EFFECTS OF MICROBIAL CULTURES AND VARIOUS OTHER ADDITIVES ON THE FEEDING QUALITY, FERMENTATION PATTERN, DRY MATTER RECOVERY AND AEROBIC STABILITY OF HIGH MOISTURE CORN

By
Frank Arthur Wardynski

A Thesis

Major Professor
Steven R. Rust

Submitted to Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Science

1991

ABSTRACT

EFFECTS OF MICROBIAL CULTURES AND VARIOUS OTHER ADDITIVES ON THE FEEDING QUALITY, FERMENTATION PATTERN, DRY MATTER RECOVERY AND AEROBIC STABILITY OF HIGH MOISTURE CORN

By

Frank Arthur Wardynski

Three experiments were conducted to evaluate the effects of lactic acid producing bacteria inoculation (LABI) on the preservation, nutritive quality and aerobic stability of high moisture corn. In experiment one, large concrete upright silos were utilized to evaluate control (CON) vs LABI. Experiment two used small laboratory silos to evaluate CON vs LABI and whole vs ground corn in trial 1 and CON, LABI, reconstitution with water, nitrogen addition and acid addition in trial 2. Experiment three evaluated corn stored at four dry matter (DM) contents (78; 74; 66; 63% DM), three storage temperatures (4; 12.5; 20 °C), and treated with CON or LABI. Dry matter recovery was decreased with LABI in two trials, similar in two trials and increased in one trial. Corn treated with LABI was less stable after aerobic exposure as compared to CON ($P < .05$). Feeding quality was similar between LABI and CON ($P > .10$). Experiment results indicate inoculant use is not consistently beneficial or cost effective.

ACKNOWLEDGEMENTS

The reported research could not have been finished without the help of many people. I greatly appreciate everyone for their efforts.

I offer my warmest thanks to Dr. Steven Rust, who served as my advisor and friend. I appreciate Dr. Rust's uncompromising principles and work ethic. Dr. Rust was supportive and offered encouragement when needed. I also would like to thank Steve and his family for their care and closeness.

Special thanks goes to Dr. Harlan Ritchie and Dr. David Hawkins for serving on my committee. Their shared expertise and experience in the beef industry and their friendship will be treasured indefinitely.

Dr. Melvin Yokoyama deserves thanks for serving on my committee, his expertise in microbial fermentation, the use of his microbiology lab and technical assistance.

Thanks also goes to Dr. Oran Hesterman for serving on my committee and his special knowledge from the agronomic viewpoint.

I owe a great debt to many faculty, staff and students. Sincere thanks goes to all who assisted in the many hours of microbiological work. I also appreciate the assistance of technicians Sue Hengemuehle and Elaine Fink. Special thanks are also offered to Kaye Hillock for typing the manuscript and to Tadd Dawson in preparing the graphics. I also need to offer great thanks to David Main, David Lust and Peter Anderson for their

unselfish help and friendship.

I can never express the thanks which my parents deserve. Their love, support and guidance will always be appreciated. My values, morals and accomplishments are a direct result of them. I am truly grateful and I love you. Other members of my family, my sister and grandparents also are recipients of my love and gratitude.

I also would like to thank Kathy, my wife. She has been a special inspiration and worked diligently on the manuscript.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

PAGE

Experiment 1

Experiment 2

Experiment 3

INTRODUCTION

Corn is the most common grain fed to livestock in Michigan. The popularity of corn grain can largely be attributed to the energy density and cost of production of other energy sources. Livestock producers are accustomed to feeding corn, have a good knowledge base of its feeding characteristics and usually have on-farm storage capabilities.

Corn, for all practical purposes, is harvested for storage as dry or high moisture corn. Corn is often harvested wet and dried to 15% moisture. This allows the product to be stored in a relatively stable state, allowing minimal mold and microbial growth to occur. Dry storage of corn has advantages over high moisture corn. Some of the advantages include: 1) can be moved through existing marketing channels; 2) can be used by the milling industry; 3) easily transported; 4) is an excellent energy source for cattle and maintains condition for extended periods of time in the feedbunk.

However, certain disadvantages associated with dry corn can be overcome by harvest as an ensiled feedstuff. Fermentation removes the problems associated with insects and mold contamination and may lessen the negative impact of mycotoxins. Other advantages of high moisture corn storage include: 1) an earlier and extended harvest season allowing for more efficient labor utilization; 2) less harvest loss as opposed to harvesting dry corn; 3) improved digestibility. Likewise, drying corn to an acceptable moisture content is expensive due to high energy costs.

Unfortunately, storage of high moisture corn as ensilage has certain draw backs

as well. Ten to twenty percent of the dry matter in high moisture corn can be lost during storage and feeding. This problem can be minimized by proper harvest, storage and feeding practices.

Filling the silo or storage structure should be rapid to minimize respiration losses. Feeding rate should be rapid to reduce aerobic deterioration on exposed surfaces. Storage structures must be air tight or well-consolidated to ensure an anaerobic environment.

To minimize losses in the silo structure, several amendments have been added to the crop material before ensilement. One of these amendments has been the addition of starter cultures to redirect the fermentation pattern toward less carbon dioxide production and hence more carbon retention in the silo.

This thesis project was designed to evaluate the ability of starter cultures to enhance the fermentation process in a low-moisture silage. Trials one and two were designed to evaluate the benefit of a microbial inoculum to high moisture corn in field-scale silos. Once it became evident the commercially available inoculants had little benefit in high moisture corn, experiment three was designed to characterize the effects of environmental factors on the fermentation process. It was expected this characterization would provide the necessary background to initiate studies to develop a more efficacious inoculant for high moisture corn.

LITERATURE REVIEW

Physical Factors Which Affect The Efficiency Of Silage Fermentation

Oxygen exclusion is an important factor in the efficient fermentation of feedstuffs. Oxygen may be excluded by compaction during silo filling, enzymatic respiration or through bacterial fermentation. Compaction is the firmness and tightness with which feedstuffs are packed into the silo. A well compacted silo creates the environment for rapid lactic acid accumulation. Silo type, particle size and moisture content influence consolidation, compaction and oxygen depletion in silages.

Feedstuffs in upright silos are firmly packed by the weight of feed mass, whereas compaction is achieved mechanically in bunker silos. Dry matter (DM) losses are greater from bunker than upright silos (Voelker et al., 1985). Typically, the dry matter recovery from bunker and oxygen limiting upright silos is 85% and 98.6% respectively (Williams et al., 1979).

Losses during feedout and from surface exposure due to aerobic deterioration varies among silo structure type. Bunker silos have greater surface exposure, allowing more time for air to penetrate the feed mass than in upright silos. Typical energy losses attributed to aerobic deterioration during storage and after unloading range from 0-10%

and 0-15%, respectively (McDonald, 1981).

Particle size reduction will enhance compaction and reduce trapped air during silo filling which allows fermentation to develop more rapidly. Decreasing chop length or particle size increases fermentation rate, which results in lower pH values and greater lactic acid accumulation (Kroulik et al., 1955; Gordon et al., 1959). Increased fermentation rate due to smaller particle size results from the release of juices, consolidation and air exclusion (Woolford, 1984c).

Stage of maturity and moisture content can influence degree of compaction and fermentation rate. The optimum moisture content for fermentation of high moisture grains is between 25 and 30% (Fox, 1976; Aguirre et al., 1983). Grains ensiled with adequate moisture compact more completely. Moisture content is more critical in grains stored in bunker silos as the opportunity for air exposure is greater. Low moisture, ensiled grains are difficult to consolidate adequately which enhances the risk of an extended aerobic exposure period. During ensilement of low moisture grains, mold, yeast and aerobic bacteria dominate the fermentation which increases fermentation losses and may reduce aerobic stability.

Likewise, high moisture grains can be ensiled too wet which results in excessive fermentation (Goodrich and Meiske, 1976). An extended fermentation increases dry matter and energy losses and reduces palatability. Dry matter consumption of wet ensiled grains is reduced which coincides with elevated levels of soluble nitrogen (Prigge et al., 1976) and acids (Wilkinson et al., 1976; Shaver et al., 1984).

Varietal differences (genetics) and external factors (environment) affect the

maturity, yield, nutrient accumulation and epiphytic microbial populations present. A readily available source of carbohydrate is needed for an adequate fermentation to occur. Variety differences in soluble carbohydrate composition and response to stressful environmental conditions can influence the efficiency of the fermentation.

Corn reaches physiological maturity at 30-44% moisture (Goodrich and Meiske, 1976). Maturity can be determined by presence of a black layer at the tip of the kernel. Once the black layer has developed, nutrient deposition is near completion. Immature seeds are high in cell wall, crude fiber, ash, protein and water (Copeland and McDonald, 1985). Starch, sugar and lipids are the last chemical constituents to be deposited (Daynard and Duncan, 1969). Later maturing corn varieties generally produce greater yields, however, these varieties contain higher moisture levels later into the harvest season. Consequently, harvest of corn at higher moisture allows the use of later maturing and higher yielding varieties. Another advantage of harvesting corn at higher moisture levels is 10% less field loss (Goodrich and Meiske, 1976).

Genetics and environment have been shown to alter nutrient composition of grains (Jones et al., 1981; Kofoid et al., 1982). Protein content can be increased by nitrogen fertilization, low plant density and dry weather. High protein content during dry weather is a function of less carbohydrate deposition in the kernel. In other crops such as soybeans, elevated temperatures have increased lipid content. Lipids are minor constituents of cereal grains (Copeland and McDonald, 1985) and of small importance during fermentation.

Environmental stresses can greatly influence the number and species of

microorganisms present on field crops. Stresses which limit lactic acid bacteria are ultraviolet light, temperature and available nutrients (McDonald, 1981). Lactic acid bacteria accumulate on areas of the plant which have available sap and increase in numbers as plants become more mature (Daeschel et al., 1987).

Effects Of Chemical Constituents Of The Plant On Silage Fermentation

Preservation of corn through fermentation has been described as a four stage process (Barnett, 1954). The first stage involves continued plant respiration through the action of enzymes. During this stage carbon dioxide and heat are produced. Plant respiration involves enzymatic oxidation of soluble carbohydrates to carbon dioxide and proteolysis of protein to less complex forms of nitrogen. During stage 2, aerobic metabolism is initiated which yields primarily acetic acid. Management techniques which minimize the duration of stages 1 and 2 should increase the efficiency of nutrient conservation in the ensilage process. Stage 3 involves the initiation of lactic acid production and anaerobiosis. The final phase involves a rather quiescent period where lactic acid production reaches its peak and pH remains stable at 4.0.

A simplified chemical equation of respiration is $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$ + 2870 Kilojoules. Cells from intact and freshly harvested plants respire. During growth, plants capture and use respiration energy as ATP (adenosine triphosphate) for biosynthesis of new plant material. A portion of captured energy is lost as heat to the environment (Salisbury and Ross, 1969). Respiration continues in harvested plant material which liberates larger amounts of energy from oxidized glucose as heat with

virtually no biosynthesis (McDonald, 1981).

Plant respiration is accomplished primarily by three sequential pathways (McDonald, 1981). First, simple sugars are converted to pyruvate by glycolysis. Secondly, pyruvate is oxidized to form acetyl Co-A which is oxidized in the Krebs cycle to produce water, carbon dioxide, $FADH_2$ and $NADH_2$. The $FADH_2$ and $NADH_2$ are used in the electron transport system as electron donors to phosphorylate ADP to ATP.

The primary concern of plant respiration during ensiling involves substrate depletion and proteolysis. Several factors influence the extent of plant respiration during the early stages of ensilement. Ruptured plant cells and elevated ambient temperature have been shown to increase the rate and velocity of respiration (McDonald, 1981). Duration of plant respiration depends upon oxygen supply, cellular pH and temperature. Firmly and rapidly consolidated silage undergoes less plant respiration because of less available oxygen (McDonald et al., 1966). In inadequately consolidated silos, silage temperature can increase and reduce nutritional value (Henderson et al., 1982).

Fermentation is a process in which microorganisms metabolize substrates for energy and produce organic acids as endproducts (Salisbury and Ross, 1969). Fermentation accelerates as plant respiration ceases. Available substrate is metabolized by aerobic coliforms and heterolactic microorganisms. As oxygen is depleted and pH decreases, homolactic fermentation dominates which establishes a relatively quiescent forage mass. During the aerobic phase, the primary end products are acetic and lactic acids and ethanol. The homolactic phase yields primarily lactic acid (Woolford, 1984c).

Plant carbohydrates can be classified into 3 types; consisting of soluble, storage

and structural. Water soluble carbohydrates or simple sugars are readily available and fermented by microorganisms. The chemical linkages in storage and structural carbohydrates limits the availability of these compounds as substrates for microbial growth. Starch, which is the most abundant storage carbohydrate in grains, can be utilized by lactic acid bacteria (Lingren and Refai, 1984) and more readily than structural carbohydrates; however, many of the desirable microorganisms lack an amylase enzyme (McDonald, 1981). Structural carbohydrates are of minor importance in silage fermentation because of the low cellulase activity in Lactobacillus species. Enzymes such as cellulase (McHan, 1986) and hemicellulase (Dewar et al., 1963) may increase soluble sugar content of grass and legume silages.

Hexoses are fermented by either homolactic or heterolactic fermentation. Fermentation type is determined by type of sugar fermented and microorganisms present. Homolactic fermentation is a more efficient pathway for acid production (McDonald et al., 1987). Homofermentative organisms convert one mole of 6-carbon sugar into 2 moles of lactic acid.

Heterolactic fermentation has several different pathways in which 1 mole of hexose is converted to 1 mole of lactate and other end products (McDonald, 1981). During heterofermentation, 1 mole of glucose or fructose can be fermented to 1 mole each of lactate, ethanol or acetate and carbon dioxide. Other microorganisms, in the presence of fructose, a hydrogen acceptor, convert 2 moles of fructose to 1 mole of mannitol and 1 mole of glucose. The glucose is further metabolized to lactate, acetate and carbon dioxide. Heterofermentative bacteria may use oxygen as a hydrogen acceptor

to convert acetyl phosphate to acetate which yields an additional mole of ATP. Final end products produced are lactate, acetate, water and carbon dioxide.

Pentoses can be fermented by both homolactic and heterolactic microorganisms with similar efficiencies as hexoses. One mole of pentose is converted to 1 mole each of lactate and acetate. Clostridial bacteria can metabolize sugars to butyrate, carbon dioxide and heat (Gibson et al., 1958). Clostidial fermentation is inefficient in terms of energy conservation and is, therefore, undesirable.

Lactic acid bacteria have a small role in the fermentation of organic acids such as malic, succinic and citric acids (Woolford and Sawezyc, 1984 a,b; Grazia and Suzzi, 1984). Organic acids can be substrates for lactic acid bacteria but are used readily by clostridia (Leibenspergen and Pitt, 1987). Clostridia can ferment 2 moles of lactate to produce butyrate and carbon dioxide.

Organic acids serve as buffers in silage. Organic acids tend to buffer between pH 4 and 6. Buffering capacity is commonly expressed as milliequivalents of alkali needed to increase the pH of 1 kg of silage from 4 to 6. During fermentation, organic acids are replaced with fermentation acids. Fermentation acids, such as lactic or acetic acid, have stronger buffering capacities than organic acids originally present such as malic and citric acid (Woolford, 1984). Accumulation of fermentation acids lower the pH and tend to buffer at a lower pH.

Structural relationships and composition of nitrogenous compounds change during fermentation (Bergen et al., 1974). Proteolysis involves the solubilization and cleavage of proteins into polypeptides. Much of the proteolytic activity in forage crops occurs

in the first few hours and is caused by enzymatic activity (Bergen et al., 1974). After the initial enzymatic proteolysis, microorganisms may continue the proteolytic process. Soluble nitrogen content has been shown to increase between day 28 and 56 in ensiled high moisture corn (Prigge et al., 1976) while microbial populations remained constant. Baron et al. (1986) showed little proteolysis occurred in high moisture corn during the early phases of ensilement but demonstrated a significant increase in the later fermentation period. The authors concluded the low acidity may have contributed to the increase in soluble nitrogen.

Lactic acid bacteria have limited proteolytic capabilities, however, clostridial organisms do possess proteolytic fermentation pathways (Leibensperger and Pitt, 1987). Clostridia can deaminate and decarboxylate amino acids to form ammonia, amines, amides and organic acids (Edwards and McDonald, 1978). Another proteolytic pathway used by Clostridia is the Stickland reaction (Woolford, 1984) which is an oxidation-reduction reaction. One amino acid is oxidized and another is reduced to form organic acids.

Many factors affect the velocity and extent of proteolysis. Moisture content has a large effect on proteolysis. Proteolysis is positively correlated with moisture and negatively with rate of acidification in forages (Brady, 1965). However, it has been shown that corn ensiled at high moisture content undergoes extensive fermentation with greater amounts of acid and soluble nitrogen produced (Thornton et al., 1977a). High sugar to protein ratio has been shown to improve protein stability. High moisture corn is relatively low in sugar, protein and moisture. Proteolysis activity is lower in high

moisture corn than forages, as indicated by lower soluble nitrogen contents (Baron et al., 1986). Proteolytic activity increases the proportion of soluble nitrogen to 60% of the total nitrogen in grass silage (Oshima and McDonald, 1978), 50% in whole plant corn silage or ear corn silage (Buchanan-Smith, 1982; Aumaitre and Zelter, 1975), and 40% in high moisture corn (McKnight et al., 1973). The limited fermentation that high moisture corn undergoes can lessen the stability upon exposure to air as compared to other silages. Yeast, molds and aerobic bacteria begin to respire as oxygen is introduced into the silage mass. Oxygen can enter the silage mass by infiltration through silo walls, exposed surface areas or during feedout. As aerobic organisms begin rapid growth, heat is generated which increases the temperature of the silage mass. An elevated temperature is positively correlated with dry matter lost as carbon dioxide (Woolford, 1984b). These organisms primarily utilize fermentation acids and sugars as substrates to produce carbon dioxide, ammonia and water. Aerobic activity results in dry matter loss and concentration of crude fiber (Woolford, 1984).

Harvest And Storage Losses Associated With High Moisture Grains

Storage of grain in high moisture form results in greater DM and nutrient losses. Management systems which minimize the extent of these losses would be beneficial. Nutrient losses can occur during several stages of preservation which include harvest, storage or feedout. The primary focus of this review involves losses encountered during storage and feedout.

During storage or the ensiling process, nutrients may be lost in effluent, through fermentation by microorganisms and losses due to oxygen infiltration (Pitt,1986). Microorganisms and enzymes degrade soluble nutrients such as carbohydrates and proteins to simpler chemical compounds. Fermentation losses as carbon dioxide are believed to contribute 1-2% of the total dry matter loss (McDonald, 1981). Fifty percent of the DM and 18% of the energy losses occur as carbon dioxide (Woolford, 1984c).

