorbic Acid

Sorbic acid is a short-chain, α -, β -unsaturated trans-trans, 2,4-hexadienoic monocarboxylic aliphatic acid which has shown to exhibit fungistatic properties in foods and packaging materials (Sofos and Busta, 1981). It seems to be more active against fungi than bacteria in preventing or delaying growth (Emard and Vaughn, 1952). When used for the preservation of fermented vegetables, it suppresses growth of yeasts and molds, but inhibits only slightly the desired lactic acid fermentation (Costilow et al., 1955). Sorbic acid has also been employed in the wine industry to stabilize wine against refermentation, since the normal concentration of sulfur dioxide in wine has only a low efficacy against yeasts and the refermentative action they cause. Sorbic acid is used as a preservative in cheeses (Lueck, 1980).

Mode of action of sorbic acid. Although the antimicrobial action of sorbic acid was discovered in Germany by E. Mueller more than 50 years ago (Lueck, 1980), its mechanism at the molecular level is not yet understood. Freese et al. (1973) proposed that lipophilic acid food preservatives, like sorbic acid, may owe part of their

effectiveness to their solubility in the cell membrane and subsequent action as 'proton ionophores'. This would allow protons to enter cells more rapidly and thereby increase energy requirements of the cell to maintain its alkaline pH. In effect, lipophilic acids increase proton flux across the cell membrane, so that the leakage of protons becomes equivalent to that found at lower pH in their absence. Interference with the proton gradient across the membrane disrupts many of the chemoosmotic-related functions of the cell, such as amino acid transport (Eklund, 1980). For sorbic acid to be able to exert its action within the microbial cell, it must penetrate the cell membrane. This occurs at a low pH, when most of the acid is in its undissociated form (Davidson and Juneja, 1990). Its action depends therefore on the pH of its medium (Lueck, 1980). Additional specific effects include inhibition of various microbial enzymes such as enolase or lactate dehydrogenase (Gould et al., 1983). Sorbic acid forms covalent bonds between SH-groups of enzymes and its own double bonds, thereby inactivating the enzymes.

Use of sorbic acid in silage fermentation. Most research on action of sorbic acid deals with various human food products rather than with its more recent use as a silage additive. Hence, there is very little information concerning its effects on the course of fermentation and production potential of the resulting silage. Leahy (1988)

reported improved nutrient preservation and more aerobically stable silage when sorbic acid was added to ensiled whole-plant corn. Unlike mineral acids, which have been proven to only acidify ensiled material, many organic acids elicit selective antimicrobial properties. Sorbic acid inhibits primarily yeasts and molds, including aflatoxin-forming molds in foods (Lueck, 1970). As demonstrated by Beck and Gross (1964), fungi play an important role during plant respiration and aerobic deterioration of silage. Since they compete with lactic acid bacteria for fermentable carbohydrates, and oxidize preserving fermentation acids, they cause a rise in pH and temperature, and large losses in feeding value (Middelhoven and Franzen, 1986). Because of O₂ inclusion, molds can produce surface spoilage in the silo during the ensiling process after opening the silo or feedout in the feedbunk, resulting in large nutrient losses of the ensiled feed. Because of their potential production of mycotoxins, presence of fungi in feed may be hazardous to the animal's health (Ruxton et al., 1975; Woolford et al., 1977; Cole et al., 1977). Activity of fungi and some aerobic bacteria was restricted when sorbic acid was added as a selective fermentation inhibitor to the ensiled plant material (Salunke, 1956; Alli et al., 1985; Leahy, 1988). Due to inhibition of fungi, greater concentrations of substrate (glucose) were preserved (Alli et al., 1985; Leahy, 1988). These researchers also reported reduced

surface spoilage with sorbic acid treatment, but no effect on DM loss during ensiling.

3. EVALUATION OF ENSILED FEEDSTUFF

The economic value of silage as feed depends on the level of animal production it supports. This in turn depends on silage quality and nutritive value (Barber et al., 1989).

Silage Quality

The term 'silage quality' is generally used to indicate the success of fermentation (McCullough, 1978). There are many possible parameters one could measure to determine outcome of fermentation. Bolsen and Hinds (1984) summarized a large number of criteria which are used to assess silage quality and its production potential in livestock. This list includes the chemical composition and microbial profile, ensiling temperature, nutrient losses, aerobic stability and nutritional value of the ensiled forage. Not all aspects are usually measured for any one silage.

Woolford (1984) stated that chemical determination of the principal fermentation acids provides a clear basis to judge silage quality. Several systems have been developed in which scores are assigned according to the relative proportions of lactic and acetic acids to butyric acid. The greater this proportion, the higher the quality of the silage (Flieg, 1952; Zimmer, 1966). Whereas Flieg (1952)

and Zimmer (1966) consider butyric acid, the main metabolite of saccharolytic clostridial fermentation, to be of importance in categorizing a bad silage, other workers emphasize production of ammonia from proteolytic clostridial fermentation in their evaluation. Their schemes are based on the amounts of ammonia/volatile nitrogen (VN), pH and content of lactic acid (Archibald et al., 1954). Silage with less than 10% (of total nitrogen) VN, pH lower than 4.5 and more than 1.5% (fresh wt) lactic acid is considered a good quality silage.

Van Soest (1987) noted that pH and DM content are the simplest criteria of silage quality. Low DM silages should have a pH below 4.4 to be of good quality. Higher pH indicates a proteolytic fermentation and formation of amines and butyric acid which are metabolites of a badly fermented silage. However, high pH silages with higher DM content (water content less than 65%) may be of good quality. In silages with high DM, pH appears to be less useful in predicting silage quality, since deficiency of water restricts fermentation and acid production (Van Soest, 1987). Since there is no unified system for evaluating the quality of ensiled forage, Woolford (1984) recommended that reference to the scheme employed should be given in any description.

Nutritive Value

The process of ensiling is associated with major changes in composition of the original crop which generally reduce its nutritive value (Wilkinson, 1976). Nutritive value is classified in four general components: nutrient composition, voluntary feed consumption, nutrient digestibility and energetic efficiency (Raymond, 1969; Woolford, 1984; Van Soest, 1987). Since efficiency and intake show greater inter-animal variation, digestibility is more often determined. However, feed consumption and efficiency are more responsible for total animal responses (Van Soest, 1987).

The nutritive value of silages is dependent on the nutrient composition of the standing crop and the type of fermentation occurring in the ensiled plant material. A fermentation process dominated by lactic acid bacteria generally results in lactate silages which have low pH (3.7 to 4.2) and contain large amounts of lactic acid (8 to 12%, DM). Crops rich in fermentable carbohydrates usually result in this most preferred type of fermentation (McDonald and Edwards, 1976). Acetate silages which are dominated by coliform bacteria, have a relatively high acetic/lactic acid ratio and subsequently a higher silage pH (McDonald, 1981). Clostridial silages are badly fermented and are characterized by a pH of 5.2 to 7.0 (McDonald et al., 1962; Ohshima et al., 1979). If saccharolytic clostridia

dominated, butyric acid was the primary fermentation acid (3.5% DM), and only low concentrations of lactic acid (.10% DM), soluble carbohydrates (.5% DM) and high ammonia-N content (24.6% of total nitrogen) are noted (McDonald and Edwards, 1976). If proteolytic clostridia are very active, large amounts of acetic acid (11.4% DM), ammonia-N (29.2% of total nitrogen) and relatively low butyric acid (.8% DM) are observed.

Voluntary consumption of silage is a very important factor. However, the type of fermentation occurring in the ensiled crop can affect intake. For example, chemical compounds are generated during fermentation or are added as fermentation aids to improve silage quality. Some researchers have attributed depressed intake to organic acids such as the primary fermentation acids and to products of protein degradation such as ammonia (McLeod et al., 1970; Wilkins et al., 1971, 1978). The mechanism for this reduction is not yet known. It was suggested that there are chemo-receptors in the rumen, which are sensitive to silage constituents or that after rapid absorption of nutrients, chemo-receptors in the brain or liver are stimulated (Gill et al., 1986). Barry et al. (1978) found in experiments with alfalfa silages that depression in feed consumption was more related to products of decarboxylation reactions (i.e. acetic acid) rather than to products of deamination (i.e. amines). A clostridial type fermentation reduces silage

intake because of the fermentation products ammonia and acetic acid. High concentrations of lactic acid are also associated with reduced intake of silages. Although ruminal metabolism of lactic acid is very rapid, there seems to be a clear limit to the capacity of the rumen to metabolize lactic acid. If lactic acid concentrations exceed 20% (DM), voluntary intake is depressed and indigestion can occur (Chamberlain, 1987; Newbold et al., 1986). Addition of formic acid to ensiled feed reduces intake of the silage (McLeod et al., 1970). In contrast, propionic acid has been found to restrict fermentation and to increase silage intake (Huber and Soejono, 1976).

The digestibility of good quality silage is similar to that of the fresh crop (Forbes, 1986). A small increase in digestibility of NDF was noted due to ensiling, which seems to compensate for a higher proportion of NDF in silage (Wilkins, 1988; Woolford, 1984). According to McDonald (1981) this increased amount of NDF may be a result of losses of soluble nutrients in gaseous form or via effluent. However, type of fermentation appears to affect digestibility of the ensiled feed by animals. Wilkinson and Wilkins (1980) found digestibility to be 4-percentage units lower in alfalfa silages which had undergone clostridial rather than lactic acid fermentation. The addition of fermentation aids can affect digestibility of treated silage. Wilkins et al. (1974) noted decreased protein and fiber digestibility when

the fermentation inhibitor formaldehyde was added prior to ensiling. The reaction of formaldehyde with amino groups forms insoluble complexes with proteins that dissociate under acidic conditions of peptic digestion. However, the possibility of further reaction can occur which may lead to a strong binding of carbohydrates and proteins (Van Soest, 1987).

Ensiling is an energetically efficient process in spite of high DM losses, which occur during fermentation. If one considers the desired fermentation pathway from water-soluble carbohydrates to lactic acid, the efficiency of energy retention is approximately 100% (Woolford, 1984). Ensiling can yield fermentation metabolites with a higher GE value than the original substrates (McDonald et al., 1973; Woolford, 1984). For instance, butyric acid and ethanol have GE values of $5.96 \text{ kcal}\cdot\text{g}^{-1}$ and $7.13 \text{ kcal}\cdot\text{g}^{-1}$, respectively, compared to the original substrate glucose which has a GE content of $3.73 \text{ kcal}\cdot\text{g}^{-1}$ (McDonald, 1981). However, higher GE values do not necessarily result in higher metabolizable energy (Woolford, 1984). Rumen microorganisms convert ethanol and butyric acid primarily to acetic acid, which generates some energy for microbial growth and protein synthesis.

4. RUMEN FERMENTATION

Rumen Ecology

Survival of microorganisms in the rumen environment depends on their ability to scavenge nutrients. Microbial growth in the rumen is ultimately dependent on nutrient acquisition, efficiency of nutrient transport systems and substrate affinity and specificity (Russell et al., 1990).

A variety of transport systems aid rumen bacteria in taking up nutrient components. Ammonia has been recognized as an important source of nitrogen for rumen microbial growth (Boggs, 1959). Its uptake or assimilation may be the rate-limiting step in the synthesis of microbial protein (Mackie and White, 1990). Very limited information is, however, available on the transport of ammonia. At alkaline pH, ammonia can pass across the bacterial membrane by passive diffusion (Russell et al., 1990). However, at physiological pH, ammonia is present in its ammonium ion form and requires an active transport to cross the cell membrane (Mackie and White, 1990).

Many substances which are hydrophilic or too large to pass freely across the bacterial membrane, may be taken up by a transport mechanism involving a carrier protein (Russell et al., 1990). Most soluble carbohydrates are taken up by active transport or facilitated diffusion, and must be phosphorylated prior to undergoing glycolysis.

Streptococcus bovis, Selenomonas ruminantium and Megashera

elsdenii transport sugar by a phosphotransferase system.

Among the multitude of rumen microorganisms, numerous interrelationships exist which affect their growth rates and metabolic activities. These relationships can be neutral, commensal, mutualistic, competitive or amensal. Growth rates, substrate affinities, nutrient requirements and pH tolerance of rumen microorganisms can affect their competitiveness and define their niche within the rumen microbial ecosystem (Russell and Hespell, 1981).

In spite of the importance of starch in ruminant diets, the mechanism of starch hydrolysis by rumen microorganisms has not been studied extensively. The major microorganisms involved in its degradation are Bacteroides amylophilus, Streptococcus bovis and Succinimonas amylolytica (Russell and Hespell, 1981), some entodiniomorph protozoa and fungi (Pearce and Bauchop, 1985). Although protozoa and fungi are not very important in starch utilization, it has been suggested that the engulfing of starch granules by protozoa limits the amount of starch available for bacterial fermentation and thus, prevents a lowering of rumen pH (Mackie et al., 1978). The microbial enzymes isolated seem to be α -amylase (McWethy and Hartman, 1977). The predominant route of starch degradation by these microbial amylases involves hydrolysis of this polysaccharide to glucose, followed by phosphorylation of glucose to glucose-6-

phosphate (Coleman and Laurie, 1976). Protozoa ferment starch slowly to H_2 , CO_2 , acetic and butyric acid.

The primary cellulolytic rumen microbes (Bacteroides succinogenes, Ruminococcus albus and Ruminococcus flavefaciens) are restricted in terms of the ecological niche they occupy (Baldwin and Allison, 1983). They are limited to di-, tri- and oligosaccharides released from cellulose hydrolysis as their sources of carbon and energy. They also require B-vitamins, branched-chain fatty acids and ammonia for their growth which makes them dependent on other rumen microbes, such as saccharolytic bacteria, for supply of these nutrients. Cellulolytic species in turn, secrete extracellular enzymes which hydrolyze cell wall components, thereby providing saccharolytic microbes with substrate. This phenomenon has also been called 'crossfeeding' (Russell and Hespell, 1981). Adherence or spatial closeness of rumen bacteria to cell wall components seems to be obligatory for degradation of an insoluble substrate, such as cellulose (Morris and Cole, 1987). The biological mechanism of attachment is still unknown. However, cell-associated cellulases, cellulose-binding proteins or the glycoprotein coat that encapsulates most cellulolytic microbes have been suggested to mediate attachment (Mackie and White, 1990).