Effluent losses from silages can range from 0% in grains and wilted forages to 7% in unwilted forages (Pitt, 1986). Total effluent produced is determined by moisture content and force applied to the forage mass. Rate of effluent production is a function of time required for plant cell membrane breakdown and rate of cell contents to be forced out of the cell (Pitt and Parlange, 1987).

Oxygen can migrate more readily into silage from exposed surfaces and improperly consolidated silage. Oxygen can move through silo walls and into the silage, however, greatest air movement into ensiled feeds occurs with uncovered or unsealed surfaces (Ruxton et al., 1975). Unsealed bunker or trench silos filled with high moisture fodder (50% DM) resulted in 70% less usable DM as compared to sealed silos (64 vs 90% DM recovery; Brown and Kerr, 1965). Losses due to oxygen infiltration during the fermentation process are similar to losses associated with air exposure during feedout. Maintenance of an anaerobic environment in ensilage is critical to minimize storage losses. Dormant aerobic organisms become active and utilize a vast array of readily available substrates upon exposure to air.

During feedout, soluble carbohydrates and fermentation acids provide substrates

for aerobic microorganisms to proliferate. Yeast organisms are primary contributors to aerobic deterioration (Woolford and Wilkie, 1984), however, bacteria also contribute to the process (Woolford and Cook, 1978). Reviews of aerobic deterioration in research trials have shown DM losses of 16.1% (Watson and Nash, 1960) as cited by Ashbell and Lisker (1988), whereas on farm losses due to aerobic deterioration range from 8-71% (Zimmer, 1967) as cited by Ashbell and Lisker (1988).

Yeasts have been shown to metabolize both sugars and acids (Middelhoven and Franzen, 1986). Metabolism of lactic acid to carbon dioxide by yeast elevates the pH and allows bacterial growth to begin (Lindgren et al., 1985b).

Dry matter content influences retention during fermentation and feedout. Lower DM content at ensiling will cause more rapid and extensive fermentation (Goodrich et al., 1975). Higher acid, ethanol and soluble nitrogen content, greater gas production and lower pH result from enhanced fermentation (Goodrich and Meiske, 1976; Prigge et al., 1976). The amount of DM lost increases as gas production increases. Soluble nitrogen content is greater in low DM silage. Consequently, moisture content and nutrient losses are positively correlated. Silages ensiled at less than 30% DM result in excessive effluent production, however, high moisture grains are not ensiled at these levels. Therefore, effluent loss is not a problem with high moisture grains. Ensiling feedstuffs with high DM content may have deleterious effects on recovery, since the limited fermentation may increase aerobic instability.

Amount of DM loss is influenced by the type of silo structure. Oxygen-limiting, concrete tower and horizontal silos are commonly used to store silages. Oxygen limiting

silos do not allow oxygen exposure as readily as other silo types; consequently moisture content is less critical (Fox, 1976) for adequate preservation. Concrete tower silos consolidate more thoroughly than horizontal silos (Pitt and Parlange, 1987) causing greater oxygen depletion, less oxygen infiltration and more effluent production in excessively wet forages. Horizontal silos have relatively large surface areas as compared to upright silos. Large surface areas allow greater oxygen penetration into the silage mass and greater DM losses.

Different methods have been used to evaluate the losses and changes which occur during the ensiling process. Ensiled feeds are characterized by the conversion of soluble carbohydrates to organic acids and the solublization of protein to soluble nonprotein nitrogen (Bergen et al., 1974). Under favorable conditions, pH will decrease and lactic acid content will be high relative to other fermentation acids produced (Thornton, 1976). An unfavorable fermentation is characterized by high butyric acid content and excessive proteolysis.

Large silos are frequently used to measure nutrient losses during fermentation. Large silos give an accurate assessment of fermentation losses, however, cost and size of scale limit replications. Multiple dacron bags filled with plant material and buried within each silo can be used to estimate DM recovery. Unfortunately, DM recovery estimates from buried bags have low correlations with large silo DM recovery estimates (McDonald, 1981). Location of buried bags within the silo will influence the DM recovery estimates as well.

Small scale or laboratory silos have been used to evaluate the fermentation

process. Small silos are relatively inexpensive and easily replicated. Laboratory silos have been criticized (Mayne and Gordon, 1986) for lack of similarity with types of fermentations in field scale silos. These silos are frequently more anaerobic than farm scale silos. However, laboratory silos have been used frequently to evaluate fermentation (Lindgren et al., 1985a; Baron et al., 1986). Aerobic stability can be evaluated by exposure of silage to air in insulated containers. Temperature rise, microbial growth and dry matter loss are commonly reported parameters to quantify the extent of deterioration.

Microbiology Of Silage

Epiphytic microorganisms present on plants before ensiling are extremely variable (Daeschel et al., 1987). Facultative anaerobes living in an aerobic environment are important for adequate fermentation. Numbers of epiphytic microorganisms are dependent upon several factors. Bacterial populations vary according to location on the plant. Microorganisms are more numerous on damaged or decayed foliage with few organisms found on intact inflorescence or seeds (McDonald, 1981). Bacteria are found on damaged areas because nutrients are readily available at these points. Since the lower portions of the plants are more likely to contain higher numbers of bacteria, the amount of stubble left in the field can influence the resulting fermentation. Secondly, plant maturity and climatic conditions can influence the number of organisms present on growing plants (Woolford, 1984c). As plant maturity and season progresses, the number of lactic acid producing bacteria (LAB) increase (Daeschel et al., 1987). Warm, humid weather and cloudy skies are generally associated with elevated populations of epiphytic

bacteria. In addition, calm days with little air movement reduce evaporation and consequently, increase bacteria numbers. Clostridial species are not normal inhabitants on growing plants but are present in soil. These organisms are influenced little by weather conditions (Woolford, 1984c).

Numbers of LAB species may be small on the growing plant, but larger populations have been routinely reported at the silo (Muck and Specherd, 1984; Muck and O'Connor, 1985). This increase may be explained by extended time periods between cutting and placement in the silo which allows the populations to multiply. Secondly, the harvest machinery may accumulate plant material which allows epiphytic populations to multiply and serve to inoculate plant material. It is likely that the increased population at the silo is a combination of both (Fenton, 1987).

Clostridial and Bacillus species are present in low numbers on fresh crops. These organisms are usually of soil origin and high plant levels pre-ensiling are usually due to contamination during harvest (Woolford, 1984c). Tractor and implement tires may be a source of these organisms during the consolidation of bunk or trench silos.

Lactic acid producing microorganisms are gram positive, microaerophilic, non-spore forming and usually non-motile organisms (Beck, 1978). The genera of microorganisms comprised in LAB are Lactobacillus, Streptococcus, Pediococcus and Leuconostoc. Lactobacillus belong to the family Lactobacillaceae. These organisms are rod shaped and possess species of both heterofermentative and homofermentative characteristics. The other three genera belong to the family Streptococcaceae and are spherical in shape. Leuconostoc species are heterofermentative while Streptococcus and

Pediococcus species are homofermentative (McDonald, 1981). Domination of the fermentation by lactic acid bacteria is important to ensure sufficient amounts of lactic acid production and a rapid pH decline..

Sugar is utilized by LAB as the main substrate, but organic acids can also be used. Lactic acid producing microorganisms have been shown to ferment lactate (Beck, 1978) under adverse conditions. Proteolytic activity on plant proteins is small in LAB.

Lactobacillus can survive in pH from 4.0-6.8 with 6.0 being optimum. Although, certain strains may have lower pH optimums. Optimum temperature for Lactobacillus is 30C. These microorganisms grow slowly above 45C. Pediococci organisms are thought to grow best at pH 5.5 (Woolford, 1984c). Clostridia are more heat tolerant than LAB with optimum temperature at 37C. Excessive respiration activity increases silage mass temperature and favors Clostridial fermentation. Clostridia cannot tolerate high acid levels and have an optimum pH range of 7.0-7.4. This emphasizes the importance of lactic acid production and pH reduction.

Clostridia are members of the family Bacillaceae. Most clostridia are obligate anaerobes with some aerotolerant species. Additionally, most clostridia are gram positive bacteria but may switch cell wall structure to become gram negative in older cultures. There are two types of clostridia involved in fermentation; 1) saccharolytic bacteria which utilize sugars and lactic acid with weak proteolytic activity and 2) proteolytic bacteria which degrade protein with weak saccharolytic activity (Leibensperger and Pitt, 1987). Clostridia perfringens have both saccharolytic and proteolytic capabilities. Silage dominated by clostridia is characterized by elevated levels of butyric acid, ammonia and

pH (Leibensperger and Pitt, 1987). Clostridia ferment lactic acid to produce butyric acid. Butyric acid is weakly acidic which hampers pH decline.

Another bacterial species which is commonly present in silages are Enterobacteriaceae. The organisms are rod shaped facultative anaerobic bacteria. Many enterobacteria are pathogenic, however, those found in silage are thought to be non-pathogenic (McDonald, 1981). The role of enterobacteria in silage fermentation is minor provided LAB growth is sufficiently rapid and pH is lower than 5.5. Since the pH optimum for enterobacteria is 7.0, activity is greatest early in the fermentation process. Enterobacteria, commonly referred to as coliform bacteria, are inefficient fermenters as evidenced by the end-products of metabolism. Sugars are fermented to lactate, acetate, succinate, ethanol, 2,3-butanediol and formate. Formic acid is reduced to hydrogen ions and carbon dioxide under acid conditions. Coliforms have proteolytic activity and are responsible for a portion of protein degradation in silage.

Other microorganisms present include: yeasts, molds, and other aerobic bacteria. Under normal silage fermentation conditions, these organisms contribute only a minor role during anaerobic fermentation. However, these microorganisms can be extremely prolific upon exposure to oxygen. In general, these microorganisms have a detrimental effect on the nutritional value of silages.

Yeasts are oval or elliptical in shape and reproduce by budding or binary fission, often forming chains of elongated cells. Certain species of yeasts grow in anaerobic conditions and are acid tolerant. Under anaerobic conditions, yeast ferment sugars to ethanol and compete with LAB for soluble sugars.

Yeasts are more active in ensilage after oxygen exposure. Two physiological types of yeasts are present which attribute to silage instability. Bottom or ground growing yeast ferment sugars to carbon dioxide and water. Another type, top growing yeast, consume lactic acid resulting in decreased energy content and preservation of the feedstuffs (Woolford, 1984c).

Filamentous fungi or molds contribute little to the fermentation process under anaerobic conditions but are associated with decomposition of fermented feedstuffs and mycotoxin production (Lee et al., 1986). Molds and fungi are more prevalent in silage which has undergone an inadequate fermentation. Molds can utilize cellulose, cell wall material, sugars or lactic acid as substrates. The most prominent by-product of mold and fungi fermentation is carbon dioxide.

Aerobic bacteria such as Bacillus species are involved with aerobic instability as well. Some are facultative anaerobes. Little is known about Bacillus, but are thought to be significant contributors to aerobic deterioration in well preserved silages (Lindgren et al., 1985b).

Additives and Inoculants

There are several management techniques to improve silage fermentation and minimize nutrient losses. Previous studies of the chemistry and microbiology of silage have suggested that methods to enhance the rate and extent of lactic acid production would be beneficial. Several strategies have been utilized to enhance lactic acid production such as microbial cultures, enzymes, nutrient amendments, fermentation inhibitors and reconstitution. Current technology allows the production of

microorganisms to add as starter cultures to fermenting biomass to facilitate lactic acid production. The array of microorganisms present in the unfermented crop material has a major impact on the efficiency of the fermentation (Muck and O'Connor, 1985). Beneficial microorganisms, whether epiphytic or added as an inoculant, must be abundant and dominate over deleterious organisms for a favorable fermentation to occur.

Addition of microbial cultures can be beneficial when epiphytic LAB populations are low (Muck and O'Connor, 1985). Addition of microbial cultures containing LAB have been shown to favorably influence fermentation patterns in ensiled crops (Ely et al., 1981; Lindgren et al., 1983; Lindgren et al., 1985a; Kung et al., 1987).

The criteria established for a successful microbial inoculant (Whittenburg, 1961, as cited by Woolford, 1984) are: 1) high growth rate, competitive and must be able to dominate the fermentation; 2) homofermentative; 3) acid tolerant with activity to pH 4.0; 4) ferment glucose, fructose, sucrose and also preferably fructosans and pentosans as well; 5) inability to convert dextrose to sucrose or fructose to mannitol; 6) limited ability to ferment organic acid; 7) survive at temperatures exceeding 40C; and 8) no proteolytic activities.

Two Lactobacillus species, L. plantarum and L. acidophilus, meet the majority of these criteria (Seale et al., 1986). Streptococcus faecalis, S. faecium and Pediococcus acidilactia possess many of the beneficial characteristics as well (Lindgren et al., 1985a). Lactobacillus plantarum has been identified as possessing most of the qualities identified by Whittenburg for an ideal inoculant, however, this organism tends to grow slowly during the early stages of fermentation (Bryan-Jones, 1969 as cited by McDonald, 1981).

Therefore, the addition of inoculants containing multiple species or strains may be advantageous. Streptococcus may be a candidate as a second organism to an inoculant containing Lactobacillus plantarum. Streptococcus grows rapidly under aerobic conditions and endures a wide temperature range. Secondly this organism is less acid tolerant and tends to disappear at pH 5.0 which is a condition optimal for L. plantarum (Bryan-Jones, 1969 as cited by McDonald, 1981). Another possible companion organism is Pediococcus acidilactici. It is acid sensitive and has an optimum temperature of 40C (Buchanan and Gibbons, 1974).

Addition of enzymes may increase substrate for fermentation, but the enzymes are active shortly after ensiling but are inactivated by anaerobic conditions and low pH (Ruxton et al., 1975). Mineral acids added to ensiled feeds can prevent enzymatic activity, however consumption by livestock may be reduced (Thomas, 1978).

Since many Lactobacillus organisms lack amylase enzymes and crop materials such as corn and alfalfa contain starches, the addition of amylase has been evaluated (McDonald, 1981). Amylase addition breaks down polysaccharides which are otherwise unavailable to lactic acid bacteria. Likewise, cellulose is a major carbohydrate fraction in forages and under certain climatic conditions (McDonald, 1981), soluble carbohydrate may be limiting. Therefore cellulase addition has been evaluated (Huhtanen et al., 1985). Cellulose is degraded by cellulase addition which provides more substrate for growth of lactic acid bacteria (McHan, 1986). Hemicellulose is another carbohydrate reservoir which can be hydrolyzed by addition of enzymes to fermenting plant material (Dewar et al., 1963). These enzymes have shown some benefit, however, the magnitude

has rarely been large enough to justify the cost.

Nutritional amendments added to ensilage may contribute nutrients or provide substrate to allow a more efficient fermentation. Nutritional amendments evaluated previously include grains (Jones, 1988), molasses (Singh et al., 1985), limestone (McDonald, 1981) and whey (Thomas, 1978). Increased supply of available sugars to substrate-deficient forages has shown an improvement in silage characteristics especially in high protein feeds (Svensson and Treit, 1964; Seale et al., 1986; Jones, 1988).

Grain has been added to alfalfa and grass silages as a nutritional amendment. These amendments were effective in enhancing the energy value of the resulting silage, but did not affect efficiency of fermentation (Jones, 1988). McDonald (1981) suggested malt extract would be a more suitable amendment because the carbohydrate fractions present would have less complex structures. Another source of soluble carbohydrate added to silage has been molasses or molasses by-products. Under certain conditions, favorable responses have been recorded (Woolford, 1984c). Clostridial-type fermentations have been reported in molasses treated silage which may result from excess substrate and reduced competition between LAB and clostridial organisms.

Whey, a major by-product of cheese production, has been used to increase soluble carbohydrate content in ensiled feedstuffs. Lactose, the primary sugar component in whey, is unavailable to LAB organisms (Thomas, 1978). Whey contains greater than 90% water which can be useful with forages lacking adequate moisture for fermentation.

Nitrogenous compounds have been added to silages to enhance the crude protein content and as a preservative (Alaspaa, 1986). Most nitrogenous amendments are

comprised of non-protein nitrogen such as urea, ammonium hydroxide and anhydrous ammonia. Ammonia compounds increase nitrogen content, buffer ensilage and exhibit fungicide properties (Russell et al., 1988). Ammonia buffers the pH decline and thereby extends the fermentation period which increases lactic acid accumulation. On a wet matter or as is basis, current recommendations suggest application of 0.2-0.8% anhydrous ammonium hydrate or 0.5%-1.5% urea to low protein feedstuffs (Owens et al., 1981).

Limestone (Ely, 1978) has been used as a nutritional amendment to corn silage and high moisture corn. Limestone and other calcium salts tend to buffer the pH decline and extend the fermentation. Corn silage and high moisture corn contain minimal amounts of calcium. Addition of calcium salts fortify the calcium density in the feedstuffs. Since corn silage has low concentrations of crude protein and calcium, addition of NPN and calcium salts provides a more complete feedstuff in addition to the fermentation benefits (Ely, 1978).

Several researchers (Siddons et al., 1984; Woolford, 1984c; Murphy, 1986; Voelker et al., 1989) have attempted to improve the fermentation process by addition of fermentation inhibitors such as: acids, esters, salts, sterilants and antibiotics. Mineral and organic acids have been used to inhibit fermentation by maintenance of high hydrogen ion concentration. Sulfuric acid has strong acidifying potential and is effective as a preservative, however, feed consumption may be hindered (Murphy, 1986). In addition, corrosive properties make it an unattractive alternative.

Organic acids inhibit fermentation by reduction in the hydrogen ion concentration

and antimicrobial activity (Woolford, 1984). As carbon chain length increases, acidic properties decrease and antimicrobial characteristics increase. Formic acid is widely used in the United Kingdom as a silage preservative. It is a strong acid (pk_a = 3.75; Waldo, 1978), has bactericidal properties, inhibits coliform bacterial growth and enhances lactic acid fermentation (McDonald, 1981). However, yeast organisms are tolerant of formic acid (Thomas, 1978). Combinations of formic acid and formaldehyde have been used to preserve direct cut grass silages (Haigh et al., 1987).

Acetic acid is commonly used for preservation of foods for human consumption. Acetic acid is classified as a weak acid; has a pk_a of 4.64 (McDonald, 1981) and inhibits enterobacteria and yeast. Acetic acid has been shown to increase residual soluble carbohydrates and decrease soluble nitrogen content and dry matter loss (Mann and McDonald, 1976). Acetic acid has been shown to improve aerobic stability (Moon, 1983). Improved stability may be attributed to antimicrobial properties against yeast.

Propionic acid and esters of propionic acid have antifungal characteristics and have been benefical for low dry matter silages (Voelker et al., 1989). Silage pH influences the antimicrobial effects of propionic acid. Aerobic stability is improved in silages with high propionate levels and Driedger (1976) suggests this effect is due to the antimicrobial aspects of propionic acid. Attempts to utilize propionic acid producing bacteria as a fermentation enhancer have been unsuccessful (McDonald, 1981).

Long chain volatile fatty acids (valeric, caproic and heptanoic acid) have inhibitory action against molds, yeast and enterobacteria (Thomas, 1978). Some other acids which have received investigation are acrylic, sulfamic, benzoic, sorbic, citric and

glycolic acid (McDonald, 1981). The effects of these acids on microorganisms have been variable (Thomas, 1978).

Another form of additive used to restrict or inhibit fermentation is sterilants. Extent of fermentation restriction is highly dependent upon application rate of the sterilant. Formaldehyde is the most commonly used sterilant and is usually applied as formalin (40% formaldehyde in aqueous solution). Another form, paraformaldehyde, is a crystalline material which contains 82-92% formaldehyde. Formaldehyde is a bacteriostat that suppresses fermentation and results in less acid and ammonia production (Henderson et al., 1982). Formaldehyde treated silage can become less stable than untreated silage as formaldehyde is lost through volatilization (Kung et al., 1986).

Many antibiotics have been used as fermentation inhibitors. Bacitracin has been used to inhibit gram positive microorganisms (McDonald, 1981). Antibiotics such as clindamycin, tylosin and pimaricin (Woolford et al., 1975; Woolford and Wilkins, 1975 as cited by Thomas, 1978) have been shown to reduce fermentation through antibacterial, antiprotozoa and antifungal properties, however, it has not proven to be cost effective.

Fermentation of dry feedstuffs can be enhanced by addition of water (Hinders, 1976). Crop materials with less than 40% moisture undergo limited fermentation because of low epiphytic bacteria, limited substrate and inadequate moisture for anaerobic bacterial growth (Kung et al., 1987). Crop material ensiled too dry may undergo excessive heating, carmelization and nutrient loss. Reconstitution of dry corn has been shown to enhance fermentation (Goodrich and Meiske, 1976). Grains harvested at 24% moisture reconstituted to 30% have similar fermentation characteristics as corn harvested

and ensiled at 30% moisture (Aguirre et al., 1983).

Feeding Value of High Moisture Corn

Preservation and storage of HMC has several advantages. Harvesting and storing of corn in the high moisture form allows an earlier and extended harvest season. Secondly, less field loss occurs (Fox, 1976; Goodrich and Meiske, 1976) with harvest of high moisture versus dry corn. Another benefit of high moisture as compared to dry corn involves the increased digestibility (Buchanan-Smith, 1976) and feed conversion efficiency (Perry, 1976). The fourth advantage of HMC involves the elimination of drying costs. Currently, the cost to dry one bushel is $.02 with a 1.4% shrink per percentage unit of moisture removed. In addition, transportation cost is minimized as the corn is used on the farm instead of transported to a terminal market. Several disadvantages are associated with high moisture harvest and storage. Grain stored as high moisture cannot be sold through commercial channels. Secondly, harvest and filling rate must be rapid to minimize respiration, thus storage facilities should be relatively close to harvest site. Rate of removal once feeding begins must also be rapid to minimize aerobic deterioration and high moisture feeds have a shorter bunk life as compared to dry feeds.

Previous research has suggested corn must be ground before feeding (Baker, 1973). However, weight gains are similar for whole and ground corn in high concentrate diets with less than 15% roughage (Dexheimer, 1973). Prigge (1976) showed that soluble nitrogen concentrations are higher in ground versus whole ensiled corn. Soluble

nitrogen concentration is commonly associated with depressed animal performance (Sprague and Bremiman, 1969) Studies from 1971-1973 reported by Guyer and Farlin (1976) indicate efficiency and rate of gain are improved by 5 and 3% respectively for cattle fed ensiled whole corn versus ground HMC. Gill et al. (1982) showed a 15% improvement in feed conversion efficiency with ground ensiled corn as compared to a mixture of whole and ground blended corn prior to ensilement.

Dry matter content has a direct impact on preservability and feeding quality. Acceptable preservation has been observed with dry matter content as high as 79% dry matter in oxygen-limiting silos (Goodrich et al., 1975), however, carmelization can occur in excess of 76% dry matter (Fox, 1976). Corn stored at low dry matter content will have elevated acid and soluble nitrogen content (Fox, 1976) which can decrease dry matter consumption (Goodrich and Meiske, 1976). Dry matter intake has been shown to be reduced with ensiled corn as compared to dry corn (Wilkinson et al., 1976). Palmquist and Conrad (1970) demonstrated that intake, milk fat percentage and acetate to propionate ratio within the rumen were decreased with high moisture corn diets for dairy cows. In growing-finishing cattle fed high moisture corn, conversion of metabolizable energy into tissue gain was more efficient than for cattle fed dry corn (Goodrich and Meiske, 1976). Henderson and Bergen (1970) and Klosterman et al. (1960) suggested the improved efficiency was due to the high availability of acids in high moisture corn. Burroughs et al. (1970) reported decreased dry matter intake and improved feed conversion efficiency as high moisture corn replaced dry corn in the diet. Tonroy et al. (1974) reported a 3-13% reduction in dry matter intake with high moisture

corn diets, however, weight gains were similar.

Buchanan-Smith (1976) reviewed 17 studies which compared high moisture and dry corn in diets of growing-finishing cattle. Cattle fed HMC gained faster or equal to cattle fed dry corn in 11 of the trials. In addition, in 16 of the 17 trials, feed conversion efficiency was improved with HMC as compared to dry corn. Digestibility of HMC tended to be greater than dry corn as well. Aguirre et al. (1984) demonstrated that total tract starch digestion increased as moisture content increased, whereas ruminal fiber digestion decreased. A quadratic effect was seen in organic matter (OM) and dry matter (DM) digestion. Digestion of OM and DM was highest at 65% DM and lowest at intermediate levels (75%). Thornton et al. (1978) showed that digestibility was enhanced in corn stored at 30% moisture as compared to 23% moisture corn and suggested the difference was due to greater starch availability. Aguirre et al. (1984) showed total nitrogen digestibility was greater at the highest moisture content (35%).

The type of protein fed with HMC is a concern. Martin et al. (1980) summarized 10 trials conducted in Oklahoma that compared soybean meal (SBM) and urea as supplemental protein sources for HMC diets and reported an advantage in intake, rate of gain and feed conversion efficiency with SBM. However, these trials indicated an advantage for urea over soybean meal with dry corn, though these results were more variable. The benefit of urea may be less with dry whole corn than with dry processed corn.

Elevated levels of soluble nitrogen can adversely affect animal performance primarily through depressed intakes (Bergen, 1971). Several factors can affect soluble

nitrogen content of the diet as listed by Sprague (1976): grain type, level and type of roughage in the diet and type of supplemental protein. Each of these factors may influence the rate of proteolysis in the rumen.

High dry matter content of ensiled corn has been associated with inadequate oxygen expulsion which allows mold growth to occur. Elevated levels of mold in the diet may depress animal performance (Goodrich et al., 1974).

Microbial inoculants have been added to HMC prior to ensilement (Bolsen et al., 1984) to enhance the fermentation. Dry matter recovery was improved by more than 5% with the addition of lactic acid producing microorganisms. Cattle fed the inoculated corn gained weight more efficiently than cattle fed untreated corn. However, cattle fed the control corn had greater dry matter intake and weight gain.

Deterioration that occurs after oxygen exposure results in DM loss; increased pH; reduced lactic acid; increased yeast, mold and aerobic bacteria populations (Stevenson, 1976). Yeast may cause the initial microbial deterioration in forage silages and aerobic bacteria in corn silage (Woolford, 1984b). Well preserved silages, as defined by low pH and minimum residual soluble carbohydrate, (Woolford, 1984c) tend to be stable upon air exposure. Certain researchers have suggested rapid fermentation with high lactic acid levels are necessary for a stable silage. However, Ohyama et al. (1975) states the only two chemical constituents which constantly create stable silages are elevated ammonia and butyric acid content. In addition, Ohyama et al. (1980) suggests acetic acid has a higher antimycotic activity than lactic acid. Inoculation with rapidly growing lactic acid producing bacteria has been shown to decrease stability of silages even though pH was

below 4.5 and lactic acid levels were high (Rust et al., 1989). All silages that are stable upon air exposure are not desirable. For instance, silages which have undergone a clostridial type fermentation are stable because of the high ammonia and butyric acid levels. Aerobic deterioration may also be critical in the feedbunk. Feedbunks must be managed to prevent accumulation of stale and moldy feed. The longer the stability period of HMC, the easier bunk management becomes. Grains which are fed within 24 hours of initial oxygen exposure should have minimal loss and be of high nutritional quality (Stevenson, 1976). Ensiled grains exposed to oxygen for relatively long periods of time before feeding will have increased microorganisms present, elevated temperatures and decreased nutritive quality (Henderson et al., 1979).

THE EFFECTS OF MICROBIAL INOCULATION FOR THE PRESERVATION OF HIGH MOISTURE CORN ON CATTLE PERFORMANCE, FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY

ABSTRACT

Two trials were conducted to evaluate the effects of microbial inoculation for preservation of high moisture corn (HMC). In trial 1, three concrete upright silos were filled with 83 t of HMC that was treated with either a dry microbial inoculant (DI), prefermented liquid microbial inoculant (PFLI) or an untreated control (CON). Prefermented liquid microbial inoculant or control untreated HMC were placed in two silos in trial 2. Inoculants were applied to supply $2X10^6$ cfu/g of wet corn. Cattle feeding trials were performed to evaluate nutritive value of the HMC in trials 1 and 2. Probe samples were collected from the bottom two doors of each silo and during feedout to evaluate fermentation changes over time. Buried dacron bags at 4 elevations within each silo were recovered during feedout to determine dry matter losses and chemical composition changes during the fermentation period. In both trials, concentrations of soluble nitrogen (SN), lactate, acetate and lactic acid producing bacteria (LAB) increased over time ($P < .05$). Soluble carbohydrates and pH decreased through d 21 but increased ($P < .05$) by time of feedout in trial 1. Acetate content was lower with PFLI as compared to CON or DI (trial 1) indicating a more homolactic fermentation. In trial 2, SN and LAB concentrations were greater while pH was lower with PFLI treated corn ($P < .05$). Dry matter recovery tended to be greater with CON ($P < .05$) than PFLI treated HMC in trial 2. Aerobic stability was evaluated on corn from silos in trial II by placement in styrofoam containers. Temperature and microbial numbers increased ($P < .05$) over the 5 d incubation period for both HMC treatments. Microbial enumeration

was greater for PFLI on d 3 as compared to CON. Average daily gains were similar for all treatments in both trials, however, the DI treated HMC was consumed to a greater extent ($P < .05$) than PFLI corn in trial 1.

Key words: High Moisture Corn, Lactic Acid Bacteria, Inoculant

INTRODUCTION

Moisture conditions during harvest season encourages livestock producers to store corn in a high moisture form. However, losses during storage and feedout can be substantial (McDonald, 1981). Fox (1976) and Goodrich and Meiske (1976) have previously reported 5-20% of ensiled dry matter may not be recovered from the silo. In addition, the low moisture content of high moisture corn (HMC) limits the extent and type of fermentation. Consequently, most ensiled HMC begins deterioration in less than 24 h post-exposure to oxygen (Stevenson, 1976).

Microbial inoculants have been shown to successfully shift fermentation patterns toward more efficient metabolic pathways resulting in less dry matter loss during the fermentation process with whole corn plant and sorghum silages (Bolsen et al., 1984) and grass silages (McDonald, 1981). Microbial inoculation to stimulate a homolactic fermentation has been shown to decrease aerobic stability in whole corn plant silage (Rust et al., 1989). Since the use of microbial inoculants in high moisture corn has not been extensively investigated, this study was conducted to evaluate the potential for microbial inoculants to enhance preservation of the nutritional value of the fresh crop and reduce deterioration upon air exposure. A secondary objective was to evaluate the effects of inoculation of HMC on dry matter recovery from upright silos.

MATERIAL AND METHODS

Trial 1

High moisture corn (HMC) was harvested at approximately 72% dry matter and

ensiled in one of three upright concrete silos (3.6 m x 13.5 m). An alternate load sequence was used to fill the three silos by placement of two loads in a silo (approximately 12.6 t) before rotation to the next silo. A separate silage blower was used for each silo. High moisture corn was placed through a New Holland 355 portable grinder-mixer fitted with a 1.6 cm screen. Silo filling was completed in 6 d and approximately 83 t (72% DM) were placed in each silo. The three treatments allocated to silos were: control (no inocula, CON); dry granular inocula (DI); and prefermented liquid inocula (PFLI). The inoculants[1] (DI and PFLI) were comprised of Lactobacillus plantarum, Streptococcus faecium and Pediococcus acidilactici. The PFLI was reconstituted by mixing 280 g of commercial soluble inoculant (565 x 10^9 cfu/package) in 9.5 L of distilled water and allowing the mixture to set at room temperature for 24 h prior to use. The application rate of DI and PFLI was 1 kg and 2.1 L per t of harvested corn (as is basis), respectively. The respective inoculation rates were used to supply 2 x 10^6 cfu/g of corn. Inoculants were added at the silage blower. A Gandy applicator[2] was used to apply DI while PFLI was applied with a sprayer apparatus equipped with two nozzles using 1.76-2.11 kg/cm^2 of pressure. A thermocouple and two dacron bags were placed at each of four elevations (2.4, 4.8, 7.2 and 9.6 m) in each silo. Temperatures were recorded daily for the first 32 d post-ensiling. Dacron bags were filled with a known weight of HMC and buried. Strips of colored plastic were placed

---

1 Medipharm, USA Des Moines, IA

2 Gandy Company Manufacturers, Owatonna, MN

45-60 cm above the buried bags to identify bag location during feedout. On appearance of plastic strips, bags were uncovered, weighed and samples frozen for chemical analysis. Differences in DM weights of buried bags between placement and removal from silos were utilized to estimate dry matter losses during ensilement. In addition, dry matter recovery from each of the upright silos was determined from the difference in weight of dry matter placed and dry matter fed.

Each load of HMC placed into each silo was subsampled and stored at 4 °C until the end of each day. Samples were composited to provide a single sample for each silo per day and frozen for laboratory analysis. Two 5 cm holes were bored in the bottom two doors of each silo and fitted with rubber stoppers. A 500 g sample was obtained from a single door from each silo on 0, 1, 2, 3, 4, 5, 6, 11, and 21 d post-ensilement. A Penn State forage sampler[3] was utilized to collect samples through bore holes. One hundred fifty g of each bore sample were taken to the laboratory for microbial analysis and the remainder frozen at -19 °C. During the feed out period for each silo, samples were collected every two weeks and analyzed for DM content to calculate DM intake.

Thirty pens of cattle consisting of 12 pens of steers (382 kg) and 18 pens of heifers (342 kg) were randomly assigned to the three treatments to evaluate dry matter intake, weight gain and feed conversion efficiency of the three corn treatments. One hundred eighteen steers were placed in the twelve pens and 104 heifers in 18 pens. Cattle were fed the respective treatments for 56 d.

---

[3] Nasco Agricultural Sciences, Fort Atkinson, WI.

Two initial and final weights were obtained on consecutive days. Cattle were previously adapted to HMC diets and allowed a 5 d period to adapt to the addition of treated HMC in the diets before the trial was initiated. Diets consisted of 85% HMC 10% corn silage and 5% supplement (Table 1).

Trial 2

Two upright concrete silos were filled with approximately 90 t of HMC (75% DM). Corn was ground through a 1.6 cm screen on a New Holland 390 tub grinder prior to ensilement. Each silo was assigned one of two treatments, either CON or PFLI. Silos were filled in a similar load sequence and PFLI applied as previously described. A thermocouple wire and two dacron bags filled with HMC were placed at four elevations within each silo as in Trial 1. Temperatures were recorded from thermocouples during the first 28 d post-ensilement. Samples were collected from the bore holes in the bottom doors on O, 1, 2, 4, 8, 16 and 40 d and stored in a similar manner as described in Trial 1. Samples were collected every 2 wk from each silo during the feedout period for dry matter analysis to determine dry matter intake.

Twenty styrofoam containers, ten per treatment, were filled with 4.8 kg of ensiled HMC and maintained at 20 °C after exposure to air. Cooking thermometers were placed into the HMC in each container to record daily temperature changes. Duplicate containers for each treatment were evacuated on 1, 3, 5, 8, or 14 d and weighed for calculation of DM recovery. Subsamples were collected as containers were evacuated and microbial and chemical analyses performed.

One hundred seventeen crossbred steers (328 kg) were allotted to 18 pens and fed

Table 1. Composition of Diet Fed To Cattle (Expressed as a percent of dry matter)

	Trial 1	Trial 2
High Moisture Corn	85	85
Corn Silage	10	--
Alfalfa-Grass Hay	--	10
Supplement[a]	5	5

[a] Supplements were balanced to provide 11% crude protein, .5% calcium, .35% phosphorous, .7% potassium, .25% sodium chloride and 250 ppm monensin.

one of the two HMC treatments for 126 d. Two weights obtained on consecutive d were recorded for initial and final weight determination. Steers were adjusted from an all corn silage diet to the treated corn diets over a 14 d period. The diet consisted of 85% HMC, 10% chopped hay and 5% supplement (Table 1).

Laboratory Analysis

Frozen samples were allowed to thaw at 20 °C and chopped in a Hobart Macerator[4]. A 100 g sample of chopped wet material was placed in a 60 °C forced air oven for DM determination (AOAC, 1984). Total nitrogen concentration was determined on wet tissue with a micro-kjeldahl procedure (AOAC, 1984) using a Technicon Autoanalyzer[5]. A 10% homogenate of the chopped wet material and distilled water was diluted 50-fold and analyzed for soluble carbohydrate content (Dubois et al., 1956). Soluble nitrogen was measured by placing 20 g of chopped wet material into 80 ml of Ohio buffer (Johnson, 1969) and 5 ml of the homogenate was analyzed for nitrogen using micro-kjeldahl analysis with a Technicon Autoanalyzer (AOAC, 1984). Fifty ml of the homogenate were strained through cheesecloth and pH determined with a combination electrode (Baertsche et al., 1986). Volatile fatty acid analysis in trial 1 was performed by gas chromatography. Twenty ml of strained homogenate was acidified with 4 ml of 25% metaphosphoric acid solution (w/v). After mixing, the extract was centrifuged at 27000 x g for 15 min. Two ul of supernatant were injected into a Hewlett Packard

---

[4] The Hobart Manufacturing Company, Troy, OH

[5] Technicon Instruments Corporation, Tarrytown, NY

Gas Chromatograph (5840A)[6] equipped with a flame ionization detector and microprocessor. The column (2 ml x 2 mm id) was packed with 10% SP - 1200 and 1% H_3PO_4 on chromosorb WAW (80/100). Lactic acid concentration (Trial 1) of the strained homogenate was determined by the procedures of Barker and Summerson (1941). In trial 2, lactic acid, volatile fatty acids and ethanol concentrations were analyzed with high pressure liquid chromatography (HPLC) using a modified procedure from Siegfried et al., (1984). One ml of 20.92% (w/w) calcium hydroxide and .5 ml of 10% (w/w) cupric sulfate were added to 1 ml of the acidified homogenate. Samples were vortexed after addition of each reagent. Cupric sulfate reagent contained .4% (w/w) crotonic acid which served as an internal standard. After reagent addition, samples were allowed to stand at 5 °C for 30 min followed by centrifugation at 10,000 x g. The supernatant was acidified with 25 ul of concentrated sulfuric acid and frozen and thawed twice to remove any remaining protein. Samples were centrifuged at 10,000 x g and filtered through .22 um nylon filters.[7] Standards were prepared in an identical procedure as samples. Thirty-five ul were injected into the HPLC system with a 30 min run time. A Biorad ion exchange column HPX-87H (Cat no. 125-0140)[8] was heated to 45 °C for succinate, lactate, formate, acetate, propionate and butanediol separation and 30 °C for ethanol and butyrate separation. A Biorad guard column (cat.