Manipulation of Rumen Fermentation

Nutrient components supplied in feed are subjected to fermentative reactions by rumen microorganisms and hydrolytic digestion by the animal's own enzyme systems (Chalupa, 1980). Fermentation and outflows of nutrients from the rumen can be manipulated favorably by inhibiting breakdown of certain dietary components, such as proteins, and by increasing hydrolysis of rather slowly digestible polymers, such as cellulose and hemicellulose. Protein degradation can be reduced by protecting proteins from microbial hydrolysis, while fiber digestion may be enhanced by favoring or depressing certain microbial species and their metabolic activities (Chalupa, 1984). During rumen fermentation, short-chain fatty acids and microbial cells are formed from dietary nutrients to serve the ruminant animal as sources of energy and protein, respectively. Methane, heat and urea are also generated, representing a loss of energy and nitrogen for the animal (Mackie and White, 1990). The balance of these fermentation products is regulated by the types, numbers and activities of rumen microorganisms. The quantity and quality of fermentation metabolites therefore, largely determine the efficiency of nutrient utilization and ultimately, the productive performance of the animal (Russell and Hespell, 1981; Mackie and White, 1990).

Volatile fatty acids resulting from carbohydrate

fermentation are the primary energy source for animal metabolism (Nocek and Russell, 1988). Animal performance appears to be highly related to the proportion of acetate to propionate in the rumen. Greater propionate proportion is usually coupled with decreased methanogenesis and reduced acetate and/or butyrate percentage with no change in total VFA concentration (Van Nevel et al., 1974). The increase in propionate production in the rumen was found to have a protein-sparing effect (Schelling, 1984). Feed efficiency and ADG of ruminants were improved (Goodrich et al., 1984). Amylolytic microbes produce considerably more propionate than do cellulolytic organisms (Baldwin and Allison, 1983). Thus, diets high in starch stimulate growth of amylolytic microbes which compete favorably for soluble sugars and products of starch and hemicellulose hydrolysis (Baldwin and Allison, 1983). Increased propionate production after feeding cereals is not only caused by high starch fermentation, but also by alteration of fermentation products formed from other carbohydrate sources. The types and concentrations of end products in rumen fermentation are dependent on the activities of rumen microorganisms and their ability to adapt to dietary changes (Russell et al., 1990). Maximal animal production can be achieved when rumen fermentation and microbial protein synthesis are optimized, provided that most end products can be utilized by the animal.

Manipulation of Protein-Energy Balance

Manipulating proportions of protein and energy-yielding nutrients in a diet can have significant effects on the nutrient utilization by the animal (Oldham, 1984). Relative amounts, form and rate of availability of protein and carbohydrates in feeds are likely to determine the partition of available nutrients between useful products such as milk or tissue and wasteful products such as heat, methane or urea (Oldham, 1984). The carbohydrate proportion in the diet provides energy for the formation of the high-energy bond in ATP, which is utilized by rumen microorganisms for maintenance and growth. ATP is also required for synthesis of microbial protein from amino acids, ammonia and glycolytic intermediates (Baldwin and Allison, 1983). The energy-generating and energy-utilizing processes in the rumen need to be coupled if continued production of fermentation products with concomitant microbial growth is to be obtained (Russell and Hespell, 1981) and optimum energy and protein is made available for the ruminant animal.

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II. EFFECT OF SORBIC ACID OR AN AMYLASE-STERILANT PRODUCT ON ENSILING EFFICIENCY OF CHOPPED WHOLE-PLANT CORN

1. ABSTRACT

Effects of additives on changes in chemical composition during ensiling chopped whole-plant corn were tested in a 2 x 2 factorial arrangement of sorbic acid (SA) and amylase-sterilant preparation (AS) using 208-1 steel drums as experimental silos.

Addition of SA preserved 43.7% more water-soluble carbohydrates (17.1 vs 11.9%, DMB, $P < .01$) during fermentation. Concentrations of lactic and acetic acids were not affected, but butyric acid, ethanol and ammonia-N were 97.4, 34.2 and 6.4% lower, respectively, with SA treatment than without SA ($P < .02$). Treatment with SA also resulted in greater amounts of hemicellulose and cellulose at the end of ensiling ($P < .05$).

Treatment with AS resulted in silages with lower pH ($P < .07$), 9% increased lactic acid concentration and reduced butyric acid concentration (52.7%; $P < .07$) by d 43 of ensiling. Concentrations of ethanol (33.1%) and ammonia-N (6.8%) were increased in AS-treated silages compared to those treated without AS ($P < .10$). More ethanol may be indicative of increased utilization of glucose by yeasts. Larger amounts of ammonia-N may have come from breakdown of nitrates or nitrites. At the end of ensiling, amounts of N-

free extract (3.1%), NDF (5.5%) and ADF (6.7%) were lower in AS-treated silage than in other silages ($P < .03$). Although additives need to be tested under farm conditions, SA showed more potential for improving silage quality and nutritive value than AS or SA+AS treatments.

2. INTRODUCTION

Many silage additives are marketed with the claim that they will improve silage quality and reduce nutrient losses during fermentation. Extent of nutrient loss depends upon the microorganisms dominating the fermentation. Generally, lactic acid fermentation reduces losses of DM and energy in silage (McDonald, 1981). Early dominance of lactic-acid producing bacteria may be established by adding anti-microbial agents, such as sorbic acid, to the ensiled plant material which inhibit proliferation of yeasts, molds and some aerobic bacteria (Alli et al., 1985; Woolford, 1984).

Another approach to increase lactic acid production is to improve availability of fermentable carbohydrates to lactic acid bacteria (Woolford, 1984). Since most of the carbohydrate fraction in corn silage is starch, addition of an amylolytic enzyme may accelerate mobilization of glucose and maltose from starch hydrolysis which can be easily utilized by lactic acid bacteria.

Objective of this study was to compare extent and type of nutrient transformation due to treatment with sorbic

acid, amylase-sterilant or both during the ensiling process using laboratory-scale silos.

3. MATERIALS AND METHODS

Silage Production

Whole-plant corn (Pioneer Hybrid 3147) was harvested at late dough to early dent stage, chopped into approximately 6.4 to 12.7 mm long pieces and immediately transported in a wagon less than 1 km to a staging area. Chopped corn was quickly unloaded into 208-liter steel drums lined with plastic bags and stored for a short time at 4.4°C to reduce excessive respiratory heat until silage additives were added. Approximately 150 kg of chopped whole-plant corn was mixed for about 2 min in a Marion Mixer. Silos were assigned randomly to a 2 x 2 factorial arrangement of 0 or 0.10% sorbic acid (Monitor, Monsanto, St.Louis, MO) and 0 or 0.05% commercial product consisting of α -, β -amylases, a sulfur-containing sterilant (at least 21% Sulfur) and dextrose (Silo Guard II, ISF Corp., Waverly, N.Y.)

Chemical Parameters

Eight experimental silos (208 l) were lined with large polyethylene bags and filled with five smaller plastic bags each containing approximately 13 to 15 kg of chopped plant material. Plant material in each bag was compacted to expel as much air as possible, and bags were sealed tightly. This

ensiling technique permitted sampling of corn silage from the same laboratory silo at different times without air penetrating the entire silo. Two samples (400 g) of plant material were taken 1,2,3,7,13,19, 25,31,37 and 43 d post ensiling and stored (-20°C) until analyzed for proximate constituents (AOAC, 1980) and NDF, ADF and ADFN (Goering and Van Soest, 1970), water-soluble carbohydrates (WSC), lactic acid, pH, ethanol, VFA, and ammonia-N. Aqueous silage extracts were prepared by immersing frozen silage in liquid nitrogen, macerating and homogenizing with a Waring blender (Parker, 1978).

Water-soluble carbohydrates were determined on aqueous extracts (El Hag et al., 1982) according to the phenol-sulfuric acid method of Dubois et al. (1956). Simultaneous analyses of lactic acid, ethanol and VFA were performed on aqueous silage extracts using a Hewlett-Packard gas chromatograph (Model # 5890A). Four ml of aqueous silage extract were mixed with 1 ml of 0.03 M oxalic acid, centrifuged ($10,000 \times g$) for 10 min, and $0.2 \mu\text{l}$ of supernatant liquid was injected onto a 1.83 m x 2 mm ID glass-lined column packed with 80/120 Carbopack B-DA/4% Carbowax 20M (Supelco, Inc., Bellefonte, PA). The column was conditioned for 19 h at 245°C . The gas chromatograph was operated isothermally at 175°C (column) and 200°C (inlet and detector). The carrier gas was nitrogen with a flow rate of 24 ml min^{-1} . Silage pH was measured by glass electrode after

mixing a sample (25 g) with 250 ml of deionized water for 10 min. Ammonia-N was measured by an ion-specific electrode (Corning, Medfield, MA).

Temperature Measurement

Eight experimental silos were lined with two large polyethylene bags and filled with untreated or additive-treated chopped whole-plant corn. Three thermocouples were inserted at different heights in the center of the plant mass, the material was compacted, and both plastic bags were sealed carefully. Temperature changes were measured with an Atkins Digital Thermocouple Thermometer during fermentation in 4-h intervals for the first 24 h, then every 12 h for 6 d, followed by daily temperature readings to 43 d post ensiling. Ambient temperature over each drum was recorded also.

Statistical Analysis

Fermentation criteria measured over time were analyzed by an ANOVA using the model:

$$Y_{ij} = \mu + S_i + A_j + SA_{ij} + E_{ij};$$

changes in ensiling temperature were determined by a split-plot in time using the model:

$$Y_{ijk} = \mu + S_i + A_j + SA_{ij} + D_k + SD_{ik} + AD_{jk} + E_{ijk}$$

where μ = mean; S_i = sorbic acid level i ; A_j = amylase-sterilant level j ; SA_{ij} = interaction of main effects; D_k = day of ensiling; SD_{ik} = interaction of sorbic acid with day

of ensiling; AD_{jk} = interaction of amylase-sterilant with day of ensiling; E = residual error.

Contrasts were made to test main effects and interaction of treatments (SAS, 1985). Probability of $P < .10$ was used to designate significance.

4. RESULTS

Crop Composition

Nutrient composition of the experimental corn silages after 43 d of ensiling is given in Table 1. Treatment with SA resulted in 2.5% lower DM (31.7 vs 32.5%, $P < .01$), whereas NDF and ADF were 6.8% (53.1 vs 49.7% DMB; $P < .01$) and 2.2% greater (29.8 vs 27.6% DMB; $P < .01$), respectively, due to greater amounts of hemicellulose ($P < .05$) and cellulose ($P < .01$) in SA-treated silages. Silages treated with AS contained 2.8% more DM (32.6 vs 31.7%), 3.1% lower N-free extract (46.5 vs 48.0% DMB; $P < .03$), 5.5% lower NDF (49.9 vs 52.8% DMB; $P < .01$) and 6.7% lower ADF (27.7 vs 29.7% DMB; $P < .01$) concentrations than silages without AS, the latter primarily caused by lower cellulose content ($P < .01$). In contrast, significantly more N-free extract in AS-treated corn silage was reported by Leahy et al. (1990). Positive relationships of NDF with hemicellulose ($r = .90$) and of ADF with cellulose ($r = .92$) were noted.

Crude protein content was not affected by treatments. Sorbic acid did not change ADFN content in silage, which

TABLE 1. NUTRIENT COMPOSITION OF SORBIC ACID AND AMYLASE-STERILANT
TREATED CORN SILAGE AT 43 DAYS POST ENSILING

Item	Silage additive ^a					Contrasts ^b		
	None	SA	AS	SA+AS	SE ^c	SA	AS	SAXAS
DM, %	31.8	31.6	33.3	31.8	.26	.01	.01	.01
N-free extract, %DMB ^d	49.8	46.1	45.0	48.0	.61	.45	.03	.01
NDF, %DMB ^d	51.9	53.6	47.4	52.5	.91	.01	.01	.03
ADF, %DMB ^d	29.2	30.2	26.0	29.4	.63	.01	.01	.05
Hemicellulose, %DMB ^d	22.7	23.4	21.4	23.1	.32	.05	.13	.36
Cellulose, %DMB ^d	25.7	26.4	23.0	25.8	.51	.01	.01	.02
CP, %DMB ^d	9.05	8.91	8.92	8.84	.06	.48	.52	.84
ADFN ^e	9.97	9.90	8.39	8.82	.28	.56	.01	.42

^a None = Control; SA = Sorbic acid at .10%; AS = α -, β -Amylases and sterilant at .05%; SA+AS = both additives combined at individual rates.

^b Probability of a significant effect of sorbic acid (SA), amylase-sterilant (AS) or interaction (SAXAS).

^c Standard error of treatment means; n=2.

^d DMB = dry matter basis.

^e ADFN = acid detergent fiber nitrogen as percent of total N.

contrasts with data of Leahy (1988) in corn silage treated with SA. However, AS treatment decreased ADFN by 13.4% (9.94 vs 8.61% TN; $P < .01$).

Ensiling Temperature

Profiles of ensiling and ambient temperatures are illustrated in Figure 1. During the first three days, SA-treated corn showed an average 1.8° lower ensiling temperatures than corn ensiled without SA ($P < .01$). Thereafter, temperature curves did not differ among treatments. Ambient temperature was positively correlated with ensiling temperature ($r = .68$).

Changes in Chemical Composition

Water-soluble carbohydrates. Treatment with SA had 43.7% more WSC remaining in corn throughout fermentation (17.1 vs 11.9% DMB; $P < .01$) than control (Table 2). Greater retention of WSC from the originally ensiled plant material after treatment with SA was also reported by Alli et al. (1985) and Leahy (1988). Concentrations of WSC in AS-treated corn silage were not different from the control which agrees with findings of Leahy (1988), but contrasts with results of Alli and Baker (1982) who reported greater amounts of WSC in corn silage treated with an amylase product. When compared to untreated silage, SA addition to corn resulted in 60.3% more WSC, AS treatment in 17.4% more and SA+AS treatment in 50.1% more WSC on 43 d post ensiling.

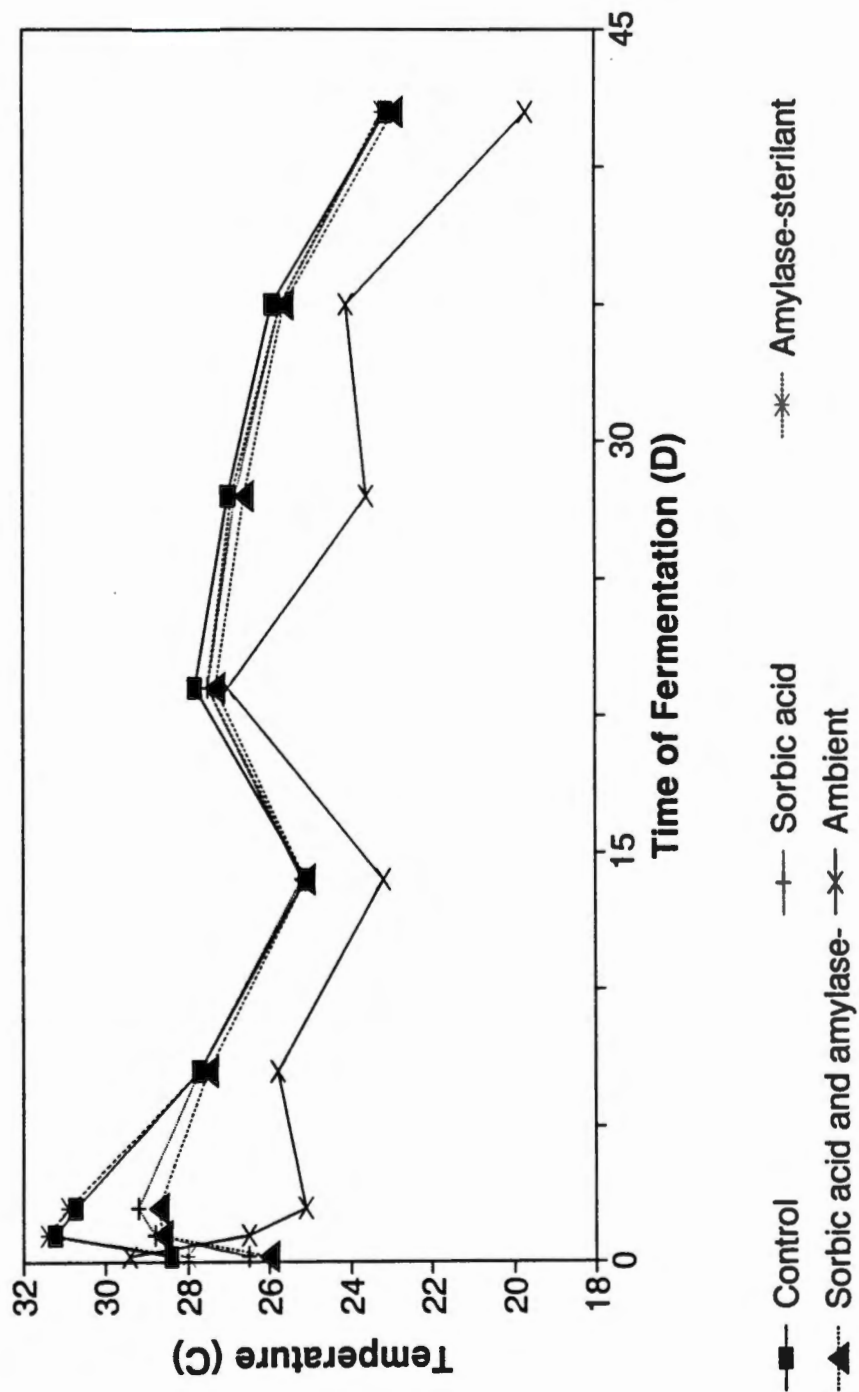


FIGURE 1. ENSILING TEMPERATURES IN CORN SILAGES TREATED WITH SILAGE ADDITIVES

TABLE 2. CHANGES IN WATER-SOLUBLE CARBOHYDRATES, LACTIC ACID AND PH OF CORN SILAGES OVER TIME

Day of ensiling	Silage additive ^a				SE ^c	Contrasts ^b		
	None	SA	AS	SA+AS		SA	AS	SAXAS
	----- WSC ^d , % DMB ^e -----							
1	14.6	25.4	14.8	24.9	2.17	.01	.95	.89
2	12.2	16.3	11.7	17.3	.99	.01	.81	.48
37	10.6	12.8	11.9	12.2	.54	.34	.77	.48
43	8.86	14.2	10.4	13.3	1.09	.10	.87	.55
Mean	11.6	17.2	12.2	16.9	.88	.01	.90	.78
	----- Lactic acid, % DMB ^e -----							
1	5.24	4.47	4.76	4.43	.18	.19	.49	.56
2	5.74	5.16	5.32	5.57	.12	.41	.94	.10
37	5.53	5.53	5.83	5.61	.09	.62	.40	.61
43	3.75	5.05	4.97	4.62	.22	.13	.19	.03
Mean	5.07	5.05	5.22	5.06	.12	.69	.71	.76
	----- pH -----							
1	4.30	4.20	4.20	4.40	.06	.70	.70	.29
43	4.50	4.10	4.15	4.15	.07	.03	.07	.03

^a None = Control; SA = Sorbic acid at .10%; AS = α -, β -Amylases and sterilant at .05%; SA+AS = both additives combined at individual rates.

^b Probability of a significant effect of sorbic acid (SA), amylase-sterilant (AS) or interaction (SAXAS).

^c Standard error of treatment means; n=2.

^d WSC = water-soluble carbohydrates.

^e DMB = dry matter basis.

Lactic acid and pH. Changes in lactic acid concentrations and pH over time are also shown in Table 2. By d 7, concentrations of lactic acid had progressively increased to $7.42\% \pm .07$ (DMB), whereas silage pH had fallen to $3.85 \pm .03$, with no effect of treatments noted (data not shown). Thereafter, lactic acid concentrations decreased causing silage pH to rise. Silages treated with SA or AS did not contain significantly more lactic acid, although their pH was somewhat lower ($P < .10$) than the control. A negative correlation of lactic acid production with silage pH was observed ($r = -.85$). Similar lactic acid contents in SA- or amylase-treated silages to control during fermentation were reported by Alli et al. (1985) and Leahy (1988).

Ethanol and acetic acid. From the first day of fermentation, ethanol was present in all ensiled plant material and was consistently lower in SA-treated silages than control (Table 3). By 43 d, ethanol content was 34.2% lower in SA-treated silage ($.72$ vs 1.09% DMB; $P < .01$) than in silages without SA. Treatment with AS did not affect ethanol production immediately. From d 7, however, ethanol production became greater in AS-treated silage and on d 43, there was 33% more ethanol in AS-treated silage (1.03 vs $.77\%$ DMB; $P < .03$) than in silages not treated with AS.

Acetic acid (Table 3) increased with time in all experimental silages. Treatment with SA reduced acetic acid concentration during the first 19 d of ensiling (1.86 vs

TABLE 3. CHANGES IN ETHANOL AND ACETIC ACID CONCENTRATIONS
OF CORN SILAGE OVER TIME

Day of ensiling	Silage additive ^a				Contrasts ^b			
	None	SA	AS	SA+AS	SE ^c	SA	AS	SAXAS
----- Ethanol, % DMB ^d -----								
1	.671	.647	.689	.604	.02	.07	.60	.23
2	.631	.664	.658	.586	.01	.43	.31	.08
19	.928	.595	1.06	.801	.07	.01	.03	.47
37	.968	.817	1.21	.814	.07	.04	.25	.24
43	.817	.731	1.36	.703	.11	.01	.03	.02
Mean	.803	.691	.995	.702	.03	.01	.10	.13
----- Acetic acid, % DMB ^d -----								
1	1.32	1.10	1.30	1.14	.04	.01	.32	.04
2	1.46	1.31	1.52	1.27	.04	.01	.82	.16
19	1.97	1.27	1.75	1.53	.10	.01	.72	.02
37	1.88	1.91	1.79	1.84	.03	.57	.27	.91
43	1.82	1.96	1.90	1.86	.04	.62	.91	.34
Mean	1.69	1.51	1.65	1.53	.05	.12	.93	.78

^a None = Control; SA = Sorbic acid at .10%; AS = α -, β -Amylases and sterilant at .05%; SA+AS = both additives combined at individual rates.

^b Probability of a significant effect of sorbic acid (SA), amylase-sterilant (AS) or interaction (SAXAS).

^c Standard error of treatment means; n=2.

^d DMB = dry matter basis.

1.40% DMB; $P < .01$). Thereafter, amounts were not different from the control. Addition of AS to ensiled whole-plant corn did not affect acetic acid content at any time. This agrees with results reported by Alli et al. (1985) and Leahy (1988).

Butyric acid, ammonia-N and isoacids. Minimal concentrations of butyric acid (Table 4) were detectable in all experimental silages by d 3 of fermentation. Treatment with SA inhibited formation of butyric acid throughout the ensiling period (.078 vs .002% DMB; $P < .02$). Addition of AS also lowered concentration of butyric acid, particularly on day 43 (.055 vs .026% DMB; $P < .07$).

Only a small percentage ($1.01 \pm .02$) of total nitrogen in corn was ammonia-N (Table 4). By 43 days, its concentration in silage was increased twofold. Addition of SA to corn reduced ammonia-N formation after 2 d ($P < .01$) and ammonia-N remained lower throughout fermentation. Use of sorbates also led to significantly reduced volatile N content during fermentation as reported by Alli et al. (1985). Throughout ensiling, isobutyric acid concentration was lower in SA-treated silages (.057 vs .050%, DMB; $P < .02$), but isovaleric acid production was not affected by this treatment (data not shown).

Silages treated with AS contained somewhat greater amounts of ammonia-N during ensiling. However, AS addition to corn did not affect concentration of either isoacid in

TABLE 4. CHANGES IN BUTYRIC ACID, AMMONIA-N AND ISOACID CONCENTRATIONS OF CORN SILAGE OVER TIME

Day of ensiling	Silage additive ^a					Contrasts ^b		
	None	SA	AS	SA+AS	SE ^c	SA	AS	SAXAS
----- Butyric acid, % DMB ^d -----								
1	ND ^e	ND	ND	ND				
2	.013	ND	.009	ND	.002	.01	.23	.23
19	.015	.009	.017	.005	.002	.02	.78	.34
37	.015	ND	.018	ND	.003	.01	.09	.09
43	.109	ND	.048	.005	.017	.01	.07	.05
----- NH ₃ -N, % TN ^f -----								
1	.966	1.00	1.06	1.02	.016	.99	.11	.28
2	1.44	1.14	1.43	1.19	.054	.01	.51	.43
19	1.77	1.70	1.92	1.67	.040	.18	.67	.99
37	1.86	1.75	1.97	1.85	.029	.01	.01	.80
43	1.92	1.91	2.17	1.91	.048	.08	.10	.10
--- Isobutyric acid, % DMB ^d ---								
43	.093	.060	.045	.057	.008	.49	.14	.18
--- Isovaleric acid, % DMB ^d ---								
43	.006	.005	.009	.005	.002	.63	.85	.77

^a None = Control; SA = Sorbic acid at .10%; AS = α -, β -Amylases and sterilant at .05%; SA+AS = both additives combined at individual rates.

^b Probability of a significant effect of sorbic acid (SA), amylase-sterilant (AS) or interaction (SAXAS).

^c Standard error of treatment means; n=2.

^d DMB = dry matter basis.

^e ND = not detectable.

^f NH₃-N, %TN = ammonia-N expressed as percent of total N.

silage.

5. DISCUSSION

Rapidly reduced pH, approximately 70% of fermentation acids as lactic acid, low ammonia and only traces of butyric acid indicated that all experimental silages had undergone the desired lactic acid fermentation. Use of SA in silage caused no effect on N-free extract content per se, but greater amounts of retained WSC. This effect of SA on WSC has been described by other workers (Alli et al., 1985; Leahy, 1988) and was suggested to be due to SA's inhibitory action on yeasts and molds which generally utilize glucose as substrate. By reducing fungal population, some WSC are spared. However, instead of being fermented by lactic acid bacteria, WSC accumulated in silage. This effect may be due to the rapid drop in pH to 4.0 which can inhibit activity of lactic acid bacteria as noted by Alli and Baker (1982). Another explanation may be that SA directly inhibited growth of some lactic acid bacteria in silage, thereby limiting fermentation of available substrate to lactic or acetic acid by homo- or heterofermentative lactic acid bacteria.

Addition of AS resulted in decreased N-free extract content, indicating that more starch was hydrolyzed to glucose and maltose in this silage which made more substrate available to epiphytic microflora than in untreated silage. It seems from these results that only little more WSC was

fermented by lactic acid bacteria to lactic acid and thus, WSC accumulated in the silage. This effect may be due to low pH conditions in silage which may have suppressed growth of some lactic acid bacteria.

Ethanol in the silage is most likely produced by yeasts, since the most common heterofermentative lactic acid bacterium in corn, Lactobacillus brevis, does not ferment glucose to ethanol under anaerobic conditions (Edwards and McDonald, 1978). Because treatment with SA inhibits growth of yeasts (Salunke, 1956; Lacey et al., 1981), ethanol production is limited, which explains lower ethanol contents in these silages compared to control. Larger amounts of fiber were probably also associated with SA's inhibitory effect on fungi.

Use of AS in corn silage generated somewhat greater amounts of ethanol, indicating that some of the WSC available from starch hydrolysis were fermented by yeasts. Addition of glucose to ryegrass and alfalfa was reported to greatly increase concentrations of ethanol in silage (Seale et al., 1986; Chamberlain, 1988). Thus, presence of great amounts of glucose allows yeasts to proliferate and to result in formation of ethanol in silage which does not contribute much energy to rumen fermentation when fed to ruminants (Van Soest, 1987).

Because the steel drums used as laboratory silos were poor insulators, ensiling temperatures were affected by

changes in ambient temperature. Storage temperatures, however, never reached the optimum for clostridial growth (37°C). Thus, growth and metabolism by clostridia were not favored. Clostridial fermentation of glucose to butyric acid was somewhat greater in the control than the additive-treated silages, but still well within the limits of a good-quality silage (Zimmer, 1966). Proteolytic clostridia are assumed to be the major cause for ammonia formation, generally occurring in silage with low DM, high pH and high ensiling temperatures (McDonald, 1981). Isoacids along with ammonia are generated from the amino acids valine and leucine by the Stickland reactions of proteolytic clostridial enzymes (McDonald, 1981). Since concentrations of both isoacids in AS-treated silage were not different from the control, the small increase in ammonia-N must have come from breakdown of nitrates or nitrites as described by Spoelstra (1985). Addition of SA somewhat reduced ammonia-N formation along with isoacid production, probably caused by SA's ability to inhibit some bacteria.