---

[6] Hewlett Packard, Palo Alto, CA

[7] Millipore Corporation, Bedford, MA.

no. 125-0129)[8] was utilized to protect the ion exchange column. Mobile phase was prepared by diluting 1.66 ml of concentrated sulfuric acid and .41 g of EDTA to a volume of 4 L with double distilled millipore filtered water. The mobile phase was boiled to dissolve EDTA, allowed to cool and filtered through .22 um nylon filters. Mobile phase flow rate was .7 ml/min. The HPLC system[9] was comprised of a Waters 6000A pump, 712 WISP, 730 data module, 720 system controller and 410 refractive index detector. The detector was set at 8 x 10^6 refractive index units full scale. Ammonia-N was determined on the Technicon Autoanalyzer by injection of strained homogenate.

Microbial Analysis

A 100 g sample of HMC was added to 900 ml of sterile distilled water. The mixture was vigorously stirred and filtered through two layers of cheesecloth. The filterate was serially diluted in 10-fold increments to 10^{-12}, using sterile .1% peptone solution. Enumeration of lactic acid producing microorganisms (LAB) was by the spread plate method (Hungate and Fletcher, 1962). A propipettor was utilized to apply 0.2 ml on LBS (BBL Microbiological Systems, MD) agar plates of each dilution and incubated at 39 °C for 72 h. Colonies were grown, and counted and presumptively identified as lactic acid producing microorganisms. Samples from the aerobic stability study were

---

[8] Bio Rad Chemical Division, Richmond, CA.

[9] Waters Associates Inc., Milford, MA

prepared and counted in a similar manner, except the agar plates contained starch.

Statistical Analysis

Cattle performance, fermentation characteristics and aerobic stability were analyzed as a completely randomized design using the analysis of variance procedure (SAS Institute, 1987). Nonorthogonal designed contrasts were made to separate means of cattle performance using Bonferronni-t statistics (Gill, 1978).

RESULTS AND DISCUSSION

Cattle Performance

In trial 1, dry matter intakes were greater ($P < .05$) for cattle fed DI as compared to PFLI (Table 2). Weight gain and fed conversion efficiency were similar. Likewise, weight gain, dry matter intake and feed efficiency were similar for cattle fed the two treatments in trial 2. Results from these two trials suggest the nutritive value of HMC is not significantly influenced by inoculation prior to ensilement. The results of these trials support the conclusion drawn from studies which evaluate the effects of inoculation on the nutritive value of corn silage (Luther, 1986; Rust et al., 1989) or alfalfa (Mader et al., 1985) that microbial inoculation does not enhance nutrient value. Since the nutritive content and acceptability is not altered, the beneficial effects of an inoculant for HMC preservation would have to be manifest in improved bunk stability or enhanced nutrient preservation.

Table 2 Effects of Inoculation of High Moisture Corn On Efficiency of Utilization for Growing-Finishing Cattle

	Treatments			
Trial 1	C[a]	DI[a]	PFLI[a]	SEM[a]
No. of Pens	10	10	10	
ADG,kg	1.24	1.26	1.21	.045
DMI,kg	9.75[c]	10.05[c]	9.03[b]	.185
Gain/feed	.127	.126	.135	.004
Trial 2				
No. of Pens	9		9	
ADG,kg	1.41		1.41	.027
DMI,kg	8.72		8.68	.155
Gain/feed	.163		.162	.003

[a] SEM=standard error of the mean; C=control; DI=dry inoculant; PFLI=prefermented liquid inoculant

[bc] Means in a row with unlike superscripts differ ($P < .05$)

Fermentation Characteristics

Barnett (1954) subdivided silage fermentation into four phases: 1) cellular respiration; (2) aerobic bacteria producing acetic acid; (3) LAB producing lactic and acetic acids; and (4) a quiescent period providing sufficient fermentation has occurred. The concept of phases was utilized by Rust et al. (1989) to study changes over time in bunker silos. In the study, phase 4 was separated into two components, d 5-21 representing the beginning and feed out representing the end (d 151-242). The concept of phases established by Barnett as modified by Rust et al. was incorporated into the analysis of chemical changes over time in the upright silos. The probe samples collected through the bored holes were composited to provide three time periods; phase 1-3 (1-4 d), beginning of phase 4 (5-21 d) and the end of phase 4 during feedout (from buried bags). Greater soluble nitrogen content was exhibited at the beginning of phase 4 over phases 1-3 and continued to increase until the end of phase 4 in trial 1 (Table 3). As in trial 1, soluble nitrogen content did not change in trial 2 until the beginning of phase 4 and continued to increase until the end of phase 4 ($P < .05$). Ammonia nitrogen concentration increased ($P < .0001$) throughout the period of ensilement in trial 2. Further calculation indicates 20.7 and 18.2% of the soluble nitrogen were in the form of ammonia at feed out time for CON and PFLI, respectively. In support of the trend observed in this study, Prigge et al. (1976) demonstrated solubilization of nitrogen occurred through 56 d. This finding suggests solubilization and deamination continue through the fermentation period which in turn suggests the true protein content of high moisture corn decreases the longer it is stored. Consequently, the type of supplemental

Table 3. Effects Of Inoculation On Chemical Changes In Ensiled High Moisture Corn

	Phase 1-3						Phase 4						Feedout						Probability		
Trial 1	CON[a]	SD[a]	PFLI[a]	SD[a]	DI[a]	SD[a]	CON[a]	SD[a]	PFLI[a]	SD[a]	DI[a]	SD[a]	CON[a]	SD[a]	PFLI[a]	SD[a]	DI[a]	SD[a]	Inoc[a]	Phase	I*P[a]
Dry Matter, %	68.5	1.1	73.8	4.34	68.5	1.15	67.7	.43	72.3	5.46	67.9	.43	72.5	3.91	75.7	1.6	74.6	1.71	.0004	.0001	.47
Crude Protein[b]	10.03	.36	8.83	.53	9.8	.26	9.83	.29	9.93	.22	9.65	.26	9.54	.67	9.48	.47	9.76	.72	.95	.28	.7
Soluble N[bc]	.18	.041	.21	.071	.16	.029	.35	.095	.235	.072	.27	.122	.52	.059	.47	.141	.4	.112	.18	.0001	.48
Soluble CHO[bc]	3.55	.92	4.7	1.55	3.89	.55	2.17	.62	2.82	.55	2.22	.2	3.74	2.15	3.18	1.07	3.44	1.07	.59	.0046	.58
pH	5.11	1.07	5.55	.59	5.19	1.1	3.9	.40	3.66	.28	3.59	.43	5.45	.92	6.34	.25	4.99	.89	.12	.0001	.28
Lactate[b]	1.45	1.07	2.35	1.58	.98	.7	3.62	.66	3.35	.40	3.04	2.88	4.23	2.13	6.93	2.51	4.72	3.7	.30	.0006	.73
Acetate[b]	1.17	.54	.62	.23	1.23	.48	2.2	.71	1.23	.33	3.14	.91	2.54	.73	1.4	.48	2.87	2.2	.01	.01	.81
Propionate[b]	.06	.039	.003	.005	.06	.024	.003	.005	.003	.005	.072	.061	.096	.075	.058	.028	.145	.106	.01	.0036	.76
Isobutyrate[b]	.103	.071	.068	.039	.178	.055	.03	.035	.07	.048	.182	.046	.104	.086	.088	.031	.153	.22	.05	.86	.86
Butyrate[b]	.105	.167	.005	.01	.02	.014	.018	.005	.02	.008	.028	.015	.134	.226	.08	.046	1.033	1.114	.21	.07	.10
Isovalerate[b]	.013	.019	0	0	.008	.005	.008	.005	0	0	.003	.005	.008	.013	.035	.026	.009	.015	.60	.03	.02
Valerate[b]	.043	.061	.003	.005	.003	.005	.01	.008	0	0	0	0	.02	.035	.068	.078	.011	.018	.31	.11	.13
LAB[bc]	9.03	.46	8.12	1.13	8.0	1.07	8.89	.29	9.32	.29	9.13	.17							.52	.02	.12
DMR, %[c]													98.08	6.84	97.48	3.44	96.93	5.1	.93		

Table 3. Continued

	Phase 1-3				Phase 4				Feedout				Probability		
Trial 2	Control	SD[a]	PFLI[a]	SD[a]	Control	SD[a]	PFLI[a]	SD[a]	Control	SD[a]	PFLI[a]	SD[a]	Inoculant	Phase	I*P[a]
Dry Matter, %	76.7	1.19	76.2	.61	76.6	1.20	75.8	.28	76.8	.91	76.0	.77	.10	.86	.95
Crude Protein[b]	9.17	.31	8.37	.61	8.95	.49	8.75	.21	9.03	1.10	9.38	.53	.54	.44	.32
Soluble Nitrogen[b]	.16	.019	.15	.02	.17	.008	.20	.389	.28	.049	.39	.079	.12	.0001	.09
Ammonia Nitrogen[b]	.018	.0023	.018	.0023	.026	.0085	.028	.0064	.058	.0139	.071	.0178	.44	.0001	.54
Soluble CHO[bc]	3.04	.21	3.25	.18	2.47	.41	2.72	.71	2.87	1.36	1.69	.64	.57	.17	.20
pH	6.27	.11	6.06	.21	5.87	.04	5.03	.28	4.75	.18	4.17	.06	.0001	.0001	.01
Lactate[b]	.61	.23	.62	.07	.72	.09	.73	.15	.88	.14	.89	.12	.90	.0055	.98
Acetate[b]	.065	.057	.031	.027	.112	.017	.093	.003	.141	.058	.11	.039	.20	.01	.96
Ethanol[b]	.054	.093	0	0	.217	.03	.172	.035	.341	.082	.325	.077	.28	.0001	.85
LAB[bc]	4.74	.72	6.92	.34	6.98	.25	7.89	.41					.0031	.0025	.10
DMR, %[c]									101.1	1.4	99.9	.5	.08		

[a] CON - control; PFLI - prefermented liquid inoculant; DI - dry inoculant; SD - standard deviation.

[b] Expressed as a percent of dry matter.

[c] Soluble N - soluble nitrogen; Soluble CHO - soluble carbohydrate; LAB - lactic acid producing bacteria; DMR - dry matter recovery.

protein may influence the efficiency of ruminal organic matter fermentation and ultimately, the efficiency of utilization of metabolizable energy. Microbial counts were not evaluated at the end of phase 4, but the elevated soluble nitrogen content may result from acid solubilization (Prigge et al., 1976) or proteolysis by microbial enzymes (Bergen et al., 1974). In trial 1, soluble carbohydrate content and pH decreased until phase 4 began and then increased during phase 4 ($P < .01$) for all treatments. Soluble carbohydrates and pH values were lowest during the beginning portion of phase 4 and increased during the remainder of the ensilement period. The pH rise suggests microbial activity occurred throughout the period of ensilement and sufficient fermentation endproducts did not accumulate to suppress microbial metabolism. A significant interaction ($P < .02$) between inoculation treatment and time was observed for pH changes in trial 2. Acidity decreased at a faster rate for the inoculated HMC as compared to CON. Soluble carbohydrate content was unchanged by treatment. The discrepancy between the two trials is not readily explained, however, PFLI in trial 1 had a very high pH at the end of phase 4 which is difficult to explain unless extensive deterioration occurred prior to the removal from the silo. Lactic acid content increased over time from the early phases until the end of phase 4 ($P < .05$) and was not affected by inoculation treatment in trials 1 and 2. Acetic acid content was greater in phase 4 than phases 1-3 ($P < .01$) in trials 1 and 2. In trial 1, PFLI exhibited lower acetic acid concentrations ($P < .01$) than DI or CON. However, in trial 2, inoculation treatment did not influence acetic acid production ($P > .2$). Lactic acid concentrations continued to increase after the initiation of phase 4, while acetic acid accumulation was not evident. The elevated lactic acid content without acetic acid increase would suggest a more homolactic fermentation occurred after the early portion of phase 4 as opposed to early

fermentation (phases 1-3). In contrast to these results, Woolford (1984) reported results which indicate the homolactic species are predominant during the early stages of fermentation while heterolactic bacteria become more prevalent as fermentation progresses to the later stages. Lactic acid content was considerably lower in trial 2 than trial 1 and values previously reported by Bolsen et al. (1984). A portion of the difference in lactic acid content between the two trials can be explained by the lower dry matter content in trial 2 and consequently the extent of fermentation may have been less. The trend of lactic acid accumulation in trial 2 follows a more typical fermentation pattern than trial 1. Another potential explanation for discrepancy between the two trials may involve the methodology. Trial one results were measured by a gas chromatography procedure whereas trial two endproducts were quantified by HPLC analysis.

In trial 1, propionic acid content increased ($P < .004$) during phase 4. A significant phase x inoculation effect was detected for butyric acid ($P < .10$) and isovaleric acid ($P < .03$) in trial 1. The control corn exhibited greater butyric acid content than PFLI ($P < .10$) while DI had an extremely high butyric acid value at the end of phase 4. Corn treated with PFLI showed a greater isovaleric content than CON or DI ($P < .02$) at the end of phase 4. Propionic acid ($P < .01$) and butyric acid ($P < .05$) content were greater with DI over CON or PFLI. The elevated concentrations of branched chain fatty acids may partially explain the pH rise during phase 4. The source of the branched chain acids may have resulted from deamination of amino acids which would produce ammonia and buffer the pH upward. Propionic acid and longer chain fatty acids were not detected in trial 2. Ethanol content was evaluated in trial 2 and was found to increase ($P < .0001$) continuously until the end of phase 4.

In both trials, LAB were found to be greater ($P < .02$) at the beginning of phase 4 as

compared to phases 1-3. Lactic acid bacteria were not measured in the buried bags, however, lactic acid content was increased which would suggest LAB levels may have been increased. A phase x inoculation interaction trend was observed in trial 2 ($P < .10$) for LAB. Lactic acid producing bacteria were greater with PFLI over CON ($P < .0031$) during phases 1-3. However, LAB populations increased after phase 1-3 with CON to similar populations as PFLI during phase 4.

Dry matter recovery as measured from the buried bags was decreased ($P < .08$) with PFLI as compared to CON in trial 2, however, DMR was similar in trial 1. Temperature of corn in silos did not appear to be affected by inoculation treatment in either study (Figure 1). In contrast, Rust et al. (1989) showed that temperature was lower in inoculated whole plant corn silage as compared to untreated silage.

Microbial counts and temperature of the corn treatments during aerobic exposure in trial 2 are shown in Figures 2 and 3. Microbial enumeration after 3 d of air exposure were greater for the inoculated corn ($P < .05$) as compared to CON. By d - 5, enumeration estimates for both treatments were similar. Temperature was greater on 3 and 5 d with PFLI treated corn as opposed to control corn. Control corn exhibited an accelerated heat production between 5 and 8 d exceeding the PFLI corn by 8 d. The elevated microbial population and temperature as seen with PFLI indicate a less stable product. Rust et al. (1989) showed that corn silage treated with a microbial inoculant adversely affected stability as measured by elevated temperature.

Previous research efforts with crops containing higher moisture levels were directed toward the control of fermentation by addition of microorganisms capable of early, rapid growth (Ely et al., 1981). Given the inability of accelerated early growth from microbial starter cultures

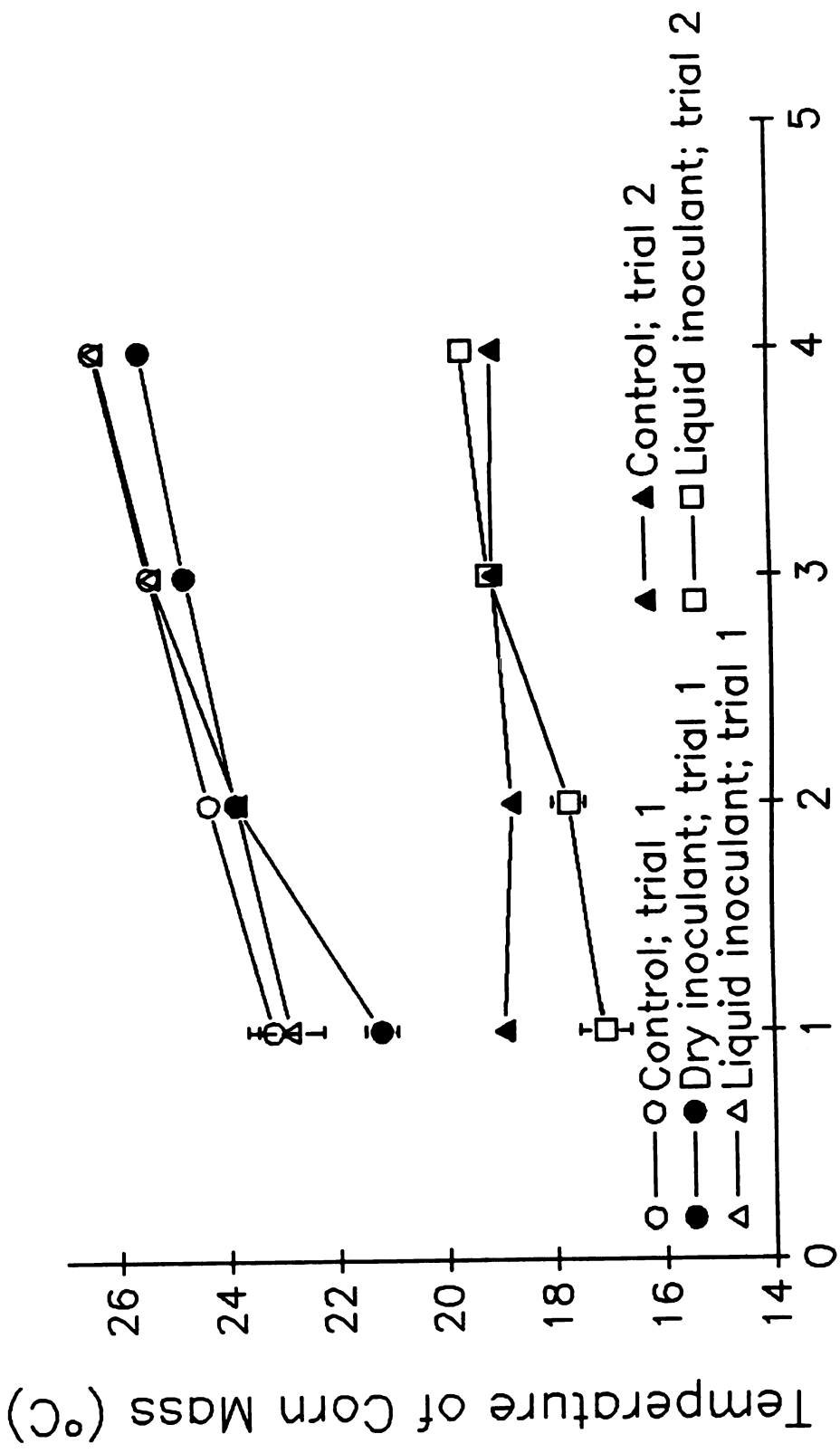

Figure 1. Change of temperature in silos during fermentation due to inoculation treatment. Bars represent standard deviation for each mean.