Larger amounts of non-fermented WSC in silage may enhance rumen fermentation activity. One mole of glucose fermented in the rumen generates 4 moles of ATP via the Embden-Meyerhof-Parnas pathway, whereas lactic acid produces only 0.5 mole of ATP via the acrylate pathway during rumen fermentation (Chamberlain, 1987). Microbial growth efficiency may be improved when energy and nitrogen

availability in the rumen are balanced (Stern and Hoover, 1979). Further investigation of effects of SA- or AS-treated silages on rumen fermentation activity are required before conclusions regarding animal performance can be drawn.

6. IMPLICATIONS

The results presented in this experiment were obtained under laboratory conditions using small-scale silos and thus, need to be confirmed with experiments under farm conditions before recommendations can be made. However, since addition of sorbic acid preserved greater amounts of WSC in silage, it may provide an additional energy source for ruminal fermentation and ultimately, enhance growth performance. Treatment with an amylolytic compound provided somewhat greater quantities of WSC, but also larger amounts of ammonia and ethanol which are generally considered to be undesired metabolites during fermentation. In this laboratory-scale experiment, treatment with sorbic acid showed more potential for improving silage quality and nutritive value for the ruminant animal than addition of amylase-sterilant or both additives combined and thus, may be economically more beneficial for the producer.

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III. NUTRITIVE VALUE AND RUMEN FERMENTATION IN SHEEP FED CORN SILAGES TREATED WITH SORBIC ACID, AN AMYLASE- STERILANT OR BOTH ADDITIVES COMBINED

1. ABSTRACT

A balance trial was conducted with sheep to study effects of treating corn silage with sorbic acid (SA), amylase-sterilant (AS) or both (SA+AS) on nutrient digestibility and nitrogen utilization. A subsequent rumen fermentation experiment with the same experimental animals investigated effects of changes in silage fermentation on rumen fermentation activity as measured by in-vitro gas production, rumen VFA production and microbial protein synthesis.

Addition of SA, AS and SA+AS increased concentrations of retained water-soluble carbohydrates by 52, 47 and 36%, respectively, compared to the control ($P < .10$). In-vitro (ruminal) gas production rate was almost twofold higher with SA- and AS- treated silages ($P < .10$). Total VFA production was greater with all additive treatments. As expected, concentration of ruminal microbial protein was increased by 125 and 133% with AS and SA addition, respectively, compared to the control ($P < .10$). Effects of SA+AS were not additive, but rather reduced changes in most parameters.

Enhanced gas (probably mainly CO_2) production and microbial protein synthesis are indicative of a more energy-

efficient rumen fermentation, and may account for improved animal performance previously seen with beef heifers fed the same experimental corn silages.

2. INTRODUCTION

Two major compositional changes occur in the silo, namely conversion of WSC to short-chain organic acids and alcohol, and degradation of plant protein to NPN compounds (Andrieu, 1976; Wilkinson, 1976). Following consumption of corn silage, these metabolites are immediately utilized by rumen microorganisms as energy or nitrogen source for growth and protein synthesis or directly absorbed into the abomasum (Russell and Hespell, 1981; Baldwin and Allison, 1983).

Nutrient digestibility of silage, rumen fermentation pattern and, ultimately, animal productivity can be positively affected by altering the type of fermentation and extent of nutrient transformation in the silo by means of fermentation manipulators. Fermentation of ensiled whole-plant corn can be manipulated by addition of a selective microbial inhibitor, such as sorbic acid, or a stimulant, such as an amylase. Sorbic acid (SA) had shown to be effective in reducing surface spoilage (Alli et al., 1985; Leahy, 1988). Improved cattle performance was reported when an amylase-sterilant (AS) was added to the ensiled corn crop (Bolsen and Ilg, 1980; Leahy et al., 1990).

Objectives of this experiment were (1) to test the

effectiveness of SA, AS or SA+AS in producing good-quality silage with improved nutritive value and (2) to try to explain improvements in cattle performance, especially with AS treatment. For this purpose, nutrient digestibility, nitrogen metabolism and rumen fermentation activity in sheep were studied with these treatments.

3. MATERIALS AND METHODS

Experimental Silages

Corn plants (Pioneer Hybrid 3147) were harvested at the late dough to early dent stage, chopped into 6.4 to 12.7 mm long pieces, and transported for about 1 km in a wagon. Chopped plant material was quickly unloaded into 208-l steel drums lined with two plastic bags and kept for a short time at 4.4°C until fermentation manipulators were added. The four experimental treatments were: 1) none (control); 2) .10% sorbic acid (SA; Monitor, Monsanto, St. Louis, MO); 3) .05% of a commercial enzyme product consisting of α -, β -amylases, sulfur-containing sterilant (at least 21% Sulfur) and dextrose (AS: Silo Guard II, ISF Corp., Waverly, N.Y.); 4) both additives combined at their individual application rates (SA+AS). Untreated and treated corn silages were mixed in a Marion Mixer for about 2 min. Experimental corn silages were ensiled in 208-l steel drums for approximately 2 mo.

Experimental Animals

Sixteen mature wether sheep, eight Dorset-Finn sired and eight Suffolk-Hampshire crosses, with an average body weight of 58 kg were placed in individual metabolism crates (Briggs and Gallup, 1949) and allotted by breed to each of the four experimental silages. Thus each experimental silage was fed to four sheep, two from both breeds.

Digestion and Nitrogen Metabolism Experiment

Sheep were group-fed untreated corn silage for 7 days prior to the start of this experiment to become accustomed to silage consumption. During a 1-wk adjustment period, each sheep's maximum feed intake was established. During the succeeding preliminary phase of 4 d and the subsequent collection period, DM intake was reduced to approximately 1.5% of the animal's body weight allowing a 10% feed refusal. A 7-d collection period followed, during which daily feed intake, refusal, feces and urine were recorded.

Silage samples (400 g) were collected daily, composited and frozen for later analyses. Proximate components (AOAC, 1980), NDF, ADF and acid detergent fiber nitrogen (ADFN; Goering and Van Soest, 1970) were determined. Water-soluble carbohydrates (WSC) were quantified with the phenol-sulfuric acid method described by Dubois et al. (1956). A simultaneous analysis of ethanol, VFA and lactic acid was performed using the gas chromatographic method described in detail in a companion study (Chapter II).

Ammonia-N was measured using an ion-specific electrode (Corning, Medfield, MA). Representative samples of refusal were also collected each day and frozen for DM determination.

Total volume of urine and amount of feces excreted during each day of collection were recorded. Ten percent aliquots of urine and feces were taken, composited and refrigerated. Urine was preserved with 6N HCL and feces with thymol crystals. Kjeldahl nitrogen analysis was conducted on urine samples. Analyses for determination of DM, proximate constituents, NDF and ADF were performed on fecal samples. Apparent digestion coefficients of each nutrient, percent TDN and nitrogen utilization parameters were calculated.

Rumen Fermentation Experiment

Nine days after the end of the total-collection digestion experiment, rumen fermentation activity of the same experimental animals was determined. Each sheep had been fed its respective experimental silage for approximately 4 wk. Two 40-ml replicate samples of rumen fluid were obtained from each sheep via orogastric tube and negative pressure approximately 5 h after their morning meal. Forty ml of fluid were used to determine in-vitro gas production, the remaining sample was quickly frozen for later analyses of rumen VFA and microbial protein.

Gas production. A constant-volume manometric technique (modified from Umbreit et al., 1964) was used for this purpose. The tube's design was based on the principle that at constant temperature and initial gas volume, any alterations in the amount of gas would be measured by changes in pressure.

In each of the 32 manometric plastic tubes (four tubes per treatment with their respective duplicates), 30 ml of artificial saliva and 0.5 g of a standard substrate (alfalfa hay) were mixed. All tubes were immersed into a prewarmed waterbath (39°C) prior to the start of the experiment (Figure 1). To account for a possible location effect in the waterbath, the placement of replicate tubes was balanced. After addition of 20 ml of rumen fluid to two replicate tubes, they were thoroughly flushed with CO₂ and tightly capped. Following a short incubation period, gas generated from rumen microbial metabolism was quantified by displacement of water within the side column for 3 h.

Rumen VFA. Sixteen frozen rumen fluid samples were thawed and centrifuged at 5,000 x g for 5 min. Aliquots of 5 ml were removed from the supernatant and acidified with 1 ml of 25% phosphoric acid. After 30 min in the refrigerator, samples were again centrifuged at 10,000 x g for 10 min. A Hewlett Packard gas chromatograph (Model # 5890A) was used to quantify rumen VFA by injecting two 2 µl

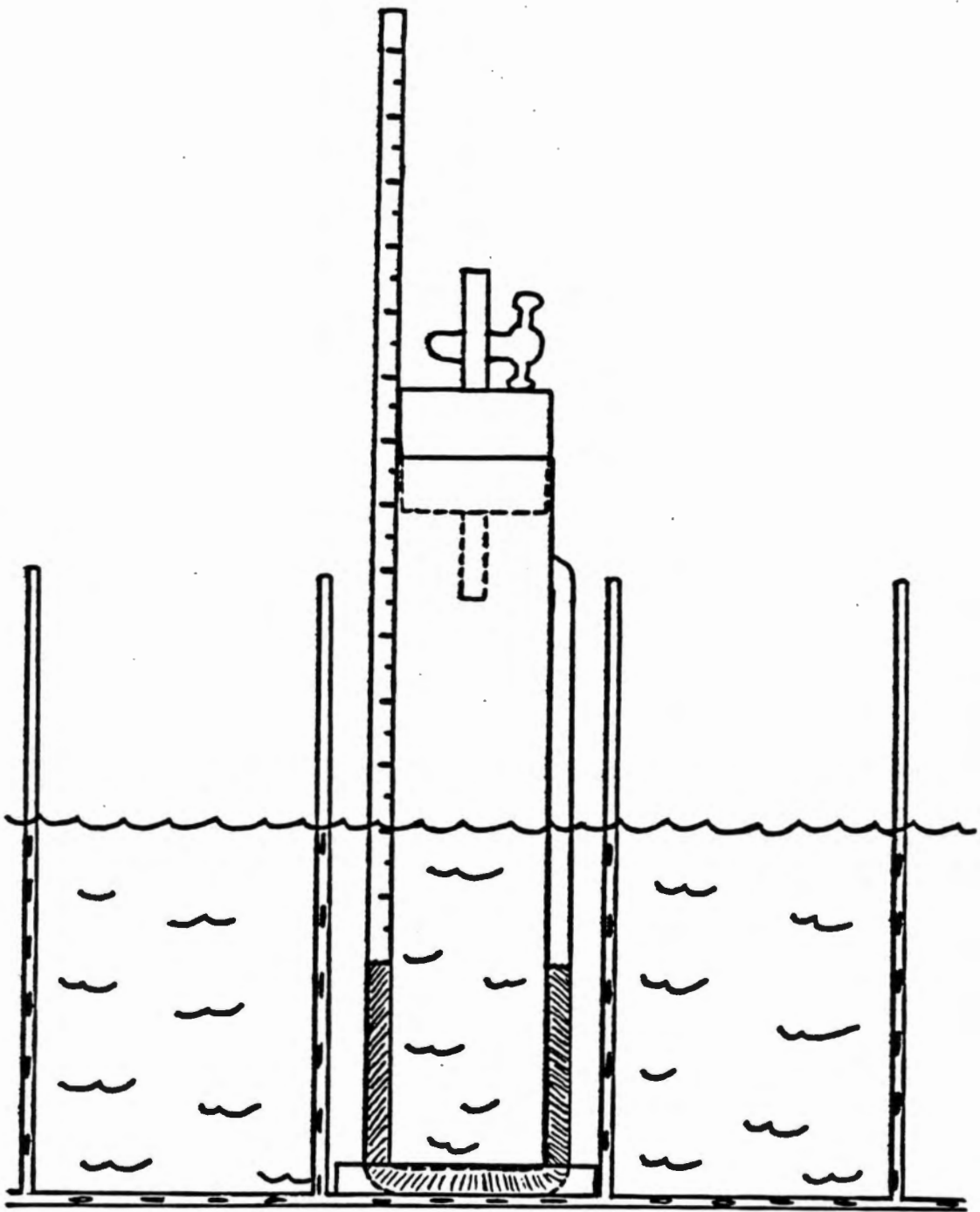


FIGURE 1. IN-VITRO GAS PRODUCTION APPARATUS

replicate samples of the supernatant onto a 10.0 x 0.53 mm silica capillary column (Model # HP-FFAP). Amounts of individual rumen VFA were calculated by a Hewlett Packard computing integrator in reference to the calibration standard curves.

Microbial protein synthesis. Concentration of microbial protein was determined on frozen rumen fluid samples using D-alanine as marker for bacterial nitrogen. Hydrolysates of the 16 rumen fluid samples were prepared by adding 10 ml of 6N HCL and 0.2 g of activated charcoal to 0.25 g of sample in screw-cap glass tubes. All tubes were thoroughly flushed with CO₂, tightly closed and placed in a 100°C oven for 24 h. After filtration through Whatman no. 5431 filter paper, sample solutions were evaporated to dryness under a ventilation hood. After diluting the dried samples to a volume of 10 ml with distilled water, two replicate aliquots (0.1 ml each) of these hydrolysates with their respective blanks were analyzed for D-alanine with the method described by Garrett et al. (1982, 1987). Concentrations of D-alanine in the sample were calculated by reference to a standard curve.

Statistical Analysis

Effects of treatments on silage characteristics, digestibility, nitrogen-utilization parameters, rumen VFA, gas production and microbial protein synthesis were

determined by an ANOVA using GLM procedures (SAS, 1985) with the model:

$$Y_i = \mu + T_i + E_i$$

where μ = mean, T_i = treatment i , E = residual error.

A Duncan's multiple-range test was used for mean separation (SAS, 1985). Significance was declared at $P < .10$ unless otherwise noted.

4. RESULTS

Digestion and Nitrogen Metabolism Experiment

Crop Composition. Fermentation characteristics of experimental corn silages are shown in Table 1. All preserved silages had a pH below 4.2 and contained less than .04% butyric acid (on dry matter basis). Sorbic acid (SA)-treated silage contained 53% more WSC than untreated silage ($P < .10$). Amylase-sterilant (AS) treatment resulted in 47% greater retention of WSC than control and somewhat greater levels of butyric acid, ethanol and isobutyric acid ($P < .10$). Treatment with both additives (SA+AS) resulted in 35.6% greater WSC concentration ($P < .10$); concentrations of fermentation acids and ethanol were not different from the control, indicating that effects were not additive.