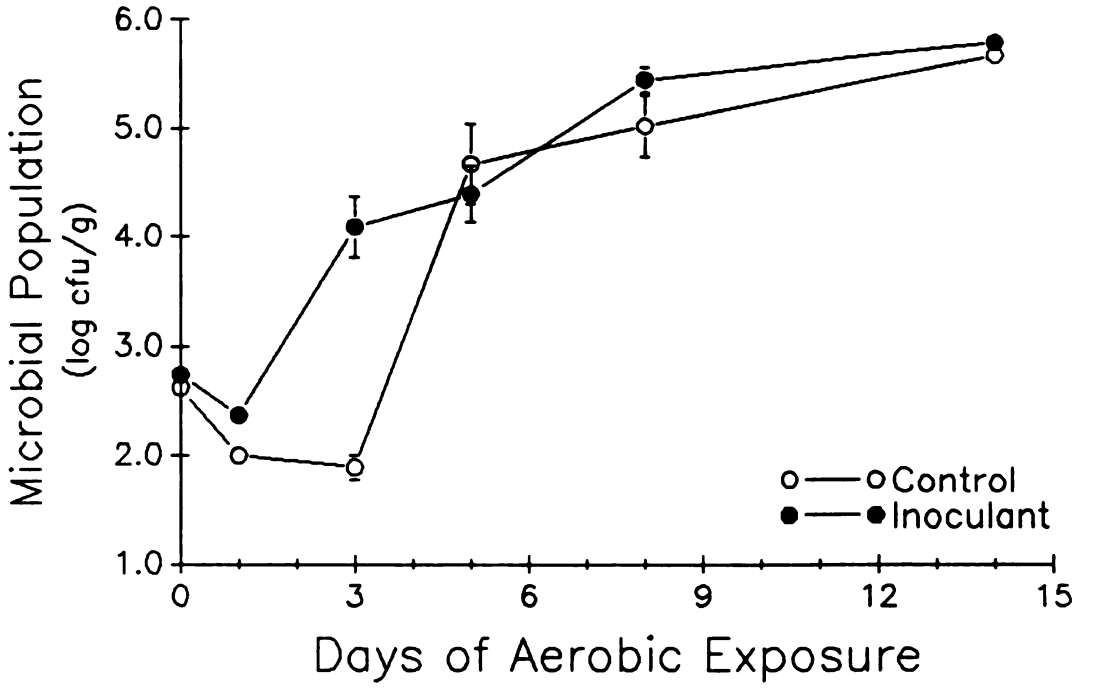

Figure 2. Change in microbial population of high moisture corn during aerobic exposure due to inoculation. Bars represent standard deviation for each mean.

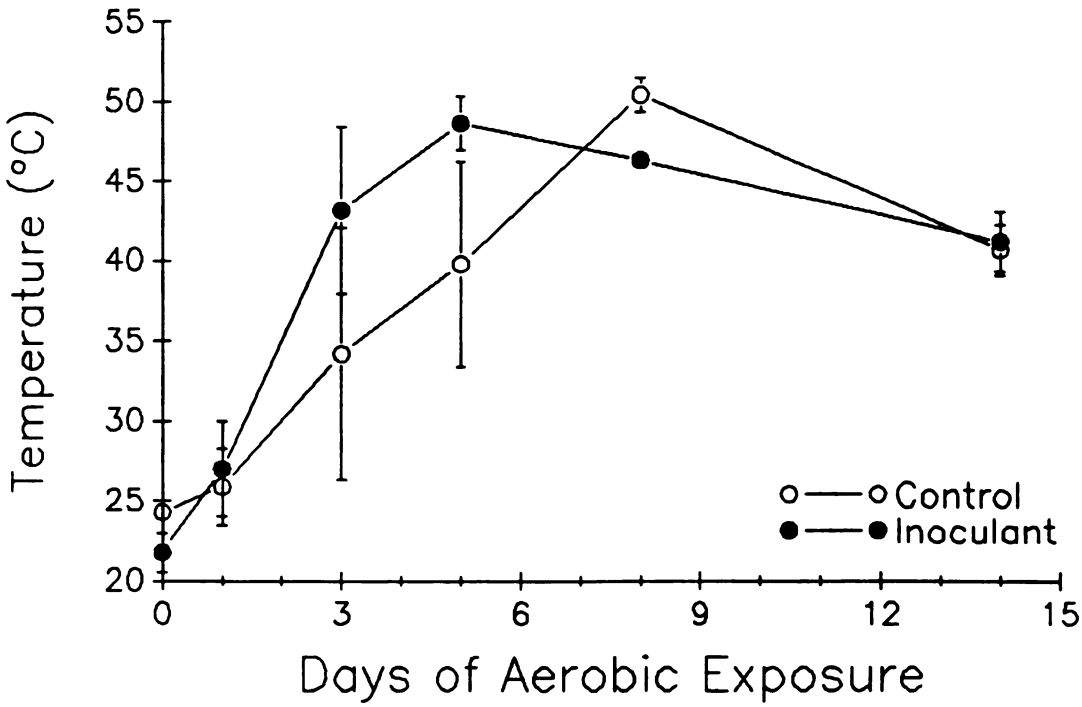

Figure 3. Change in temperature of high moisture corn during aerobic exposure due to inoculation. Bars represent standard deviation for each mean.

to control microbial activity after 4-7 d, alternative strategies to control fermentation beyond 7 d may be beneficial for high moisture corn.

THE EFFECTS OF INOCULATION, MECHANICAL PROCESSING, RECONSTITUTION, AND CHEMICAL ADDITION ON THE FERMENTATION OF HIGH MOISTURE CORN

ABSTRACT

Two trials were conducted with laboratory silos to evaluate the effectiveness of various methods of ensiling high moisture corn (HMC). In trial one, a 2X3X6 factorial arrangement of treatments was utilized to evaluate two particle sizes of HMC stored at 25% moisture (whole versus ground); three inoculation rates of lactic acid producing bacteria (LAB; 0, 1 x 10^6 and 2 x 10^6 cfu/g of wet corn); and six time periods of ensilement (1, 2, 4, 8, 16 and 40 d). Lactate, acetate, ammonia-N content and LAB enumeration were greater with ensiled ground HMC as compared to whole corn ($P < .05$); however, dry matter recovery (DMR) was greater with whole corn treatment ($P < .05$). Ground HMC had lower ethanol content and pH, but greater soluble nitrogen content than whole corn within both LAB inoculation rates ($P < .05$). Higher inoculation rates improved DMR in whole corn and depressed recovery with ground corn ($P < .05$). In trial 2, fermentation characteristics of ground corn ensiled at low moisture content (20%) was evaluated. A 9 x 4 factorial arrangement of treatments was used. The nine treatments applied to the corn prior to ensilement included no treatment or control; LAB inoculation (2 x 10^6 cfu/g); water to reconstitute corn to 75% DM; LAB plus reconstitution, 1% ammonium hydroxide; .89% urea; 1% propionic acid; .15% sorbic acid or a combination of .3% propionic and .1% sorbic acid. Duplicate silos were evacuated at four time endpoints for each treatment (1, 3, 7 or 40 d). Crude protein, ammonia-N, soluble nitrogen and pH were greater while microbial enumeration was reduced ($P < .05$) with addition of urea or ammonium hydroxide. A portion of the

added urea was not hydrolyzed in the low moisture corn, as evidenced by increased soluble N but lower ammonia N ($P < .05$) as compared to the ammonium hydroxide treated corn. Treatment with organic acid reduced the number of bacteria and pH ($P < .05$) as compared to control. Sorbic acid was a more potent inhibitor of fermentation than propionic acid ($P < .05$). Reconstitution of corn increased bacterial growth and lowered pH ($P < .05$) as compared to untreated corn. Fermentation was more extensive with water addition and restricted with nitrogen compounds or organic acids. Dry matter recovery was similar after 40 d ensiling between treatments in trial 2 ($P > .10$). As indicated by DMR, inoculation was beneficial with whole corn and deleterious with ground corn stored at 25% moisture; however, DMR was not improved by addition of amendments or additives to 20% moisture corn.

Key Words

High Moisture Corn, Fermentation, Additives, Inoculation, Particle Size, Reconstitution.

INTRODUCTION

High moisture corn (HMC) has been widely used as a feedstock for ruminant animals. Due to diversified farm enterprises, labor availability and adverse climatic conditions may limit the timeliness and efficiency of harvest. Consequently, HMC is often harvested at moisture contents less than 25% moisture which can create an environment for an unfavorable fermentation (Fox, 1976). The rate and extent of acidification through fermentation are limited by the low moisture content and limited bacterial activity. The restricted fermentation reduces the nutritive value (Owens and Thornton, 1976) and resistance to deterioration upon air exposure (Stevenson, 1976). The addition of chemical or microbial additives prior to ensiling may lessen the detrimental effects of the restricted fermentation.

Various treatments have been used during the ensilement of forage and grain silages (Bolsen et al., 1984; Russell et al., 1988; and Rust et al., 1989). Microbial inoculants have been shown to favorably influence fermentation and improve dry matter recovery (Bolsen et al., 1984). Goodrich et al. (1975) has shown a more rapid fermentation occurred with water addition to low moisture corn to a final moisture content of 33.1%. Organic acids have also been used to restrict microbial growth. Propionic acid is routinely utilized to preserve HMC stored at low moisture contents (Voelker et al., 1985). Propionic acid has caustic properties and can be hazardous. Sorbic acid, a less corrosive material, has been shown to inhibit microbial growth (Lee et al., 1986). Furthermore, combinations of organic acids may inhibit microbial activities more effectively than a single acid (Moon, 1983). Decreasing particle size has

been shown to increase consolidation and improve fermentation (Woolford, 1984c).

The purpose of this study was to determine the effects of microbial inoculation rate and mechanical treatment on fermentation characteristics and dry matter recovery (DMR) in corn ensiled at 75% DM, and secondly, to evaluate chemical and microbial additives to enhance preservation of low moisture corn.

MATERIALS AND METHODS

Trial 1

High moisture corn was harvested at approximately 75% DM and ensiled in 108 laboratory silos (45.7 x 10.2 cm dia.) constructed of PVC pipe and fitted with rubber caps. A bunsen valve was inserted in the top of each silo to allow gas escape. The 36 treatments were in a 2x3x6 factorial arrangement of treatments with three replications. Treatments included two physical forms (ground vs whole), three levels of microbial inoculation[1] (0, 1 x 10^6 or 2 x 10^6 cfu/g of wet corn) and six periods of ensilement (1, 2, 4, 8, 16 or 40 d). High moisture corn (75% DM) was ground through a New Holland 390 tub grinder fitted with 1.6 cm screen. Three kg of corn were placed and consolidated in each silo. Inoculants contained Lactobacillus plantarum, Streptococcus faecium and Pedioccoccus acidilactia organisms. The freeze dried inoculant was reconstituted by mixing 280 g of commercial inoculant (565 x 10^9 cfu per 280 g package) in 9.5 L of distilled water. The mixture was allowed to ferment at room temperature for 24 h prior to use. Prefermented inoculant was applied at the rate of 204

---

[1] Medipharm USA, DesMoines, IA

ml per t of wet corn. The number of epiphytic lactic acid producing microorganisms on the fresh corn crop was 6.8 x 10^3 cfu/g and the prefermented inoculant contained 1 x 10^8 cfu/ml at time of application. Inoculum was manually dispersed on the corn prior to ensilement. Three silos from each inoculant treatment within a physical form of corn were opened on 1, 2, 4, 8, 16 and 40 d. Dry matter recovery from each silo was determined from differences in initial and final dry weight. Two sub-samples from each silo were stored at either -19 °C for future laboratory analysis or placed on ice for less than 5 h at 4 °C before microbial enumeration.

Trial 2

Seventy-two PVC laboratory silos (45.7 x 10.2 cm dia.) were filled and consolidated with 3 kg of corn containing 80% DM. A 9x4 factorial arrangement of treatments included nine treatment amendments and four silo evacuation dates post-ensilement. The nine additive treatments were: 1) control, no treatment; 2) reconstitution with water to 75% DM; 3) inoculation with LAB (2 x 10^6 cfu/g of wet corn; 4) reconstitution to 75% DM and inoculation (2x10^6 cfu/g of wet corn); 5). 1% ammonium hydroxide; 6) .89% urea; 7) 1% propionic acid; 8) .15% sorbic acid and; 9) .3% propionic and .1% sorbic acids. Inoculant was prepared as described in trial 1. Prefermented inoculant was applied at a rate of 47.3 ml per 22.7 kg of fresh corn to supply 2x10^6 cfu/g of fresh corn. Sixty-seven ml of water per kg of corn was added to reconstitute 50 kg of corn to 25% DM. Duplicate silos of each treatment were evacuated on 1, 3, 7, and 40 d post-ensiling. A 150 g sub-sample was taken, placed on ice and

microbial enumeration was performed within 5 h. Three hundred g were stored at -19 °C for laboratory analysis.

Microbial Analysis

A 100 g sample was mixed with 900 ml of sterilized water, blended for 5 min with a magnetic stirrer and filtered through four layers of cheesecloth. The water extract was serially diluted in 10-fold increments in sterile .1% peptone solution. Enumeration of lactic acid producing bacteria (LAB) was by the spread plate method (Hungate and Fletcher, 1962) using selective LBS agar (BBL Microbiological Systems, MD). Each dilution was plated in triplicate. Two hundred ul were placed on each plate, incubated aerobically at 39 °C and colonies counted after 72 h. Colonies were presumptively identified as lactic acid producing microorganisms.

Laboratory Analysis

Samples were allowed to thaw at room temperature and chopped in a Hobart Macerator[2]. A 100 g sample was dried at 60 °C in a forced air oven for dry matter determination (AOAC, 1984). Samples were analyzed for total nitrogen using a micro-kjeldahl technique (AOAC, 1984) with a Technician Autoanalyzer[3]. A 20 g sample of chopped corn was placed in 80 ml of Ohio buffer for soluble nitrogen content determination (Johnson, 1969) by analyzing five ml of the homogenate was analyzed for nitrogen using micro-kjeldahl technique with a Technicon Autoanalyzer (AOAC, 1984).

---

[2] The Hobart Manufacturing Company, Troy, OH

[3] Technicon Instruments Corporation, Tarrytown, NY

A 20% homogenate was prepared with wet corn and distilled water and strained through four layers of cheesecloth for pH, ammonia nitrogen, soluble carbohydrate, volatile fatty acid, lactic acid and ethanol content. Determination of pH was performed on the homogenate with a combination electrode (Baertsche et al., 1986). The homogenate was diluted (1:50) and analyzed for soluble carbohydrate content (Dubois et al., 1956). The strained homogenate was centrifuged at 27,000 x g for 15 min and injected into the Technicon Autoanalyzer for ammonia-N determination. Twenty ml of the strained homogenate was acidified and deproteinized with 4 ml of 25% metaphosphoric acid solution. After mixing, the solution was centrifuged 27,000 x g for 15 min. Supernatant was prepared for lactic acid, volatile fatty acid and ethanol analysis by HPLC procedure of Siegfried et al. (1984) with modifications. One ml of 20.92% (w/w) calcium hydroxide reagent and .5 ml of 10% (w/w) cupric sulfate reagent was added to 1 ml of the corn extract. Samples were vortexed after the addition of each reagent. The cupric sulfate reagent contained crotonic acid (.4%) which served as an internal standard. Samples were stored at 5 °C for 30 min after reagent addition and centrifuged 10,000 x g for 15 min. The supernatant was acidified with 25 ul of concentrated sulfuric acid, frozen and thawed twice followed by centrifugation at 10,000 x g for 15 min to remove the remaining protein. The supernatant was filtered using a .22 um nylon filter (Millipore Products Division, MA). Standards were prepared by adding individual acids together and diluting with water. Standards were prepared in an identical procedure to samples. Thirty-five ml of prepared samples and standards were injected into the HPLC system with a 30 min run time. A Biorad ion exchange column (HPX-87H) was encased

in a water bath at 45 °C for succinate, lactate, formate, acetate, propionate and butanediol separation and 30 °C for ethanol and butyrate separation. A Biorad guard column (catalogue number 125-0129) was utilized to protect the ion exchange column. Mobile phase was prepared by diluting 1.66 ml of concentrated sulfuric acid and .40 g of EDTA to a volume of 4 L with double distilled millipore filtered water. The mixture was boiled to dissolve the EDTA, cooled and filtered through a .22 um nylon filter. Mobile phase flow rate was .7 ml per min. The HPLC system was comprised of a Waters 6000A pump, 712 WISP, 730 data module, 720 system controller and 410 refractive index detector[4]. The detector was set at 8 x 10^6 refractive index units full scale.

Statistical Analysis

Fermentation characteristics were analyzed using the analysis of variance procedure (SAS Institute, 1987). Nonorthoganal designed contrasts were made among d 40 means using Bonferroni - t statistic (Gill, 1978). Contrasts of interest, in trial 1, were whole versus ground corn within each inoculation treatment; and no inoculant versus low rate of inoculation and low versus high inoculation rate within whole and ground corn. In trial 2, contrasts included: Control versus inoculation, control versus reconstitution, reconstitution alone versus reconstitution and inoculation, control versus nitrogen compounds, ammonium hydroxide versus urea, control versus organic acid

---

[4] Waters Associates Inc., Milford, MA.

treatment and propionic versus sorbic acid.

RESULTS

Trial 1

Across both inoculation rates, the rate (Figure 1) and extent (Table 1) of fermentation, as evidenced by pH changes, were reduced with whole corn as compared to ground corn ($P < .05$). Ground corn reached a lower pH by 4 d post-ensiling than whole corn (4.4 versus 5.5), however, the low pH with ground corn was maintained only with inoculated corn. The pH increased in the untreated ground corn after d-8. Lactate (Figure 2) and acetate (Figure 3) concentrations were greater with ground corn than whole corn ($P < .05$) across all inoculation treatments. Within the ground corn treatment, lactate concentration was greater with the low inoculation rate ($P < .05$) as compared to control or high inoculation rate. Acetate was greater at the highest inoculation rate as compared to the other treatments. Inoculation treatments did not effect the acid profile with ensiled whole shelled corn. Lactate and acetate concentrations appear low in the study; however; previously reported studies utilized HMC containing more moisture (Young et al., 1984; Bolsen et al., 1984; Goodrich et al., 1975). The occurrence of lower lactate and higher acetate with the higher inoculation rate with ground corn may result from metabolism of lactate to acetate by yeast or clostridia (Woolford, 1984c). However, LAB have been shown to convert lactate to acetate in silages as well (Woolford, 1984c) which may indicate excess inoculation. Inoculated ground corn showed lactic acid accumulation by 4 and 8 d post-ensilment, however, acetic acid was not detected until 40 d post-ensilement. Whole corn contained greater

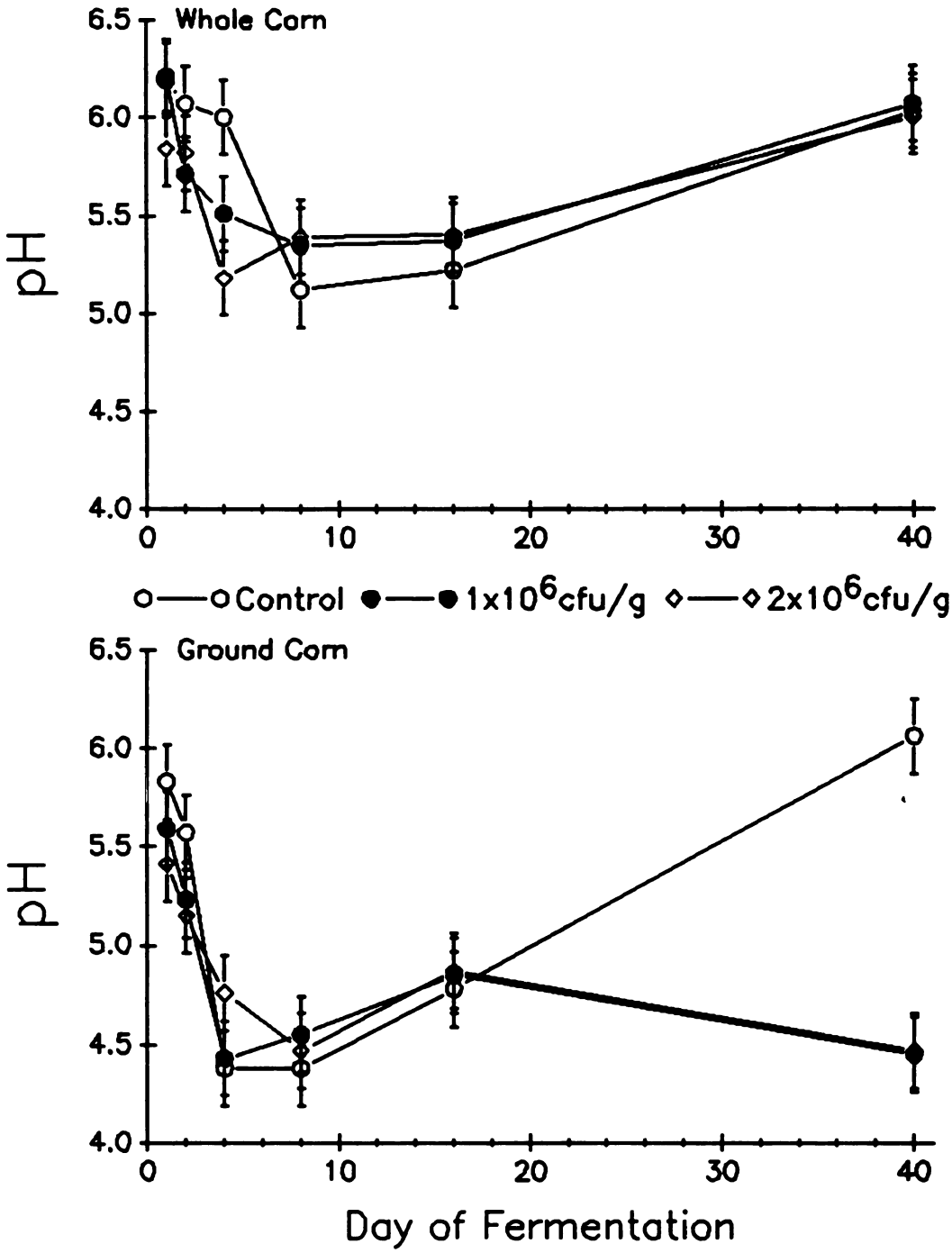

Figure 1. Effect of inoculation rate and mechanical processing on pH of high moisture corn during fermentation. Bars represent standard deviation for each mean.

Table 1. Effects of Inoculation and Mechanical Processing on the Chemical Characteristics

Of Corn After 40d Ensilement at 25% Moisture

	pH	Soluble Carbohydrate	Lactate	Acetate	Ethanol	Ammonia Nitrogen	Soluble Nitrogen	Dry Matter Recovery	LAB[a]
Whole Corn									
Control[b]	6.03	1.58	0	0	.588	.069	4.54	100.4	5.11
1 x 10^6[b]	6.07	1.41	.085	0	.588	.060	4.43	99.5	6.25
2 x 10^6[b]	6.00	1.49	.112	0	.624	.069	4.36	99.8	6.49
Ground Corn									
Control[b]	6.06	1.91	.156	.071	.449	.120	5.37	99.0	6.69
1 x 10^6[b]	4.45	1.63	1.181	.065	.409	.196	8.77	99.2	7.63
2 x 10^6[b]	4.47	1.71	.994	.133	.291	.191	7.93	98.9	7.92
SEM[b]	.135	.283	.026	.006	.037	.01	.606	.008	.172
Contrasts									
W[b] Vs. G[b] Control			*	*		*		*	
W[b] Vs. G[b] 1 x 10^6	*		*	*	*	*	*	*	*
W[b] Vs. G[b] 2 x 10^6	*		*	*	*	*	*	*	*
Within Whole Corn									
Control vs. 1 x 10^6								*	*
1 x 10^6 vs. 2 x 10^6								*	*
Within Ground Corn									
Control vs. 1 x 10^6	*		*			*	*		*
1 x 10^6 vs. 2 x 10^6			*	*				*	*

[a] LAB - lactic acid producing bacteria, log cfu/g.

[b] Control - no inoculation treatment; 1*10^6 cfu/g - inoculation rate; 2*10^6 cfu/g - inoculation rate, cfu/g; SEM - standard error of the mean; W - whole corn; G - ground corn.

[*] Asterisks identify treatment differences ($P < .05$).

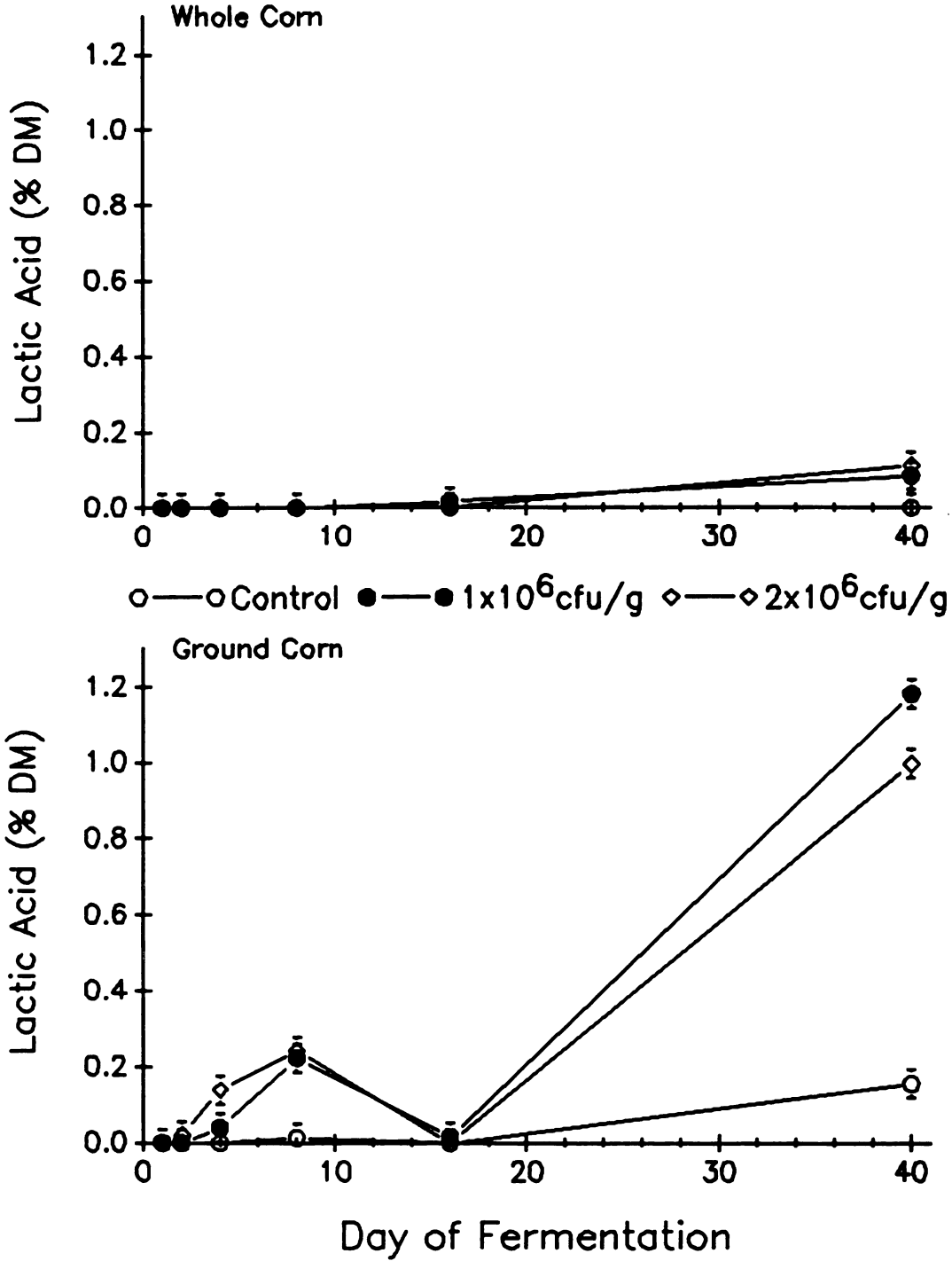

Figure 2. Effect of inoculation rate and mechanical processing on the lactic acid content of high moisture corn during fermentation. Bars represent standard deviation for each mean.

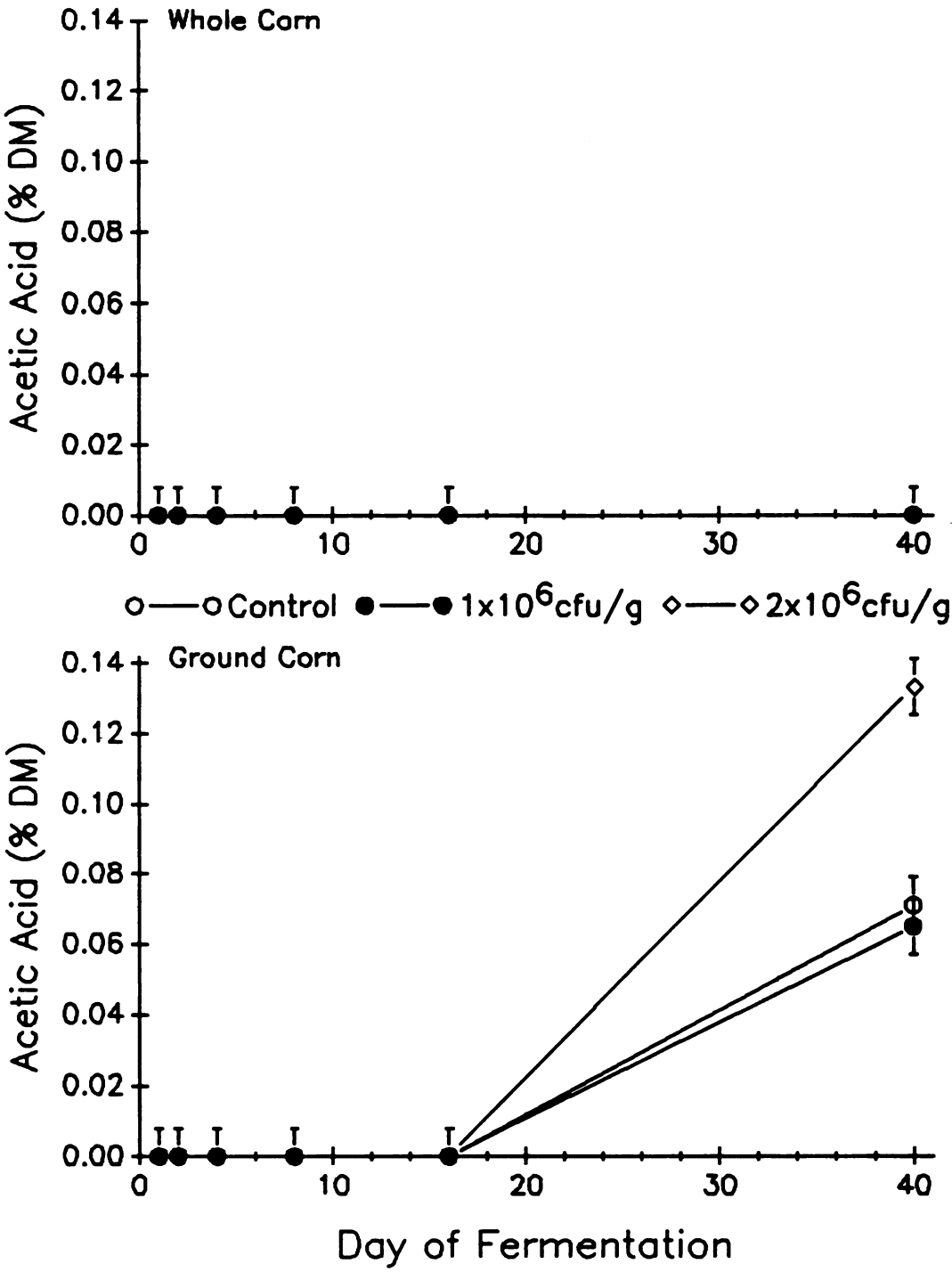

Figure 3. Effect of inoculation rate and mechanical processing on the acetic acid content of high moisture corn during fermentation. Bars represent standard deviation of each mean.

amounts of ethanol on d - 40 ($P < .05$) than ground corn (Table 1), however, ethanol accumulation was not evident until 8 d post-ensilment in the whole corn (Figure 4). Since ethanol is produced under aerobic conditions primarily by yeast organisms, (McDonald, 1981), oxygen may have been trapped between corn kernals. Soluble carbohydrates (Figure 5) were similar for all treatments.

Ensiled ground corn exhibited greater amounts of ammonia N ($P < .05$) than ensiled whole corn (Table 1). The enhanced level of deamination in ensiled ground corn may be explained by the increased availability of nitrogenous compounds in the smaller particles. Inoculation increased ammonia-N in ensiled ground corn as compared to the control. Solubilization of nitrogen was increased ($P < .05$) by ensiled ground corn but no differences were seen with ensiled whole corn. Ensiled ground corn had greater numbers of LAB than whole corn (Table 1, $P < .05$). Presumably, the particle size reduction created more substrate for microbial growth. Microbial numbers increased ($P < .05$) as inoculation rate increased with HMC from both particles sizes. It is peculiar that the population of LAB increases with levels of inoculation but the fermentation endproducts are similar in both situations. Perhaps the LAB were metabolizing fermentation endproducts.

Dry matter recovery was greater for ensiled whole ($P < .05$) as compared to ground corn (Table 1). In addition, inoculation of HMC tended ($P < .05$) to reduce dry matter recovery.

Trial 2

Dry matter content was greater ($P < .05$) in untreated corn as compared to

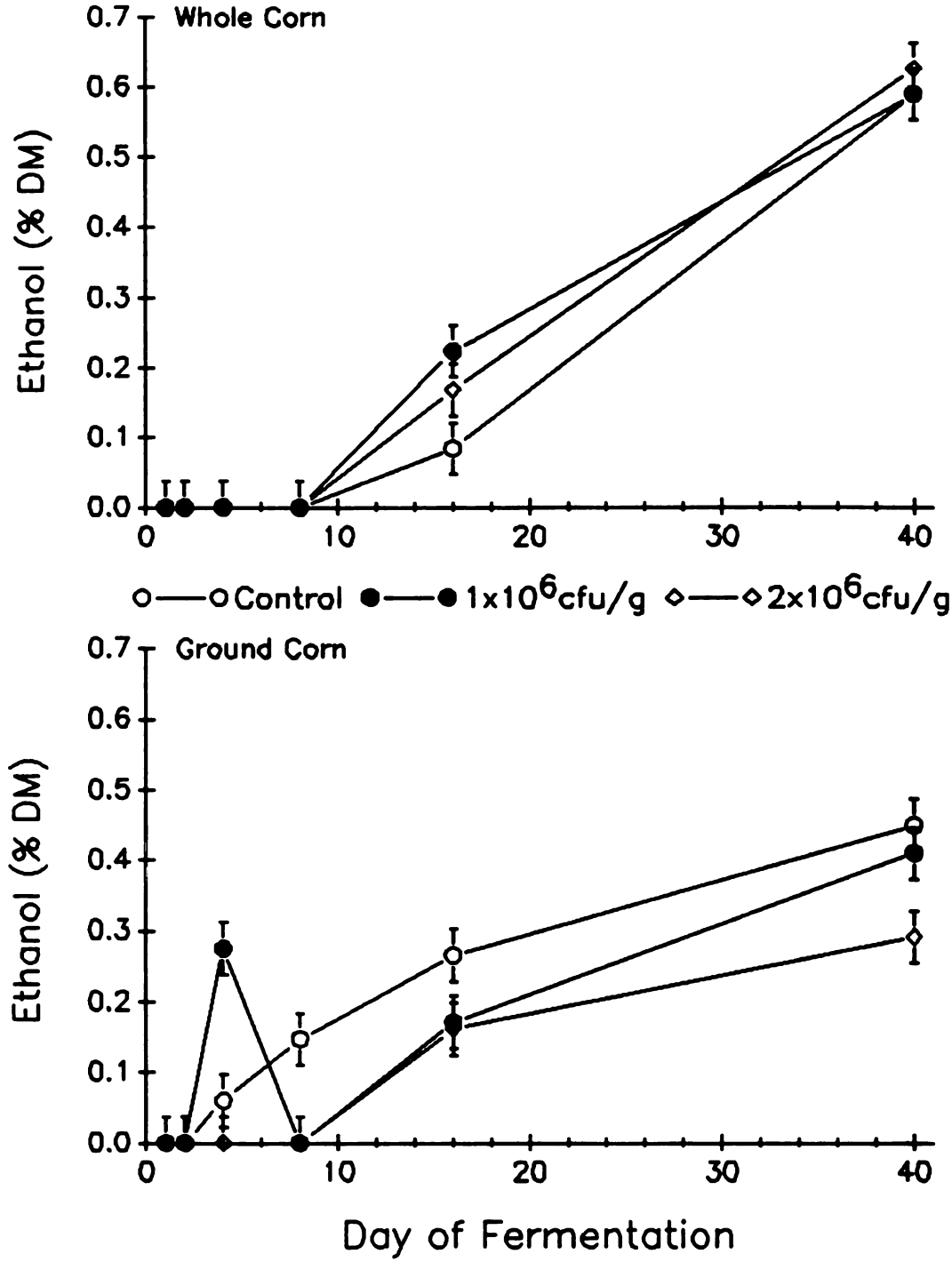

Figure 4. Effect of inoculation rate and mechanical processing on the ethanol content of high moisture corn during fermentation. Bars represent standard deviation for each mean.