Nutrient Digestibility And Nitrogen Metabolism

Nutrient composition and apparent nutrient digestibilities are presented in Table 2. Nitrogen free

TABLE 1. FERMENTATION CHARACTERISTICS OF CORN SILAGES
TREATED WITH SILAGE ADDITIVES

	Silage additive ^a				SE ^b
	None	SA	AS	SA+AS	
pH	4.03 ^{c,d}	4.00 ^c	4.07 ^d	4.00 ^c	.01
	----- % DMB ^c -----				
WSC ^f	13.2 ^c	20.1 ^b	19.4 ^g	17.9 ^d	.81
Lactic acid	5.59	5.91	6.07	5.70	.10
Acetic acid	1.99	1.96	1.99	1.92	.02
Butyric acid	.023 ^{c,d}	.021 ^c	.031 ^g	.025 ^d	.001
Ethanol	1.53 ^{c,d}	1.59 ^{c,d}	1.65 ^g	1.47 ^c	.03
Isobutyric acid	.052 ^c	.053 ^c	.067 ^g	.059 ^d	.002
Isovaleric acid	ND ⁱ	.010	ND	ND	.001

^a SA=Sorbic acid; AS= α -, β -Amylases & sterilant;
SA+AS=Sorbic acid and α -, β -Amylases & sterilant.

^b Standard error of treatment means; n=3.

^{c,d,g,h} Means in the same line with different superscript differ (P< .10).

^c DMB=on a dry matter basis

^f WSC=water-soluble carbohydrates

ⁱ ND=not detectable

TABLE 2. NUTRIENT COMPOSITION AND APPARENT NUTRIENT DIGESTIBILITIES OF CORN SILAGES

	Silage additive ^a				SE ^b
	None	SA	AS	SA+AS	
DM, %	32.0 ^c	32.4 ^d	32.3 ^d	32.6 ^c	.07
----- Composition, %DMB ^f -----					
NFE ^g	41.4 ^{c,d}	45.0 ^c	40.5 ^c	43.0 ^{d,c}	.62
NDF	52.0 ^c	48.7 ^c	51.0 ^{d,c}	50.2 ^{c,d}	.43
ADF	30.4 ^c	27.7 ^c	30.0 ^c	28.9 ^d	.35
Hemicellulose	21.6	21.0	21.0	21.3	.15
Cellulose	26.8 ^d	24.5 ^c	26.8 ^d	25.4 ^c	.33
----- Digestibility, % -----					
DM	63.9	66.2	65.5	64.8	.55
NFE ^g	85.5 ^c	87.2 ^{c,d}	85.1 ^c	88.3 ^d	.52
NDF	53.5	52.0	53.8	51.6	.78
ADF	51.5	50.4	53.6	49.4	.87
Hemicellulose	56.5	54.2	54.1	54.5	.79
Cellulose	56.7	55.2	60.0	55.7	.97
TDN	63.8 ^c	66.9 ^d	65.6 ^{c,d}	64.6 ^{c,d}	.58

^a SA=Sorbic acid; AS= α -, β -Amylases & sterilant; SA+AS=Sorbic acid and α -, β -Amylases & sterilant.

^b Standard error of the compositional treatment means; n=3. Standard error of digestibility treatment means; n=4.

^{c,d,c} Means in the same line with different superscript differ (P< .10).

^f DMB=Dry matter basis

^g NFE=Nitrogen-free extract.

extract of SA- and SA+AS-treated silages were 8.7% ($P < .10$) and 3.9% greater than the control. Treatment with SA and SA+AS also significantly reduced NDF, ADF and cellulose concentrations in silage.

Dry matter intakes were similar among the four groups of experimental animals, averaging $.87 \text{ kg} \cdot \text{d}^{-1}$. Nitrogen-free extract (NFE) digestibility and TDN were 2.0 and 4.9% ($P < .10$) greater in SA-treated silage or 3.3 ($P < .10$) and 1.3% greater in SA+AS-treated silage than the control. Treatment with AS did not significantly affect NFE, NDF, ADF or cellulose contents in silage, and no effects on digestibility of NFE, NDF or ADF were noted.

Although SA- and SA+AS-treated corn silages were 6.2 and 4.1% lower in CP ($P < .10$), no effect on ammonia-N concentrations was found (Table 3). Nitrogen metabolism was also not different with these silages compared to the control. Treatment with AS did not affect any dietary nitrogen component or nitrogen utilization with sheep.

Rumen Fermentation Experiment

Rumen VFA. Rumen fermentation parameters are shown in Table 4. Total VFA concentration was 22.8% higher with SA-treated silage ($P < .10$), and somewhat increased with AS- (9.4%) and SA+AS-treated (8.3%) silages. Molar proportion of acetic acid was 3.8% lower with SA-treated silage than with AS- or SA+AS-treated silages ($P < .10$). Acetic/

TABLE 3. NITROGEN COMPOUNDS AND NITROGEN METABOLISM OF CORN SILAGES

	Silage additive ^a				
	None	SA	AS	SA+AS	SE ^b
----- Composition, %DMB ^c -----					
Crude protein	9.19 ^c	8.62 ^d	9.14 ^c	8.81 ^d	.09
Ammonia-N ^f	.959 ^{d,c}	.957 ^{d,c}	.982 ^c	.903 ^d	.015
ADFN ^g	7.99	7.91	8.25	8.42	.11
---- Nitrogen Metabolism, % ----					
TND ^h	84.8	85.2	85.4	83.5	.88
TMBV ⁱ	61.8	61.4	64.4	62.6	1.02
NPU ^j	52.5	52.4	55.0	52.2	1.05

^a SA=Sorbic acid; AS= α -, β -Amylases & sterilant; SA+AS=Sorbic acid and α -, β -Amylases & sterilant.

^b Standard error of the compositional treatment means; n=3. Standard error of digestibility treatment means; n=4.

^c DMB=Dry matter basis

^{d,c} Means in the same line with different superscript differ (P< .10).

^f Ammonia-N expressed as a percent of total nitrogen.

^g ADFN=Acid detergent fiber nitrogen as percent of total nitrogen.

^h TND=True nitrogen digestibility.

ⁱ TMBV =Thomas-Mitchell Biological Value calculated as N-intake - [(fecal N-metabolic fecal N)-(urinary N-endogenous urinary N)]/N intake-(fecal N-metabolic fecal N). Metabolic fecal N=4.35xDM intake; endogenous urinary N=.034/body weight.

^j NPU=Net protein utilization calculated as Biological value x true N digestibility.

TABLE 4. RUMEN VOLATILE FATTY ACIDS IN SHEEP

	Silage additive ^a				SE ^b
	None	SA	AS	SA+AS	
No. of sheep per treatment	4	4	4	4	
Total VFA ^c , $\mu\text{mol ml}^{-1}$	50.9 ^d	62.5 ^c	55.7 ^{d,e}	55.1 ^{d,e}	2.11
VFA ^c , Molar %					
Acetic acid	67.9 ^{d,e}	66.2 ^d	68.7 ^c	68.9 ^c	.46
Propionic acid	18.7	19.9	18.4	19.5	.40
A/P ratio	3.63	3.33	3.74	3.53	.098
Butyric acid	10.2 ^{d,e}	10.9 ^c	9.53 ^d	9.22 ^d	.279
Others ^f	3.15 ^c	2.99 ^c	3.47 ^c	2.33 ^d	.137

^a SA=Sorbic acid; AS= α -, β -Amylases & sterilant; SA+AS=Sorbic acid and α -, β -Amylases & sterilant.

^b Standard error of treatment means; n=4.

^c VFA=Volatile fatty acids

^{d,e} Means in the same line with different superscript differ (P< .10).

^f Others=isoacids and valeric acid

propionic acid ratio was not affected by treatments. Molar proportion of butyric acid was 16.3% higher with SA-treated silage than AS- or SA+AS-treated silages ($P < .10$). Isoacid proportion was highest with AS-treated silage and the molar concentration was significantly higher than the control (1.51 vs $1.18 \mu\text{mol ml}^{-1}$).

Total Gas Production

Total gas production was increased almost twofold with SA- or AS-treated silages ($P < .10$) (Table 5). Consumption of SA+AS-treated silages did not affect gas production. Larger amounts of gas produced were associated with less body weight losses by sheep ($r = .77$, $P < .10$).

Microbial Protein Synthesis

Addition of SA or AS resulted in a 125 or 133% increase ($P < .10$), respectively, in rumen microbial protein synthesis (Table 5). Although animals on SA+AS-treated silage showed 61% greater microbial protein concentration, it was not significantly different from that of the control group. As expected, greater amounts of protein synthesized by rumen microorganisms were also associated with smaller body weight losses by sheep ($r = .91$, $P < .10$). Notable was also a positive relationship of microbial protein synthesis with gas production ($r = .67$, $P < .10$).

TABLE 5. BODY WEIGHT CHANGE, IN-VITRO GAS PRODUCTION AND SYNTHESIS OF MICROBIAL PROTEIN IN SHEEP

Treatment ^a	BW ^b , kg	GP ^c , $\mu\text{l ml}^{-1}$	MPS ^d , mg g^{-1}
None	-4.61 ^e	293.2 ^e	6.90 ^e
SA	-2.62 ^f	550.9 ^f	15.5 ^f
AS	-1.82 ^f	576.5 ^f	16.1 ^f
SA+AS	-3.07 ^f	311.6 ^e	11.1 ^{e,f}
SE ^g	.32	51.2	1.25

^a SA=Sorbic acid; AS= α -, β -Amylases & sterilant; SA+AS=Sorbic acid and α -, β -Amylases & sterilant.

^b BW=Change in body weight during entire experiment.

^c GP=Gas production measured during 3-h incubation period. Gas production was positively correlated with change in body weight ($r = .77$, $P < .10$).

^d MPS=Microbial protein synthesis. Microbial protein synthesis was positively correlated with change in body weight ($r = .91$, $P < .10$) and gas production ($r = .67$, $P < .10$).

^{e,f} Means in the same column with different superscript differ ($P < .10$).

^g Standard error of the overall treatment means; $n=4$.

5. DISCUSSION

Silage Fermentation

The magnitude of effects of silage additives on fermentation efficiency, nutritive value of corn silage and, ultimately, animal performance sustained by the silage are dependent on the quality of untreated silage, i.e. it is easier to improve a low-quality than a high-quality silage. In this experiment, high DM and WSC content of the corn crop favored good preservation. Untreated silage had more than 50% of fermentation acids present as lactic acid, a pH of 4.0, less than 1% of total nitrogen in form of ammonia and a negligible amount of butyric acid and could, therefore, be considered a good-quality silage (Wieringa, 1966; Parker and Bastiman, 1982). Consequently, there was less chance for the fermentation to be improved by the use of an additive.

In the present experiment, SA-treated silage resulted in lower reduction of NFE during ensiling, because more WSC were preserved than in the control. Higher quantities of WSC in SA-treated corn silage were also found by Alli et al. (1985) and Leahy (1988). These researchers suggested that some WSC are spared as a result of reduced fungal population in the SA-treated silage. A build-up of WSC may have been a result of reduced utilization by lactic acid bacteria. Similar contents of lactic and acetic acids between additive-treated and control silages as found in the present experiment indicate no difference in substrate utilization,

although it was available. It appears that some lactic-acid producing bacteria may have been inhibited by the activity of SA. Reduced protein degradation in silage due to SA treatment, as noted by Alli et al. (1985) was not found in this experiment.

Similar amounts of NFE, but greater WSC content in AS-treated silage seems to indicate that more starch was hydrolyzed to glucose and maltose which could be utilized by homo- or heterofermentative lactic acid bacteria to lactic acid, acetic acid or ethanol. Higher amounts of WSC in amylase-treated silage were also observed by Alli and Baker (1982). Although amylolytic enzymes occur naturally in the leaves of the corn plant (Gates and Simpson, 1968), they seem to require a longer induction period to become active (Alli and Baker, 1982). Addition of amylase to plant material rich in starch was found to accelerate mobilization of the carbohydrate reserve by starch hydrolysis (Gates and Simpson, 1968), thereby making more substrate available to silage microorganisms immediately after ensiling (Alli and Baker, 1982). However, only a small part of the available substrate was fermented by lactic acid bacteria to lactic acid, acetic acid or ethanol in this experiment. Concentration of lactic acid was only slightly higher in AS-treated silage than the control. This agrees with findings of Bolsen and Ilg (1980). Substrate utilization by lactic acid bacteria may have been limited because of the

inhibitory action on these bacteria by the sterilant component in this treatment, SO_2 , which is a powerful antimicrobial agent (Woolford, 1984).

Leahy et al. (1990) suggested that addition of the combined treatment with SA and AS (SA+AS) may decrease different degradative processes at different times during ensiling. This hypothesis, however, could not be supported by results from the present study, since greater amounts of starch remained intact and a lower concentration of WSC was found in this silage when compared to SA- or AS-treated silages.

Nutrient Digestibility

Treatment with SA, AS or SA+AS had no significant effects on most nutrient digestion coefficients. Froetschel et al. (1991) found enhanced digestibility of DM and CP in wheat silage treated with AS. In the present experiment, addition of AS increased %TDN and numerically improved DM digestibility which may have contributed to enhanced growth performance by beef heifers previously reported by Leahy et al. (1990). Addition of SA or SA+AS showed improved digestibility of NFE and TDN, probably due to greater amounts of readily fermentable carbohydrates in these silages.

Nitrogen Utilization

Nitrogen metabolism was not altered by silage treatments. Because nitrogen utilization was not affected by AS treatment, it could not be the cause for improved ADG and feed efficiency by beef heifers as noted by Leahy et al. (1990).

Effects of organic acid treatments on nitrogen retention and digestion reported in the literature are not consistent. Yu and Thomas (1975) and Wing et al. (1976) found increased nitrogen digestion and retention by propionic acid-treated silages, whereas Hills (1984) observed decreased nitrogen retention in corn silage treated with acrylic acid.

Rumen Fermentation

Greatly simplified, rumen fermentation can be expressed as the conversion of dietary fermentable carbohydrates and ammonia to microbial growth, VFA, CO₂ and CH₄ (Van Soest, 1987). A change in the amount of the dietary components alters this balance and must result in changed microbial population (numbers, profile) and amounts of fermentation metabolites. As expected, because of higher concentrations of retained WSC, silages treated with SA, AS or SA+AS resulted in higher total amounts of rumen VFA when fed to sheep. Once absorbed, short-chain fatty acids, such as acetic and butyric acids, become the primary energy source

to the host animal, while propionic acid is reserved for gluconeogenesis (Van Soest, 1987). However, molar proportions of rumen VFA were not affected by silage treatments. Similar results for molar proportions of acetate, propionate and butyrate were found by Bolsen et al. (1982) and Jaster and Moore (1988) with enzyme-treated silages.

Total gas production was suggested to predict rumen fermentation activity and microbial growth (El-Shazly and Hungate, 1965) and was found to be positively correlated with numbers of viable rumen microorganisms (EL-Din and El-Shazly, 1969). In this study, total gas produced by rumen microorganisms was increased almost twofold with AS or SA treatments. Leahy (1988) also reported an increased total gas production (in-vitro) with AS treatment and numerically enhanced values with SA or SA+AS treatments in beef heifers. This measurement does not differentiate between amounts of CO_2 , CH_4 and H_2 formed, thus increases in the total amount of gas produced do not necessarily imply enhanced microbial protein synthesis (Bartley et al., 1979; Riddell et al., 1980). However, if a molar ratio of 6:3.5 for CO_2 : CH_4 is assumed in the rumen (Wolin, 1979), together with no difference in molar proportions of VFA, it must be concluded that CO_2 contributed more to the marked increase in total gas production than CH_4 . Because gas production was highly correlated with changes in body weight of sheep, the method

used in this study to determine total gas production predicted changes in sheep performance well.

Stern and Hoover (1979) reported that nitrogen and energy availability in the rumen must be balanced to achieve maximum efficiency of microbial growth. Rumen micro-organisms are an important source of protein for the host animal. Their growth rate can significantly influence availability of amino acids to the animal for growth. Higher total rumen VFA levels with SA, AS and SA+AS indicate increased availability of energy for rumen microbial synthesis from fermentation due to retained WSC in the silage. Since dietary nitrogen was supplied adequately, although less in SA- or SA+AS-treated silages, nitrogen and energy were more efficiently utilized for microbial protein synthesis when compared to the control. In addition, AS contained sulfur-salts which may have been utilized for synthesis of methionine and cysteine in the rumen. Stern and Hoover (1979) found maximum production of microbial protein when nitrogen/sulfur ratio was optimal. Presence of sulfur in AS-treated silage may have contributed to the marked improvement in ADG and feed conversion by beef heifers reported by Leahy et al. (1990).

6. IMPLICATIONS

All experimental silages were of good quality in this experiment. All additive-treated silages maintained higher

quantities of WSC, thus more potential energy was made available to rumen microorganisms for growth and protein synthesis. Silage treated with AS also provided more dietary nitrogen and additional sulfur from the sterilant component. It not only resulted in greater production of microbial protein, but also may have provided sulfur-containing amino acids for the host animal. Treatment with SA showed some improvement in rumen fermentation activity and animal performance by sheep. From the results in this experiment, addition of SA or AS to ensiled whole-plant corn is recommendable, since they improved silage quality and enhanced rumen fermentation activity in sheep. Addition of SA+AS did not result in markedly improved rumen fermentation and performance of sheep was not enhanced, thus the combined treatment should not be used as silage additive.

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IV. FERMENTATION OF CORN SILAGE TREATED WITH AMYLASE-CONTAINING ADDITIVES: EFFECTS ON DRY MATTER LOSSES IN THE SILO AND FEEDLOT PERFORMANCE OF BEEF HEIFERS

1. ABSTRACT

Effects of three amylase-containing products (α -amylase, α -AM; α - and β -amylases, $\alpha\beta$ -AM; α -, β -amylases and sulfur-salts, $\alpha\beta$ -AS) on changes in chemical composition and microflora of whole-plant corn during fermentation were compared using small-scale laboratory silos. A feedlot trial with cattle fed amylase-treated corn silage also was conducted to investigate the causative agent responsible for increased animal performance.

Addition of α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS to corn resulted in 40.7%, 41.4% and 60.3% greater amounts water-soluble carbohydrates, respectively, than the control on d 28 post ensiling ($P < .10$). These amylase treatments had also an average 15.2% increased lactic acid concentration in corn silages on d 28 ($P < .10$). Amount of ammonia-N formed was greater in α -AM (15.0%) and $\alpha\beta$ -AS (14.3%) treated silages after 4 wk of ensiling ($P < .10$). There was no clear effect of amylase treatments on ethanol and VFA which appeared to be consistent throughout fermentation. Concentrations of NDF and ADFN were slightly reduced in amylase-treated silages compared to control. Addition of amylases did not greatly change numbers of lactic acid bacteria during

ensiling. However, there was a significant decrease in fungal count on d 28 with $\alpha\beta$ -AM (1.17 log) or $\alpha\beta$ -AS (1.10 log) treatments.

Dry matter recovery of corn silages (9-t farm-scale silos) was markedly increased (6.3 to 10.7%) with amylase treatments ($P < .10$). Improvements in wt gains of beef heifers were 6.8, 5.2 or 12.0% with α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS treatments, respectively, when compared to the control.

2. INTRODUCTION

Corn plants are rich in starch which is not directly metabolized by most lactic acid bacteria (Woolford, 1984). Although leaves of corn plants have amylases, these enzymes require some time to become active (Gates and Simpson, 1968; Alli and Baker, 1982). Lack of immediately available substrate could prevent an early dominance of lactic acid bacteria in the ensiled material. By adding amylases to ensiled corn, hydrolysis of starch to fermentable sugars may be accelerated. This could result in a homolactic fermentation of silage (Bolsen and Ilg, 1980), thereby reducing nutrient losses from the originally ensiled material (McDonald, 1981). Greater nutrient recovery, i.e. water-soluble carbohydrates (Alli and Baker, 1982; Leahy, 1988; Chapter II and III) and its ultimate importance for microbial growth and protein synthesis in the rumen, may explain improved ADG and feed efficiency by cattle with an

amylase treatment (Bolsen and Ilg, 1980; Leahy et al., 1990).

Objective of this experiment was to identify the causative agent responsible for increased growth performance with an amylase product. Effectiveness of α -amylase, α - and β -amylases or α -, β -amylases and sulfur-salts in improving fermentation and nutrient recovery of ensiled whole-plant corn was tested. The same treatments were also used in a silage feeding trial with beef heifers to compare extent of improvement in wt gains.

3. MATERIALS AND METHODS

Fermentation Study

Silage preparation. Whole-plant corn (Pioneer Hybrid 3147) was harvested in the early dent stage of maturity and cut to a chop-length of approximately 12 mm. Chopped plant material was quickly transported about 1 km to a holding area, where it was temporarily unloaded into 208-liter steel drums. Approximately 100 kg of plant material was mixed in a Marion Mixer and contained one of the following additives: 1) none (control); 2) α -amylase from a Bacillus species (α -AM: Sigma Chem. Corp., St. Louis, MO); 3) α -, β -amylases from barley malt ($\alpha\beta$ -AM: Sigma Chem. Corp., St. Louis, MO); 4) a commercial product composed of α -, β -amylases, sulfur-salts (at least 21% sulfur) and dextrose ($\alpha\beta$ -AS: Silo Guard II, ISF Corporation, Waverly, N.Y.). Alpha-amylase activity

of the three treatments was approximately 300 units·kg⁻¹ of plant material (fresh basis), one Sigma unit liberating 1 mg of maltose from starch in 3 min at pH 6.9 at 20°C. For even distribution, feed-grade dextrose was added to amylase products (α -AM: .12% dextrose on DM basis; $\alpha\beta$ -AM: .07% dextrose on DM basis; $\alpha\beta$ -AS: unknown amount of dextrose had been added by manufacturer). All treatments were added on a 0.05% fresh weight basis. Sixty-four 19-l plastic buckets were used as laboratory silos and filled with approximately 8 kg of chopped whole-plant corn. There were two replicate buckets per treatment and sampling time. Two silage samples (ca. 400g) each were taken on 1,2,3,7,14, 21 and 28 d post ensiling and frozen immediately for later microbial and chemical determination.

Chemical parameters. Proximate components (AOAC, 1980) NDF, ADF and ADFN (Goering and Van Soest, 1970) were determined on oven-dried silage samples. Silage pH, WSC, ammonia-N, ethanol, VFA and lactic acid were measured using homogenized frozen silage samples which were mixed with liquid nitrogen and blended in a high-speed Waring blender (Parker, 1978).

Silage pH was determined in aqueous extracts by glass electrode. Ammonia-N was quantified by ion-specific electrode (Corning, Medfield, MA). The phenol-sulfuric acid method of Dubois et al. (1956) was used to determine WSC in

aqueous silage extracts. Aqueous extracts, prepared as described by El Hag et al. (1982), were used for simultaneous gas chromatographic determination of ethanol, VFA and lactic acid. Four ml of silage extract were acidified with 1 ml of 0.03 M oxalic acid and centrifuged ($10,000 \times g$) for 10 min. A Hewlett-Packard gas chromatograph (Model # 5890A) was fitted with a 1.83 m x 2 mm ID glass-lined column packed with 80/120 Carbopack B-DA/4% Carbowax 20M (Supelco, Inc., Bellefonte, PA). The column was pre-conditioned for 19 h at 245°C. The gas chromatograph was operated isothermally at 175°C (column) and 200°C (inlet and detector). The carrier gas used was nitrogen with a flow rate of 24 ml·min⁻¹.

Microbiological analysis. Silage samples (25 g) were placed into a sterile plastic bag and blended with 225 ml of 0.1% peptone water in a stomacher for 3 min. Decimal dilutions of the homogenized sample in peptone water were prepared from 10⁻¹ through 10⁻⁹.

Lactic acid bacteria were quantified using a spread-plate technique with de Man-Rogosa-Sharpe agar (MRS). Plates were inverted and incubated in a CO₂ incubator at 30°C for 48 h. Yeasts and molds were estimated using a pour-plate technique with molten Rose Bengal Chlorotetra- cycline Agar (RBCA). This medium was chosen as a selective culture medium for organisms, because the antimicrobial agent

chlortetracycline and the dye, rose bengal, both inhibit bacterial growth. Both ingredients were added to the molten agar just prior to use. The solidified plates were inverted and incubated at room temperature (ca. 21°C) for 5-7 d. Coliform bacteria were estimated using the pour-plate method with Violet Red Bile Agar (VRBA). This medium exploits the ability of coliforms to tolerate bile salts (Woolford et al., 1978). After solidification of the medium, each plate was overlayed, inverted and incubated at 32°C for 24 h.

Temperature measurement. Eight 19-l plastic buckets, two per treatment, were lined with two polyethylene bags and filled with approximately 8 kg of either untreated or treated chopped plant material. A thermocouple was inserted into the center of the material prior to sealing, contents were compacted to exclude air and buckets were closed tightly. Laboratory silos were kept in a warm room (ca. 25°C) during the 28-d ensiling period to prevent great variations in ambient temperature and allow for good ensiling conditions. Ensiling temperature was measured on days 0, 1, 2, 3, 7, 14, 21, and 28. Ambient temperature was recorded at the same time.

Feeding Trial

Silage production. Corn (FFR 767) was seeded in June of 1989 at the rate of 55,000 kernels/ha¹. Fields received 596 kg of 9-18-27 fertilizer/ha¹, 2 kg of Atrazine 9-0, 5.4

1 of Lasso and 2.3 l of Lannate herbicides ha⁻¹. Ammonium nitrate (337 kg ha⁻¹) was side-dressed on the field. Corn plants were harvested in the early dent stage.

Dry matter losses. Eight upright brick silos were used to supply silage for the growth trial and to determine DM losses during primary fermentation. Two silos (capacity ca. 9 t each) were assigned to each of the four aforementioned experimental treatments. Samples of the harvested plant material were taken immediately after appropriate treatment was added. Chopped whole-plant corn was allowed to ferment for at least 100 d. Silage samples were collected when silos were first opened and at 28-d intervals thereafter. Samples were kept frozen until analyses of proximate constituents (AOAC, 1980), NDF, ADF and ADFN (Goering and Van Soest, 1970). Samples from surface spoilage were taken and frozen for DM determination. Dry matter of plant samples was determined by a two-step drying procedure (AOAC, 1980). Dry matter losses and recovery were determined for each silo by calculating DM losses which occurred during ensiling and losses due to surface spoilage. Both DM losses were added and calculated as a proportion of the DM ensiled prior to fermentation.

Growth trial. A feedlot trial with 40 Angus heifers with average initial BW of 214 kg $\pm$ 2.7 was conducted. The weanling heifers were purchased from a graded fall

feeder calf sale and placed on permanent pasture for about 2 mo. Approximately 6 wk prior to the beginning of the trial, all non-pregnant heifers and heifers pregnant less than 100 d were administered the abortifacient Prostaglandin $F_{2\alpha}$ (25 mg; lutalyse). Heifers pregnant more than 100 d were excluded from the trial. The reason for treating non-pregnant animals also, was to prevent any possible effect of the abortifacient on animal performance. Approximately 1 wk before the trial, BW, backfat thickness (sonarary measurement over 13th rib), frame and muscling scores (visual appraisal) of all heifers were determined. Results were used to allot five animals to each of eight pens. One wk before the feeding trial, all animals were fed increasing amounts of untreated corn silages as acclimation to silage feeding. The same experimental corn silages (from 9-t farm silos) which were used for determination of DM recovery were also utilized in this study.

Each pen of heifers had ad libitum access to silage. In addition, each animal received approximately 0.75 kg of hay and 0.50 kg of soybean meal to meet NRC protein requirements (NRC, 1984). Individual BW were taken twice at beginning and end of the experiment and once at 28-d intervals during the trial. Backfat thickness of heifers at beginning and end of the trial was again estimated by sonarary.

Statistical Analysis

Changes in fermentation criteria and microflora over time were assessed by an ANOVA (SAS, 1985) in a completely randomized design, using the model:

$$Y_i = \mu + T_i + E_i;$$

because of replicated measurements on the same silos, temperature data were analyzed as a split-plot design in time (Snedecor and Cochran, 1980), using the model:

$$Y_{ij} = \mu + T_i + D_j + TD_{ij} + E_{ij};$$

treatment effects on silage nutrient composition, DM recovery and growth performance of heifers (experimental unit pen) were tested by ANOVA using GLM procedures of SAS (1985) with the model:

$$Y_i = \mu + T_i + E_i$$

where μ = mean; T_i = amylase treatment i ; D_j = day of ensiling; TD_{ij} = interaction of treatment with day of ensiling; E = residual error.