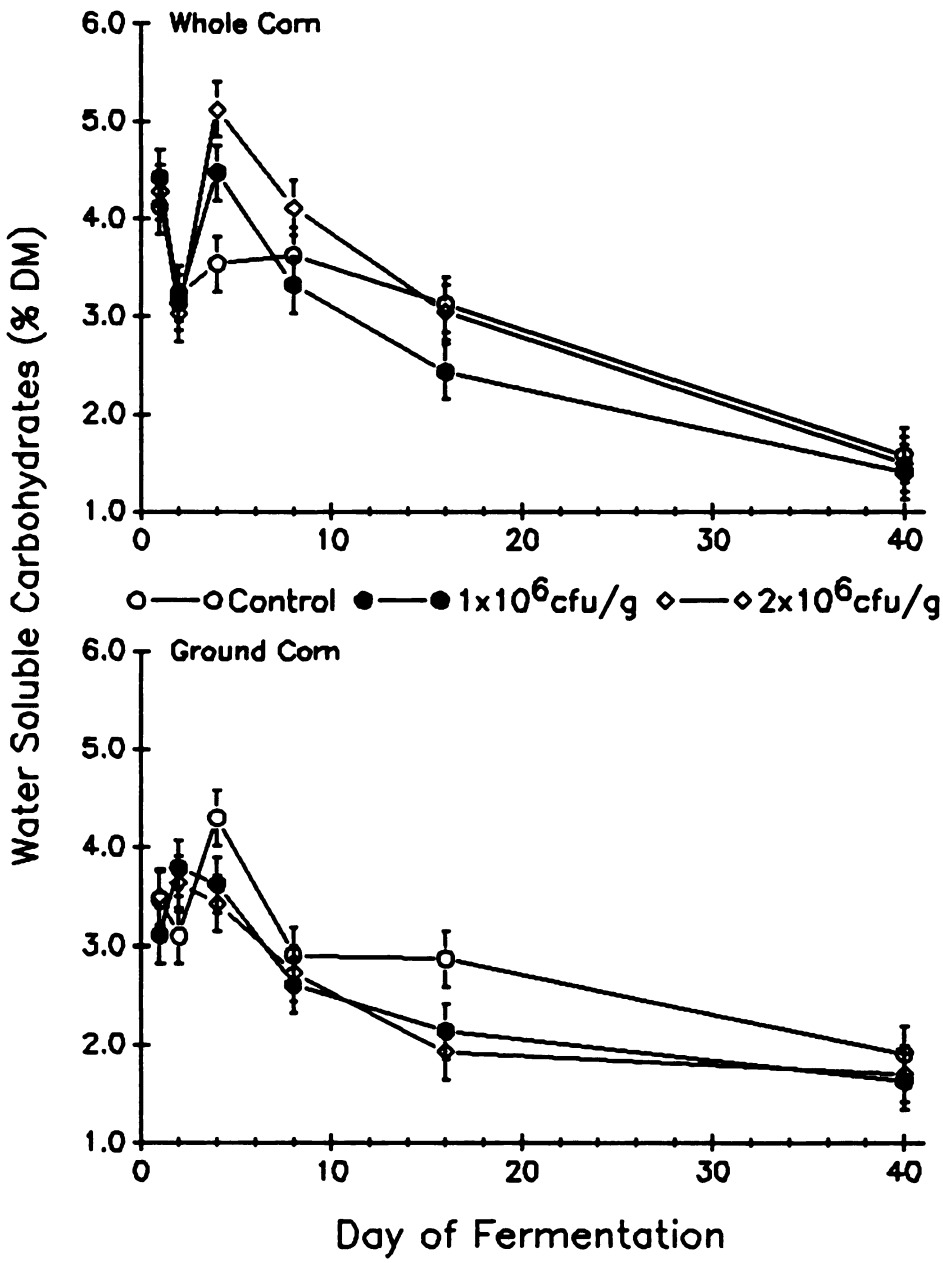

Figure 5. Effect of inoculation rate and mechanical processing on the soluble carbohydrate content of high moisture corn during fermentation. Bars represent standard deviation.

reconstituted corn (Table 2). The lower dry matter content of reconstituted corn is due

to added water. Corn treated with urea had a greater DM content than ammonium hydroxide treated corn ($P < .05$). The addition of urea and ammonium hydroxide increased crude protein, soluble nitrogen and ammonia nitrogen content ($P < .05$) over control corn after 40 d of ensilment. Urea treated corn had greater crude protein and soluble nitrogen content, but lower ammonia nitrogen content ($P < .05$) than ammonium hydroxide treated corn. Lower pH values were detected with water addition ($P < .05$) than nitrogen or acid addition. Urea treated corn had a lower pH ($P < .05$) than ammonium hydroxide treatment while propionic acid addition exhibited lower pH values ($P < .05$) than sorbic acid addition. Reconstituted corn exhibited greater LAB proliferation over control corn ($P < .05$). The addition of nitrogenous or acid compounds restricted LAB growth as compared to control corn ($P < .05$). No differences were detected between treatments for DMR or soluble carbohydrate content ($P > .05$).

Discussion

Retention of nutritional value during the ensiling process is the primary goal of silage enhancements. Dry matter recovery or retention has been the most commonly reported indicator of nutrient preservation. The purpose for ensiling feedstuffs is to allow harvest of a moist material while surrendering only a small portion of the nutritional value during fermentation and storage. Since HMC contains limited amounts of moisture, the challenge to ensure a favorable fermentation is great.

In trial 1, DMR was adversely affected by grinding and inoculation, although all

Table 2. Effects of Inoculation, Reconstitution and Chemical Addition on Fermentation Characteristics of HMC After 40d Ensilement at 20% Moisture

	Control	Reconstituted	PFLF[c]	Reconstituted and PFLF[c]	Ammonia	Propionate	Urea	Sorbate	Propionate and Sorbate	SEM[d]
Dry Matter, %	79.95	74.75	79.6	74.5	78.65	79.8	79.95	80.10	79.25	.22
DMR[a], %	100.2	99.5	99.8	99.2	98.7	100.0	99.0	100.4	99.3	.52
Crude Protein[b]	8.4	8.6	8.6	8.6	10.4	8.7	13.1	8.9	8.8	.31
Ammonia N[b]	.146	.215	.302	.234	1.847	.324	.717	.431	.268	.067
Soluble N[b]	3.98	6.43	3.82	6.68	10.76	4.83	20.47	5.02	5.08	1.32
Soluble CHO[ab]	2.65	1.84	2.70	1.78	2.63	3.92	2.55	3.62	4.31	.60
Lactate[b]	0	.273	0	.602	0	0	0	0	0	.017
Acetate[b]	0	.069	.036	.085	.185	0	0	0	0	.007
Propionate[b]	0	0	0	0	0	.969	0	0	.214	.032
pH	4.27	3.91	4.38	3.82	8.15	4.28	5.00	5.04	4.66	.087
LAB[a], log cfu/g	4.80	6.54	4.00	7.28	2.45	1.89	2.02	1.95	2.52	.341

Table 2. Continued

	Control vs Reconstituted	Control vs PFLI[a]	Reconstituted vs Reconstituted, PFLI Combination	Control vs Ammonia and Urea	Ammonia vs Urea	Control vs Propionate and Sorbate	Propionate vs Sorbate
Dry Matter	*				*		
DMR[b]							
Crude Protein				*	*		
Ammonia N				*	*		
Soluble N				*	*		
Soluble CHO[b]							
Lactate	*		*				
Acetate	*	*		*	*		
Propionate						*	*
pH	*			*	*	*	*
LAB[b]	*			*		*	

[a] PFLI - prefermented microbial inoculant; SEM - standard error of the mean; DMR - dry matter recovery; Soluble CHO - soluble carbohydrate; LAB lactic acid producing bacteria.

[b] Percent of dry matter.

treatments exhibited acceptable DMR ranging from 100.4 to 98.9 %. Bolsen et al. (1984) showed that DMR was improved with LAB addition to HMC which is in contrast to the results of this trial. In other silages such as grass, the extent of mechanical treatment did not greatly influence DMR (Steen, 1985).

In the current study, DMR was reduced under the conditions that stimulate an extended fermentation (elevated moisture content and inoculation). This is in agreement with the results of the study reported by Goodrich et al. (1975). In contrast with trial 1, a Kansas study (Bolsen et al., 1984) reported increased DMR with inoculation. In that study inoculation reduced lactic and acetic acid contents which suggests the inoculated corn had a less extensive fermentation. Though DMR was not reported, Prigge (1976) showed elevated soluble nitrogen content and lower pH with ground corn as compared to whole shelled corn which are indicators of more extensive fermentation and thereby elevated DM losses. During the ensiling process, microbial organisms and enzymes utilize plant substrates and produce carbon dioxide, as one of many endproducts (McDonald, 1981; Woolford, 1984c). In trial 1, extended enzymatic and microbial activity may have increased the amount of dry matter lost as carbon dioxide. In trial 2, no differences in DMR were detected. Recoveries were similar to those in trial 1 ranging from 100.4 to 98.7%.

The concept of extended fermentation being associated with elevated DM losses has been previously discussed (Barnett, 1954; Mcdonald, 1981), however rapid accumulation of lactic acid and decreased pH have been associated with suppressed clostridial and mold growth. Consequently, optimum fermentation is a balance between

fermentative losses and aerobic instability.

Reconstitution enhanced fermentation over control corn as indicated by elevated LAB enumeration and lower pH. However, inoculation did not greatly influence fermentation in the 20% moisture corn. The addition of nitrogen compounds or organic acids decreased microbial activity and pH.

Besides DMR, animal performance has also been used to evaluate fermentation treatments. Nitrogenous compounds have increased the crude protein content of the ensiled feedstuffs which may be beneficial to cattle performance. Young et al. (1984) reported similar cattle performance between silages treated with urea before and after ensilement. Russell et al. (1988) demonstrated similar findings with high moisture milo, however, an advantage was seen with a natural protein source (soybean meal) in the diet over urea added pre-ensiling or in the diet.

Soluble nitrogen content was greater while ammonia nitrogen content was lower in urea treated corn as opposed to ammonium hydroxide treatments. This may indicate that the urea is not fully hydrolyzed. Previous studies have reported that low moisture feedstuffs may not have adequate moisture to activate the urease activity, thus showing decreased urea hydrolysis (Ghate and Bilanski, 1979: Ghate et al., 1981; Alhadhrami et al., 1989). Ureolytic activity has been shown to come from bacterial origin (Cook, 1976) with limited urease activity being derived from plant tissue (Rode et al., 1986).

Conclusion

In this study, the production of lactic acid was increased and pH decreased with

the grinding process and microbial addition to HMC containing 75% DM, however, they proved to be disadvantageous to DMR. At 80% DM various fermentation characteristics were affected by microbial inoculation, reconstitution, nitrogen compound addition and organic acid addition with no differences in DMR. The results of both laboratory studies failed to demonstrate efficacious amendments for the preservation of HMC. Extrapolation of the results from these oxygen-limiting silos to field scale silos may be limited. These studies were performed in air-tight laboratory silos and results may differ in large scale silos.

EFFECTS OF DRY MATTER CONTENT, STORAGE TEMPERATURES AND MICROBIAL INOCULANTS ON THE FERMENTATION AND AEROBIC STABILITY OF HIGH MOISTURE CORN

ABSTRACT

High moisture corn (HMC) was ensiled in laboratory silos for 67 d in a 4x3x2 factorial arrangement of treatments to evaluate the effects of initial dry matter (DM) content, storage temperature and inoculation rate on the pattern of fermentation, dry matter recovery (DMR) and aerobic stability. Corn was harvested at 4 DM contents (78, 74, 66 or 63%), ensiled at three temperatures post-ensiling (4,12 or 20 °C) and treated with 0 or $2x10^6$ cfu/g of lactic acid producing bacteria before ensiling. Each of the 24 treatments was replicated three times. Corn was sampled for chemical and microbial analysis and DMR measured at the end of the ensilement period. Remainder of the corn from each silo was placed into a plastic lined styrofoam container to evaluate deterioration during aerobic exposure. Dry matter recovery was improved ($P < .05$) with the addition of an inoculant and the effect was greatest at the intermediate moisture levels. Storage temperature did not significantly influence DMR during fermentation or aerobic exposure ($P > .10$). Inoculation increased lactic acid and decreased acetic acid content ($P < .05$) as compared to control. Lactic and acetic acid accumulations were greatest ($P < .05$), with low DM corn. During aerobic exposure, DM losses were less ($P < .05$) at high DM content. Inoculation did not influence DM losses during aerobic exposure ($P > .10$). Temperature rise during aerobic exposure was more rapid with low DM corn ($P < .05$). Temperature of corn stored at low dry matter content and low storage temperature rose above ambient temperature ($P < .05$) within 48 h of aerobic exposure. Corn stored at 20 °C showed no change in temperature. These data indicate

that corn stored at high DM content improved DMR during fermentation and aerobic exposure. Inoculation improved DMR during fermentation and did not adversely effect stability at high DM contents. Maximum DMR can be obtained with corn stored at 78% DM and with the use of an inoculant in air tight mini-silos.

INTRODUCTION

The objective of ensilement of high moisture corn (HMC) is to preserve the nutrients present in the fresh crop as economically as possible. Unfortunately, nutrient losses occur as a result of the fermentation and subsequent exposure to oxygen may further reduce digestible nutrients. Management practices such as rapid silo filling, well sealed silos and rapid feedout have been shown to improve nutrient recovery (Fox, 1976). Several factors such as dry matter (DM) content, storage temperature and the use of a microbial inoculant or starter culture have been shown to alter fermentation patterns of whole plant corn (Luther, 1986; Rust et al., 1989) legume silage (Ely et al., 1981) and grass silages (Lindgren et al., 1985a). Limited information is available on the effects of these factors on the fermentation pattern of a low moisture crop such as HMC. Furthermore, information regarding the effects of fermentation enhancers on aerobic stability is limited in the published literature. The microorganisms involved in the deterioration process have been classified by Woolford and Wilkie (1984). In addition, microbial inoculant starter cultures have been reported to positively influence the rate of fermentation in HMC under certain ensiling conditions (Rust et al., 1987).

To accurately determine the conditions in which a starter culture would be

advantageous for HMC, a trial was designed to evaluate the effect of moisture content, storage temperature and addition of a starter culture on nutrient recovery and aerobic stability.

MATERIALS AND METHODS

General

Corn was harvested at four dry matter contents (63.4, 66.4, 74.0 or 78.1% DM) from the same field. At each moisture content, corn was placed into a roller mill, inoculated with water or prefermented liquid inocula and 3 kg consolidated in polyvinyl-chloride laboratory silos (10.2 cmdia x 45.7 cm). The inocula contained a mixture of Lactobacillus plantarum, Streptococcus facium and Pediococcus acidilactia. The inocula was reconstituted from a dry form by mixing 280 g of commercial soluble inoculant (565 x 10^9 cfu/280 g) in 9.5 L of water and allowing to set for 24 h prior to application. Inocula was applied at a rate of 47.3 ml/22.5 kg) of fresh corn to supply 2 x 10^6 cfu/g. Each moisture by inoculation treatment combination was stored at 4, 12.5 or 20 °C. For each treatment combination, triplicate silos were opened 67 d post-ensiling. Subsamples were taken for chemical and microbial analysis. The remainder of the ensiled corn was placed into plastic lined styrofoam containers to evaluate stability during oxygen exposure. Temperature of the corn mass was recorded at 12 h intervals. Corn was evacuated from containers after 48 h of aerobic exposure and subsamples were collected for chemical and microbial analysis.

Chemical Analysis

A 100 g sample of wet corn was dried at 60 °C in a forced air oven for dry matter determination (AOAC, 1984). Analyses for pH and total nitrogen were determined by procedures previously described (Baetsche et al., 1986). A 20% homogenate was deproteinized with 5-sulfosalicylic acid, diluted ten-fold and analyzed for soluble carbohydrate content (Dubois et al., 1956). For ammonia nitrogen analysis, 20 ml of the homogenate was acidified with 4 ml of 25% meta-phosphoric acid, centrifuged and analyzed on Technicon Autoanalyzer[1] for ammonia nitrogen (AOAC, 1984). Soluble nitrogen content determination (Johnson, 1968) was performed by placing a 20 g sample of chopped corn in 80 ml of Ohio buffer. Five ml of the homogenate was analyzed for nitrogen concentration using micro-kjeldahl technique with a Technicon Autoanalyzer (AOAC, 1984). Volatile fatty acids, lactic acid and ethanol were analyzed by HPLC analysis according to Siegfried et al. (1984) with modifications. The HPLC system was comprised of a Waters 6000A pump[2], 712 WISP, 730 data module, 720 system controller and 410 refractive index detector. The detector was set at $8x10^{-6}$ refractive index units full scale. A Biorad[3] exchange column HPX-87H was heated to 45 °C for succinate, lactate, acetate, propionate and butanediol separation and 32 °C for butyrate and ethanol separation. A guard-column (Biorad 125-0129) was inserted to

---

[1] Technicon Instruments Corporation, Tarrytown, NY.