Treatment means were compared by a Duncan multiple-range test (SAS, 1985). Differences were considered significant at $P < .10$ unless stated otherwise.

4. RESULTS

Fermentation Study

Crop composition during fermentation. There was no effect of treatments on DM, ADF, CP or ADFN in corn silage (Table 1). Somewhat greater N-free extract (6.8%) was found

TABLE 1. NUTRIENT COMPOSITION OF CORN SILAGES TREATED WITH AMYLASES

Item	Silage additive ^a				SE ^b
	None	α -AM	$\alpha\beta$ -AM	$\alpha\beta$ -AS	
DM, %	33.4	33.3	33.4	33.7	.16
NFE ^c , %DMB ^d	35.4	37.8	36.4	37.8	.42
NDF, %DMB ^d	50.5 ^f	46.9 ^{e,f}	48.8 ^f	46.8 ^e	.49
ADF, %DMB ^d	24.0	23.4	23.4	23.4	.21
HC ^g , %DMB ^d	26.5 ^f	23.5 ^{e,f}	25.4 ^f	23.4 ^e	.42
CP, %DMB ^d	8.01	8.09	7.97	7.91	.04
ADFN ^h , %TN ⁱ	7.73	7.28	7.53	7.58	.15

^a α -AM = α -Amylase; $\alpha\beta$ -AM = α - and β -Amylases; $\alpha\beta$ -AS = α -, β -Amylases and sterilant.

^b Standard error; n=12.

^c Nitrogen free extract.

^d Dry matter basis.

^{e,f} Means on the same line with different superscript differ (P< .10).

^g Hemicellulose. Hemicellulose is positively correlated with NDF (r= .90, P< .01).

^h Acid detergent fiber nitrogen.

ⁱ Total nitrogen.

in α -AM or $\alpha\beta$ -AS treated silages than control. A 4.1% greater N-free extract ($P < .10$) was also noted by Leahy et al. (1990) in $\alpha\beta$ -AS treated silage. Concentrations of NDF were 7.3% lower in $\alpha\beta$ -AS treated silage than the control due to a lower (11.7%, $P < .10$) hemicellulose content ($r = .90$). Neutral detergent fiber content tended also to be somewhat lower in α -AM or $\alpha\beta$ -AM treated silages. Significantly decreased NDF, ADF and cellulose concentrations were reported previously (Chapter II) in $\alpha\beta$ -AS treated corn silage after a 43-d ensiling period.

Ensiling temperature. Addition of α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS to chopped whole-plant corn had no effect on ensiling temperatures (Figure 1). Initial temperatures were similar in control and amylase-treated ensiled corn, ranging from 27.4 to 27.6°C which were also the highest temperatures recorded during ensiling. Ambient temperature was correlated with ensiling temperatures ($r = .95$).

Water-soluble carbohydrates. Treatment with amylases resulted in 16.2 to 18.3% greater WSC concentrations remaining in the corn during 28 d of ensiling when compared to control (Table 2). As early as 1 d of ensiling, amounts of WSC were greater in α -AM (37.1%), $\alpha\beta$ -AM (19.0%) or $\alpha\beta$ -AS (21.6%) treated corn than in control. By 28 d, $\alpha\beta$ -AM treated silage maintained 60.3% greater amounts of WSC than untreated silage ($P < .10$). Treatment with α -AM or $\alpha\beta$ -AS

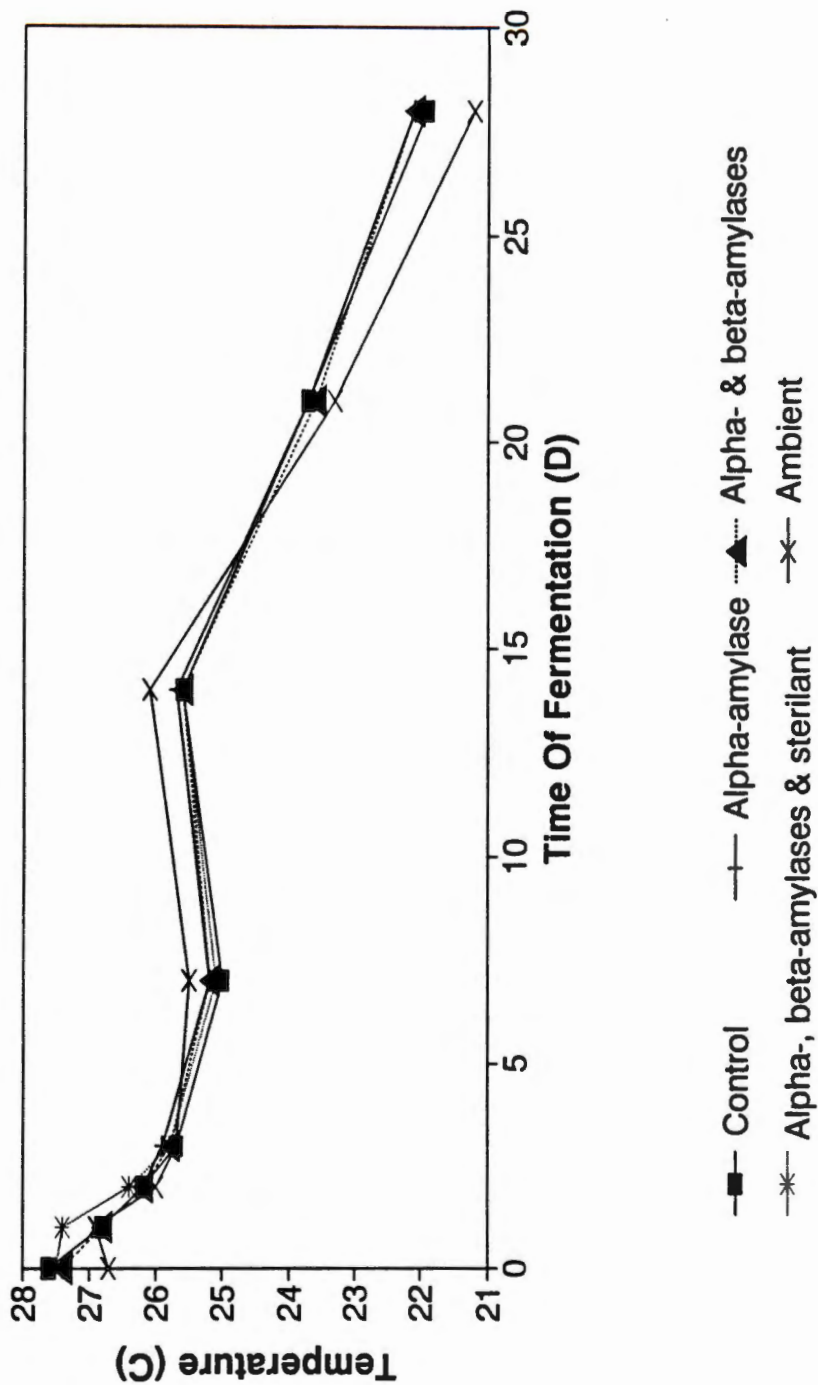


FIGURE 1. ENSILING TEMPERATURES IN AMYLASE-TREATED CORN SILAGES

TABLE 2. CHANGES IN WATER-SOLUBLE CARBOHYDRATES, LACTIC ACID AND PH OF CORN SILAGES OVER TIME

Day of ensiling	Silage additive ^a				SE ^b
	None	α -AM	$\alpha\beta$ -AM	$\alpha\beta$ -AS	
----- WSC ^c , %DMB ^d -----					
1	11.6	15.9	13.8	14.1	1.14
2	8.46	8.05	8.57	8.93	.51
21	4.90	4.93	5.32	5.66	.20
28	2.95 ^c	4.15 ^{c,f}	4.73 ^f	4.17 ^{c,f}	.32
Mean	6.98	8.26	8.11	8.22	.75
----- Lactic acid, %DMB ^d -----					
1	3.57	3.48	3.32	3.47	.06
2	3.57	3.85	3.79	3.44	.12
21	6.11 ^c	6.54 ^f	6.60 ^f	6.15 ^c	.10
28	6.47 ^c	7.55 ^f	7.42 ^f	7.39 ^f	.19
Mean	4.93	5.36	5.28	5.11	.30
----- pH -----					
0	5.25	5.25	5.15	5.10	.11
28	4.15	4.15	4.05	4.15	.04

^a α -AM = α -Amylase; $\alpha\beta$ -AM = α - and β -Amylases; $\alpha\beta$ -AS = α -, β -Amylases and sterilant.

^b Standard error; n=2.

^c Water-soluble carbohydrates.

^d Dry matter basis.

^{e,f} Means on the same line with different superscript differ (P< .10).

tended to retain somewhat greater amounts of WSC in silage.

Lactic acid and pH. Changes in lactic acid content and pH over time are also presented in Table 2. During the 4-wk ensiling period, amount of lactic acid in silages progressively increased to a mean of $7.21\% \pm .19$ (DMB), while silage pH decreased to $4.13 \pm .04$. By 28 d, increases in lactic acid concentration were 16.7, 14.7 or 14.2% for α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS treated silages, respectively ($P < .10$). Silage pH, however, did not differ among treatments. Increases in lactic acid concentration of $\alpha\beta$ -AS treated silage were greater than those reported by Bolsen et al. (1982) and in two companion studies (Chapter II and III).

Ethanol and acetic acid. On the first d, the control had more ethanol than α -AM ($P < .10$), $\alpha\beta$ -AM ($P > .10$) or $\alpha\beta$ -AS ($P < .10$) treated corn (Table 3). After 7 d, ethanol concentrations declined rapidly in all silages without effect of treatments. Somewhat lower concentrations of acetic acid were found in treated corn than control on d 1 of ensiling ($P < .10$; Table 3). Thereafter, no clear effect of treatments was found on acetic acid concentration in silage.

Butyric acid, ammonia-N and isoacids. Small amounts of butyric acid were found in all silages (Table 3), but after 1 d of fermentation, concentration of butyric acid began to decline. By 28 d, $\alpha\beta$ -AM treated silage had a somewhat

TABLE 3. CHANGES IN ETHANOL, ACETIC ACID, BUTYRIC ACID AND AMMONIA-N OF CORN SILAGES OVER TIME

Day of ensiling	Silage additive ^a				SE ^b
	None	α -AM	$\alpha\beta$ -AM	$\alpha\beta$ -AS	
----- Ethanol, %DMB ^c -----					
1	2.45 ^c	1.78 ^d	2.13 ^{d,c}	1.77 ^d	.13
2	2.72 ^{d,c}	2.02 ^d	2.89 ^c	2.64 ^{d,c}	.15
21	1.08	1.17	1.39	.97	.08
28	.44	.78	.61	.81	.15
----- Acetic acid, %DMB ^c -----					
1	1.75 ^f	1.65 ^{c,f}	1.50 ^d	1.59 ^{d,c}	.04
2	1.63	1.71	1.69	1.60	.05
21	2.75 ^c	2.63 ^{d,c}	2.30 ^d	2.73 ^c	.08
28	2.30	2.57	2.58	2.43	.07
----- Butyric acid, %DMB ^c -----					
1	.025	.032	.031	.030	.002
2	.025	.022	.028	.022	.002
21	.015	.017	.016	.014	.001
28	.013 ^d	.015 ^{d,c}	.019 ^c	.015 ^{d,c}	.001
----- NH ₃ -N, %TN ^g -----					
1	.771 ^c	.728 ^{d,c}	.680 ^d	.802 ^c	.020
2	.949 ^c	.802 ^d	.737 ^d	1.00 ^c	.042
21	1.31 ^d	1.39 ^d	1.38 ^d	1.54 ^c	.03
28	1.33 ^d	1.53 ^c	1.38 ^d	1.52 ^c	.04

^a α -AM = α -Amylase; $\alpha\beta$ -AM = α - and β -Amylases; $\alpha\beta$ -AS = α -, β -Amylases and sterilant.

^b Standard error; n=2.

^c Dry matter basis.

^{d,e,f} Means on the same line with different superscript differ (P< .10).

^g Ammonia-N as % of total N.

greater butyric acid content than the control ($P < .10$). Only a small percentage of total nitrogen in chopped corn was ammonia-N (Table 3). During ensiling, concentrations of ammonia-N increased almost twofold in all silages until 21 d, and stabilized thereafter. Silage treated with $\alpha\beta$ -AS generally had greater ammonia-N concentrations than control, particularly on d 21 and 28 (15.9%, $P < .10$). Ammonia-N concentrations in α -AM or $\alpha\beta$ -AM treated silages were lower during the first wk of fermentation ($P < .10$), but were similar to control thereafter. There was no effect of treatment on either isoacid (Data not shown).

Lactic acid bacteria. Number of lactic acid bacteria increased quickly during the first 24 h of fermentation (Table 4) and was greater in treated silages ($P < .10$); average increases were 2.03 log and 1.74 log in treated and control silages, respectively. On d 7, number of lactic acid bacteria peaked in all silages.

Fungi and coliform bacteria. Yeast and mold counts showed large variation throughout ensiling (Table 4). Fungal growth increased during the first 24 h, thereafter numbers of yeasts and molds fluctuated a great deal. By 21 d, treated silages had lower numbers of fungi than the control. Treatment with α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS resulted in 0.46, 1.06 or 0.24 log decreased fungal counts ($P < .10$), respectively. By the end of ensiling, $\alpha\beta$ -AM or $\alpha\beta$ -AS

TABLE 4. CHANGES IN LACTIC ACID BACTERIA AND FUNGI OF CORN SILAGES OVER TIME

Day of ensiling	Silage additive ^a				SE ^b
	None	α -AM	$\alpha\beta$ -AM	$\alpha\beta$ -AS	
----- Lactics ^c , log CFU ^d ·g ⁻¹ -----					
0	5.79	5.79	5.79	5.79	
1	7.53 ^c	7.80 ^f	7.78 ^{c,f}	7.87 ^f	.06
2	7.51	7.59	7.61	7.53	.05
7	8.08 ^f	7.98 ^f	7.82 ^c	8.02 ^f	.04
21	6.67	6.48	6.30	6.53	.09
28	6.27 ^{c,f}	6.29 ^{c,f}	5.81 ^c	6.39 ^f	.11
Mean	6.98	6.99	6.85	7.02	.13
----- Fungi, log CFU ^d ·g ⁻¹ -----					
0	4.68	4.68	4.68	4.68	
1	5.03	5.09	5.04	5.08	.01
2	5.06 ^c	5.09 ^c	5.16 ^{c,f}	5.23 ^f	.03
7	5.18	5.18	6.45	5.38	.29
21	5.78 ^g	5.32 ^f	4.72 ^c	5.54 ^{f,g}	.16
28	6.14 ^f	6.03 ^f	4.97 ^c	5.04 ^c	.21
Mean	5.31	5.23	5.17	5.16	.08

^a α -AM = α -Amylase; $\alpha\beta$ -AM = α - and β -Amylases; $\alpha\beta$ -AS = α -, β -Amylases and sterilant.

^b Standard error; n=2.

^c Lactic acid bacteria.

^d Colony forming units.

^{e,f,g} Means on the same line with different superscript differ (P< .10).

treated silages had significantly lower numbers of fungi, whereas α -AM treated silage did not differ from the control. No coliform bacteria were detected in any of the silages, although a small number was found in the freshly harvested corn.

Feeding Trial

Nutrient composition. There was no effect of treatments on DM, NDF, ADF and CP in corn silage (Table 5). Addition of α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS to corn resulted in 17.9, 17.9 or 11.6% lower ADFN, respectively, than in the control ($P < .10$). A 13.4% lower ADFN was found in a companion study (Chapter II) with $\alpha\beta$ -AS treated silage when compared to the control.

Dry matter losses. Dry matter losses during ensiling in farm-scale silos were greatly reduced when α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS were added to corn ($P < .10$; Table 5). Treatment with α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS resulted in 56.5, 44.0 or 45.4% lower DM losses, respectively, than the control. Dry matter loss resulting from spoilage was not affected by treatments. Therefore, total DM loss, the sum of both losses, was also lower. Total DM recovery was improved by 10.7, 6.3 or 9.7% in α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS treated silage, respectively ($P < .10$). Bolsen and Ilg (1980) also reported a 7.2% improved total DM recovery with $\alpha\beta$ -AS treated silage, and Leahy (1988) noted only a 2.7% increase with $\alpha\beta$ -AS treated silage,

TABLE 5. NUTRIENT COMPOSITION AND DRY MATTER RECOVERY
OF EXPERIMENTAL SILAGES

Item	Silage additive ^a				SE ^b
	None	α -AM	$\alpha\beta$ -AM	$\alpha\beta$ -AS	
----- Composition -----					
DM, %	26.7	27.1	27.6	28.0	.90
NDF, %DMB ^c	56.1	57.1	52.6	55.3	3.1
ADF, %DMB ^c	29.5	28.1	27.9	28.4	2.2
CP, %DMB ^c	8.30	8.35	8.12	8.69	.26
ADFN ^d , %TN ^e	5.37 ^g	4.41 ^f	4.41 ^f	4.75 ^{f,g}	.27
----- DM recovery -----					
DM loss due to ensiling	16.9 ^g	7.36 ^f	9.46 ^f	9.23 ^f	1.42
to spoilage	3.73	4.78	6.09	3.68	1.01
Total DM loss	20.6 ^g	12.1 ^f	15.6 ^{f,g}	12.9 ^f	1.46
recovery	79.4 ^f	87.9 ^g	84.4 ^{f,g}	87.1 ^g	1.46

^a α -AM = α -Amylase; $\alpha\beta$ -AM = α - and β -Amylases; $\alpha\beta$ -AS = α -, β -Amylases and sterilant.

^b Standard error; n=2.

^c Dry matter basis.

^d Acid detergent fiber nitrogen.

^e Total nitrogen.

^{f,g} Means on the same line with different superscript differ (P<.10).

due mainly to reduced spoilage.

Growth Trial. Average feed intake of heifers during the 87-d silage feeding experiment is presented in Table 6. Total DM intake was somewhat greater in treated silages due to greater DM content. Animal performance data are summarized in Table 6. Total wt gain and ADG of heifers fed treated corn silages were markedly improved, although differences were not significant. Treatment with α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS resulted in 6.8, 5.2 or 12.0% increased ADG, respectively. Leahy et al. (1990) also reported 11.8% increased ADG by beef heifers with $\alpha\beta$ -AS treated corn silage. In a digestion trial with sheep (Chapter III), less wt loss was noted with $\alpha\beta$ -AS treatment.

Gain/feed (DM) ratio was 8.0% greater with $\alpha\beta$ -AS treated silage than the control. Leahy et al. (1990) reported a significantly greater (11.0%) gain/feed ratio, and Bolsen and Ilg (1980) noted a 6.5% improved ratio with $\alpha\beta$ -AS treated silage. Although amylase addition resulted in improved gains, the ratio of gain per unit of dry feed intake was very similar among treatments due to greater DM of treated silages. However, gains per t of DM ensiled were 12.3, 3.9 or 11.7% greater with α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS treatments, respectively, than control. Estimated backfat thickness was not affected by treatments.

TABLE 6. FEED INTAKE AND ANIMAL PERFORMANCE
BY BEEF HEIFERS

Item	Silage additive ^a				SE ^b
	None	α -AM	$\alpha\beta$ -AM	$\alpha\beta$ -AS	
Average daily feed intake/animal, kg					
Corn silage, as fed	14.8	14.8	14.8	14.8	.01
Corn silage, DM	3.94	4.11	4.20	4.14	.04
Hay ^c , DMB ^d	.747	.747	.747	.747	
Soybean meal ^c , DMB ^d	.504	.504	.504	.504	
Total DM intake	5.19	5.36	5.45	5.39	.04
Average wt, gain, feed efficiency					
Initial wt ^e , kg	212	213	214	211	.88
Final wt, kg	280	285	286	287	1.75
Wt gain, kg	68.0	72.6	71.6	76.2	1.53
ADG, kg	.782	.835	.823	.876	.02
Gain/feed (DM)	.151	.156	.151	.163	.01
Gain/t DM ensiled, kg	15.4	17.3	16.0	17.2	
Backfat gain, mm ^f	3.2	2.7	2.9	3.1	.13

^a α -AM = α -Amylase; $\alpha\beta$ -AM = α - and β -Amylases; $\alpha\beta$ -AS = α -, β -Amylases and sterilant.

^b Standard error; n=2.

^c Hay and soybean meal were fed at constant levels across all treatments.

^d Dry matter basis.

^e Initial weight and initial backfat of heifers were used to allot animals to treatments.

^f Fat thickness was estimated over the 13th rib using a Scanco Scanprobe II somascope (Model No. 7310).

5. DISCUSSION

Fermentation experiment. Control and treated corn silages had undergone the desired lactic acid fermentation and were of good quality. More than 72% of the total fermentation acids consisted of lactic acid. The small amounts of ammonia-N and traces of butyric acid noted in all silages were indicative of low clostridial activity during fermentation. Clostridial growth and metabolism may have been inhibited by high DM contents and rapid rate of lactic acid production with subsequent fall in silage pH as noted in studies conducted by McDonald and Whittenbury (1973). Low ensiling temperatures may have contributed to the inhibition of clostridial growth because their optimum temperature is about 37°C (McDonald, 1981). The highest temperatures recorded during ensiling were 27.6°C which favors growth of lactic acid bacteria over clostridia. Maximum ensiling temperatures were also well below the critical temperature (above 30°C) which was suggested to cause protein fixation in silage (Wieringa et al., 1961). Ensiling temperatures, however, were affected by ambient temperatures because the walls of the small laboratory silos provided little insulation.

Although a progression in treatments was attempted, the different sources of amylases, their different pH and temperature preference and range of activity influenced the

magnitude of each treatment's effects on fermentation and animal performance. Thus, the three amylase products were compared as independent treatments. Addition of α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS to corn resulted in larger concentrations of glucose and maltose (WSC) in silage from starch hydrolysis. Significantly greater amounts of WSC in amylase-treated corn silage were reported by Alli and Baker (1982), Leahy (1988), and in a companion study (Chapter III). Although amylases exist naturally in the leaves of the corn plant (Gates and Simpson, 1968), they seem to have a lag-time of approximately 72 h until they become active (Alli and Baker, 1982). By adding amylases to a crop rich in starch, these polysaccharides are hydrolyzed immediately after ensiling, thus providing substrate quickly for the epiphytic microflora. Concentrations of lactic acid, however, were generally only numerically increased in amylase-treated silages (Bolsen and Ilg, 1980; Chapter II and III). In this experiment, their concentration was greater, although number of lactic acid bacteria was not increased in amylase-treated silages and no effect of treatments on acetic acid or ethanol contents was noted. Thus, the available WSC must have been utilized by homofermentative lactic acid bacteria with the amylase treatments.

Reduced numbers of fungi in treated silages may have been caused by increased competitiveness of lactic acid bacteria for substrate. Coliforms, which form ethanol and

acetic acid from glucose fermentation, can only grow at a silage pH of about 7.0 (McDonald, 1981). Since pH dropped to 4.5 within 24 h of fermentation, only few of these bacteria must have survived.

Ammonia-N content was somewhat greater in $\alpha\beta$ -AS treated silage which was already noted in a previous experiment (Chapter II). Since concentrations of butyric acid and isoacids were not increased in $\alpha\beta$ -AS treated silage, proteolytic clostridia were probably not responsible for this small increase in ammonia-N, but rather was a result of greater breakdown of nitrates or nitrites as suggested by Spoelstra (1985).

Feed composition and DM recovery. Generally, nutrient composition of silages was similar among treatments. Heat damage to protein, as measured by amount of ADFN in silage (Yu and Thomas, 1975), was, however, reduced by addition of amylases to corn. Heat is generated as a result of carbohydrate fermentation by fungi (McDonald, 1981). Since the number of fungi was somewhat reduced in amylase-treated silages, as noted in the previously described fermentation study, less heat was produced by their metabolic activity. This would explain lower ADFN percentages in amylase-treated silages and may contribute to greater availability of CP to the ruminant animal.

Although changes in nutrient composition during fermentation may be minimized by good ensiling techniques,

some DM loss will occur due to ensiling. Generally, a 9% DM loss during ensiling of untreated whole-plant corn is reported (Bolsen et al., 1980; Leahy et al., 1988). The magnitude of effluent loss during ensiling is dependent on the DM of the ensiled material (Van Soest, 1987). The 16.9% DM loss associated with the ensiling of untreated corn in this experiment, is greater than the percentage usually reported in the literature which may be partly due to the wetter corn crop ensiled. Although DM of treated silages were similar to the control in this experiment, greater amounts of DM were preserved during fermentation when α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS were added to the plant material. This was probably due to inhibition of clostridial and fungal activity in amylase-treated corn silages.

Growth performance. The magnitude of response in ADG of heifers fed α -AM, $\alpha\beta$ -AM or $\alpha\beta$ -AS treated corn silages is consistent with results of Leahy et al. (1990) obtained during a 3-yr feedlot study with $\alpha\beta$ -AS treated silage. The same trend in body wt change was also found during a digestion trial with wethers fed $\alpha\beta$ -treated silage (Chapter III). This improvement in growth performance with amylase treatments is most likely associated with greater preservation of nutrients from the originally ensiled plant material as noted by the improved ratio of gain per t of DM ensiled. Because more NFE was preserved in amylase-treated silages due to greater amounts of fermentable carbohydrates

retained, NFE digestibility was improved and more potential energy was available to rumen microorganisms for growth and protein synthesis. In previous experiments, rumen fermentation activity, as measured by in-vitro gas production, was significantly enhanced with the $\alpha\beta$ -AS treatment (Leahy, 1988; Chapter III). As expected, concentration of rumen microbial protein was also markedly increased with the $\alpha\beta$ -AS treatment (Chapter III). Increased rumen microbial growth and activity may have been responsible for the long-term positive effect of $\alpha\beta$ -AS on ADG and gain/feed ratio. Gains and feed efficiency continued to be greater, even after a finishing diet was fed to heifers as seen by Leahy et al. (1990). Treatment with $\alpha\beta$ -AS, containing approximately 21% sulfur, may be partly incorporated into the amino acids methionine and cysteine during synthesis of microbial protein as suggested in a previous study (Chapter III). Addition of sulfur-salts to amylases may have contributed to a greater improvement in ADG with beef heifers than amylase treatments alone. Addition of the exosplitting enzyme β -amylase which cleaves successive maltose units from the non-reducing end of the starch molecule (Whitaker, 1972), did not improve the effectiveness of α -amylase.

6. IMPLICATIONS

Use of amylases for ensiling whole-plant corn improved the lactic acid fermentation of the silages produced. Reduced DM losses during the ensiling process resulted in more fermentable carbohydrates (WSC) retained, increase in nutritive value and ultimately improved growth performance with heifers. Results from previous experiments indicate that improved ADG and gain/feed ratio by heifers during silage feeding and finishing period with the commercial product containing both amylases and sulfur-salts were associated with increased rumen microbial activity and protein synthesis. Beef heifers showed the greatest wt gains with this amylase treatment. This amylase treatment seems therefore, to be the most beneficial additive among the three amylase-containing products. A well-preserved silage and enhanced animal performance was achieved with α -amylase or α - and β -amylases, but their high production cost does not yet justify their practical application on the farm.

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VITA

Sylvia Astrid Nicklas-Bray was born in Heilbronn, Germany, on October 11, 1959. She was raised in Heilbronn and graduated from Theodor-Heuss Gymnasium in May, 1978. The following October, she began her studies in Agricultural Biology at Hohenheim University, Stuttgart-Hohenheim, Germany, and specialized in Animal Science after two years of her studies. In July 1983 she received a Diploma (Master of Science degree) in Animal Science with a concentration in Ethology. After graduation she was employed with Siemens AG, Munich, Germany, while continuing advanced studies in Ethology at the Ludwig-Maximillian University, Munich, Germany.

She was married on August 31, 1985 to James Michael Bray of Kingsport, Tennessee, and immigrated to the U.S.A. in the same year. In April 1987, she began to pursue the Doctor of Philosophy degree with a major in Animal Science and a concentration in animal nutrition at the University of Tennessee, Knoxville.

The author is a member of Gamma Sigma Delta, American Dairy Science Association and American Society of Animal Science. She will be employed by Imperial Chemical Industries in Canada after graduation.