[2] Waters Associates Inc., Milford, MA.

[3] Biorad Chemical Division, Richmond, CA.

protect the ion exchange column. The mobile phase was prepared by dilution of 1.66 ml H_2SO_4 and .4 g EDTA to 4 L of double distilled HPLC water, and filtered through .22 um nylon filter. Flow rate for the mobile phase was .7 ml/min. Injection volume was 20 ul with a run time of 30 min. A 40% honogenate was prepared with wet corn and distilled water and strained through four layers of cheesecloth for HPLC analysis. The homogenate was acidified by adding 5 ml of concentrated sulfuric acid to 20 ml of homogenate before centrifugation at 27,000 x g. One ml of 20.92% (w/w) calcium hydroxide and .5 ml of 10% (w/w) cupric sulfate were added and the sample was again centrifuged at 10,000 x g. Twenty five ul of concentrated sulfuric acid were added to the supernatant prior to a freeze-thawing to deproteinize the sample. Deproteinization process was repeated twice to ensure that a clean preparation was injected into the HPLC. Samples were centrifuged and filtered through .22 um nylon filters. Cupric sulfate solution containing .4% crotonic acid, served as an internal standard.

Microbial Analysis

Samples of high moisture corn were stored in air-tight plastic bags at 4 °C for no longer than 5 h prior to microbial enumeration. One hundred g of corn were added to 900 ml of sterile distilled water. The mixture was homogenized for 1 min in a Waring blendor and filtered through four layers of cheesecloth. The filtrate was serially diluted in 10-fold increments in sterile LBS broth (BBL Microbiology Systems, MD) for determination of lactic acid bacteria. Coliform bacteria were enumerated by serially diluting filtrate in 10-fold increments in sterile E C Medium (Difco Laboratories, Detroit, MI). The extract was also serially diluted in sterile .1% peptone solution. Aliquots of

.1 ml were dispersed on agar plates, incubated aerobically at 39 °C for 72 h and colonies counted for mold and yeast enumeration. Media for mold and yeast determination were prepared by boiling 10 g malt extract, 10 g potato starch, 1 g proteose peptone, 2 g bacto-dextrose and 16 g agar in 1 L of distilled water. After solubilization, the solution was autoclaved for 20 min.

Statistical Analysis

Fermentation characteristics were analyzed using the analysis of variance and correlation coefficients were acquired (SAS Institute, 1987). To establish relationship between specific variables, correlation analyses were performed. Mean separation was performed using Tukey's HSD (Gill, 1978).

RESULTS AND DISCUSSION

Microbial and chemical characteristics of the unensiled corn changed as the harvest season progressed (Table 1). Corn harvested at higher DM content contained greater pH and crude protein content while soluble nitrogen ammonia nitrogen and soluble carbohydrate content were lower ($P < .05$). The reason for elevated protein content at later harvest dates is unknown. Copeland and McDonald (1985) discuss the nutrient deposition into the kernel and indicate that protein and sugar content in pre-mature corn should be greater than mature corn on a DM basis. Protein and sugars are deposited relatively early during kernel development followed by starch deposition which occurs before maturity. The kernel contains no less total protein or sugar as maturity

Table 1. Initial (d-0) Chemical Characteristics Of Corn Harvested At Various DM Contents

Harvest Date	Oct. 3	Oct. 10	Oct. 31	Dec. 2	
Dry Matter, %	63.4	66.4	74.0	78.1	SEM[a]
Laboratory Analyses, % DM					
pH	6.5[d]	6.3[c]	6.6[de]	6.7[e]	.03
Crude Protein	9.6[c]	9.3[c]	10.6[d]	10.4[d]	.16
Soluble N	.115[e]	.111[d]	.104[c]	.102[c]	.003
Ammonia N	.066[f]	.040[e]	.028[d]	.025[c]	.002
Lactate	.13	.01	.02	.01	.13
Soluble Carbohydrate	7.6[d]	5.1[c]	4.9[c]	4.7[c]	.33
Microbial Analysis, log cfu/g					
Coliform	6.7	6.7	6.3	6.7	.44
LAB[b]	4.3	5.0	5.0	4.3	.37
Yeast	2.0[d]	4.3[e]	0.0[c]	1.3[cd]	.37
Mold	0.0	0.0	0.0	0.0	0.0

[a] SEM - Standard error of the mean.

[b] LAB - lactic acid producing bacteria.

[cdef] Means within a row with unlike superscripts differ ($P < .05$).

progresses, however, expressed on a percentage basis, protein and sugar contents decrease as starch accumulates. Soluble carbohydrate content over time reflects a similar sequence of events. Analysis supports the traditional view of less sugar expressed as a percent of DM as the plant matures; however, total nitrogen content does not. Microbial populations of LAB and coliform were similar across harvest dates and DM contents. Mold organisms were not present in any of the unensiled corn samples. Yeast populations appeared to be higher during the early harvest dates, although only the 66.4% DM corn had significantly greater yeast ($P < .05$).

Inoculation treatment increased DMR ($P < .05$) as compared to control (Table 2). The .5% increase of unrecoverable DM with inoculation seems small and differences that small may be difficult to demonstrate under field conditions. However, non-significant improvements in DMR of this magnitude due to inoculation of HMC have been reported in field studies (Wardynski et al., 1990). Dry matter recovery was positively correlated (Table 3) with dry matter content ($r=.86$; $P < .0001$), pH ($r=.73$; $P < .0001$), soluble carbohydrate ($r=.23$; $P < .052$) crude protien content ($r = .57$; $P < .0001$) and mold population (.28; $P < .16$). Ensiled corn with higher DM content had greater crude protein content and more mold colonies. After the 67 d ensilement period, a negative relationship was observed between DMR and yeast population (-.33; $P < .004$), soluble nitrogen (-.68; $P < .0001$) ammonia nitrogen (-.59; $P < .001$) lactic acid (-.64; $P < .0001$) acetic acid (-.77; $P < .0001$) and ethanol (-.71; $P < .001$). A significant ($P < .07$) DM by storage temperature interaction effect on DMR was evident in this study. Overall, dry matter recovery increased as DM content increased, however, 74% DM

Table 2. Effect Of Inoculation On Dry Matter Recovery After 67d Ensilement

	Control	Inoculant	SEM[a]
Dry Matter Recovery, %	98.2[b]	98.7[c]	.05

[a] Standard error of the mean.

[bc] Means within a row with unlike superscripts differ ($P < .05$).

Table 3. Table of correlation coefficients between chemical and microbial constituents

	Dry Matter	Temp.[b]	Inoc.[b]	pH	Coliform	LAB[b]	Yeast	Mold	Bacillus	DMR[b]	Crude Protein	Soluble N	Ammonia N	Lactic Acid	Acetic Acid	Ethanol	Soluble Carbohydrate
pH	.84	-.35	-.12	1.00													
	.001	.0025	.32														
Coliform	.02	-.26	.05	.15	1.00												
	.89	.028	.66	.21													
LAB[b]	-.20	-.05	.15	-.38	-1.5	1.00											
	.09	.68	.20	.001	.20												
Yeast	-.50	.23	.10	-.49	.29	.02	1.00										
	.0001	.05	.39	.0001	.013	.85											
Mold	.42	.32	-.04	.15	-.45	-.14	-.46	1.00									
	.0002	.006	.74	.20	.0001	.23	.0001										
Bacillus	.09	.07	.17	-.02	-.15	.20	-.16	.08	1.00								
	.48	.58	.14	.87	.21	.10	.17	.49									
DMR[b]	.86	.01	.21	.73	.09	-.18	-.33	.28	.01	1.00							
	.0001	.95	.08	.0001	.44	.13	.004	.016	.91								
Crude Protein	.67	-.09	-.08	.61	-.24	-.16	-.59	.29	.10	.57	1.00						
	.0001	.48	.51	.0001	.041	.17	.0001	.015	.41	.0001							
Soluble N	-.82	.48	.01	-.82	.05	.02	.64	-.29	-.16	-.68	-.69	1.00					
	.0001	.0001	.97	.0001	.69	.85	.0001	.014	.19	.0001	.0001						
Ammonia N	-.72	.59	-.04	-.78	.05	.00	.66	-.24	-.17	-.59	-.64	.98	1.00				
	.0001	.0001	.76	.0001	.67	.99	.0001	.042	.16	.0001	.0001	.0001					
Lactic Acid	-.79	.50	.08	-.85	-.03	.10	.57	-.23	-.10	-.64	-.59	.93	.91	1.00			
	.0001	.0001	.53	.0001	.83	.39	.0001	.057	.42	.0001	.0001	.0001	.0001				
Acetic Acid	-.82	.22	-.22	-.80	-.13	.24	.46	-.32	-.14	-.77	-.48	.78	.74	.81	1.00		
	.0001	.065	.066	.0001	.27	.043	.0001	.006	.24	.0001	.0001	.0001	.0001	.0001			
Ethanol	-.78	-.07	-.12	-.57	-.32	.12	.20	-.28	-.01	-.71	-.24	.49	.39	.54	.71	1.00	
	.0001	.57	.31	.0001	.006	.31	.084	.018	.93	.0001	.04	.0001	.0007	.0001	.0001		
Sol CHO[b]	-.39	-.29	.01	-.05	-.01	-.41	.13	-.14	-.21	-.23	-.09	.22	.13	.20	.11	.48	1.00
	.0006	.012	.92	.69	.92	.0003	.27	.23	.07	.052	.47	.062	.26	.10	.34	.0001	

[a] Values expressed as r with probabilities on line below.

[b] Temperature (Temp); inoculation (Inoc); lactic acid producing bacteria (LAB); dry matter recovery (DMR); soluble carbohydrate (Sol CHO).

ensilage stored at 12.5 °C had similar DM recovery as ensilage stored at 4 °C. The results of this study suggest storage of ensiled HMC at 12.5 °C may allow preservation over a wider moisture range with less DM loss.

The trends for improved DMR as moisture level decreases is in agreement with previous results reported by Goodrich and Meiske (1976). Dry matter losses associated with the HMC in this study were less than 5% which is similar to published results for other fermented feedstuffs (McDonald, 1981; Woolford, 1984c).

Comparison of the mean squares for storage temperature by the DM by storage temperature interaction mean square, indicates the majority of the variation in DMR is associated with DM content. In support of this premise, the correlation between storage temperature and DMR was nearly zero ($r = .01$; $P > .95$). The effects of storage temperature on DMR with HMC in this study are in agreement with previous results reported by Garcia et al. (1989) with ensiled alfalfa.

A DM x storage temperature interaction ($P < .05$) was observed for pH, lactic acid, acetic acid, ethanol, soluble nitrogen, ammonia nitrogen, LAB, coliform, bacillus, yeast and mold population (Figures 1-14). Across all storage temperatures, there was a trend for pH to increase ($P < .05$) as DM content increased (Figure 1). One discrepancy from this overall trend occurred with the driest corn. Storage at 12.5 °C yielded a lower pH than similar HMC stored at 4 or 20 °C. The reason for the discrepancy is unknown. Lactic acid content increased ($P < .05$) as DM content decreased with all three storage temperatures, however, the amount of lactic acid production per unit of DM increased more with highest storage temperature (Figure 1).

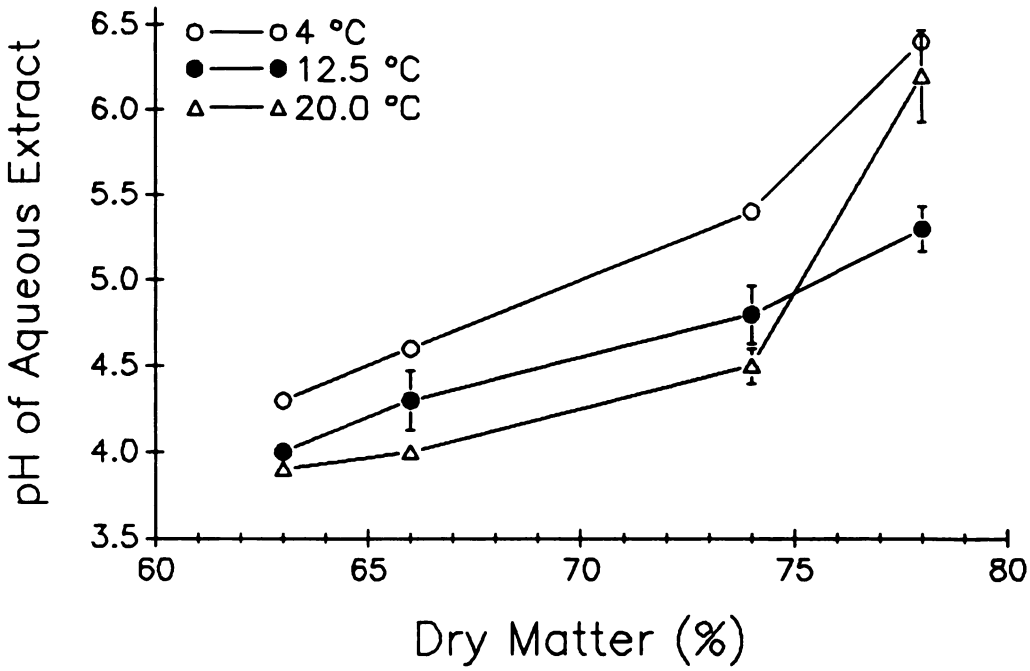

Figure 1. Effects of storage temperature and dry matter content on pH decline. Bars represent standard deviation for each mean.

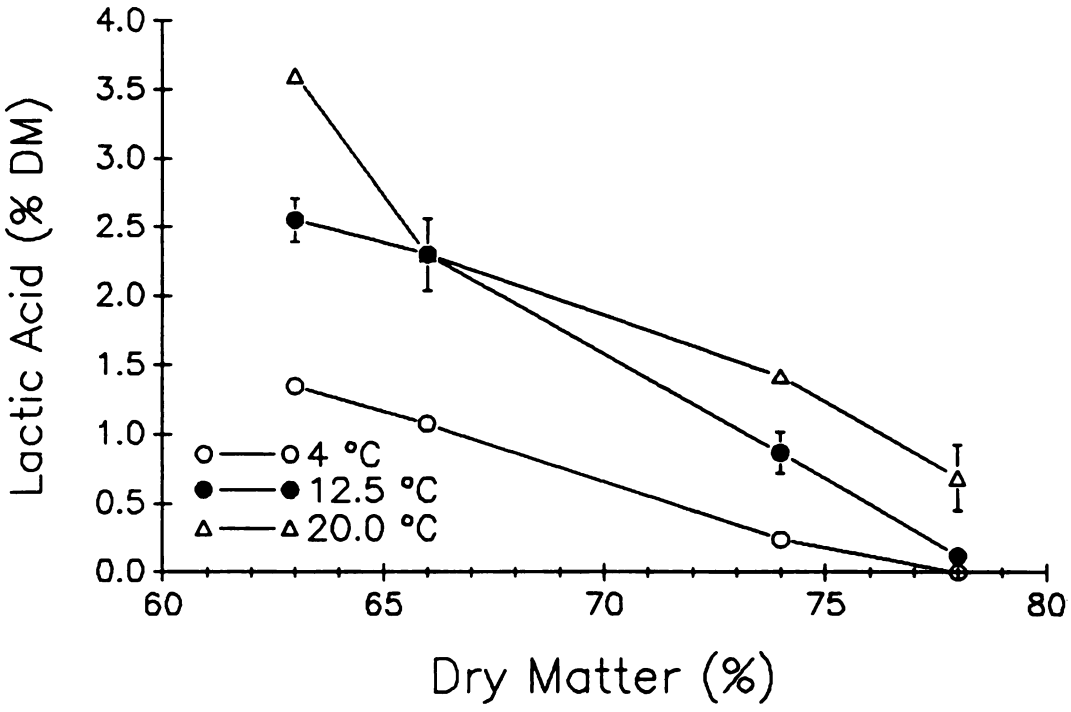

Figure 2. Effects of storage temperature and dry matter content on lactic acid content. Bars represent standard deviation for each mean.

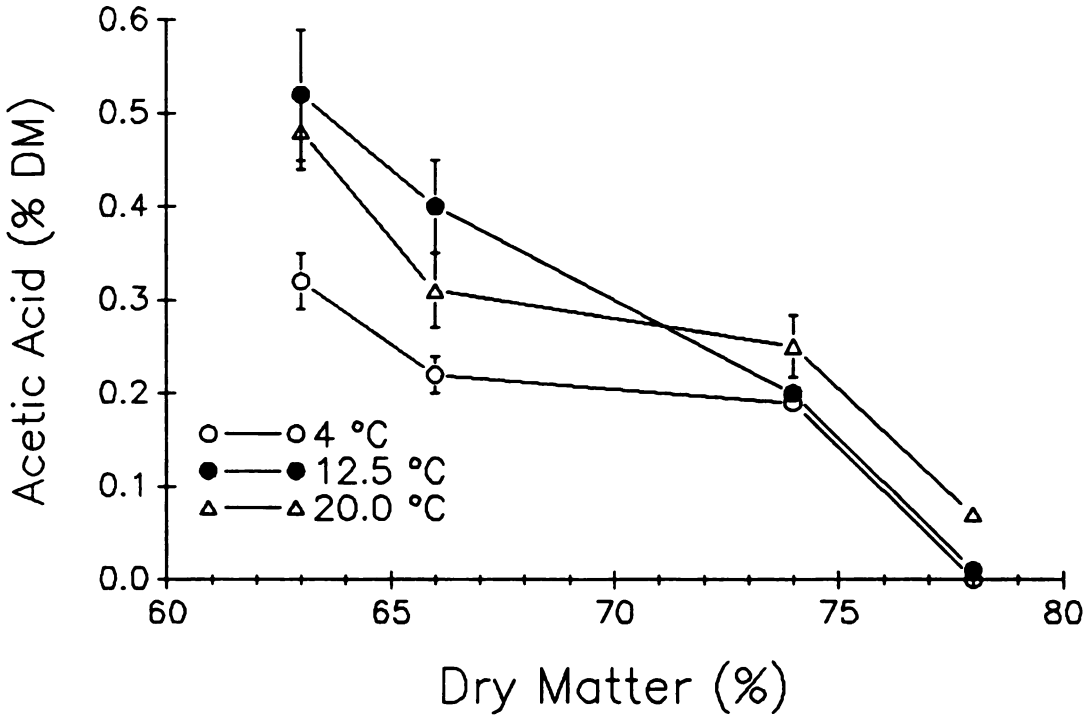

Figure 3. Effects of storage temperature and dry matter content on acetic acid content. Bars represent standard deviation for each mean.

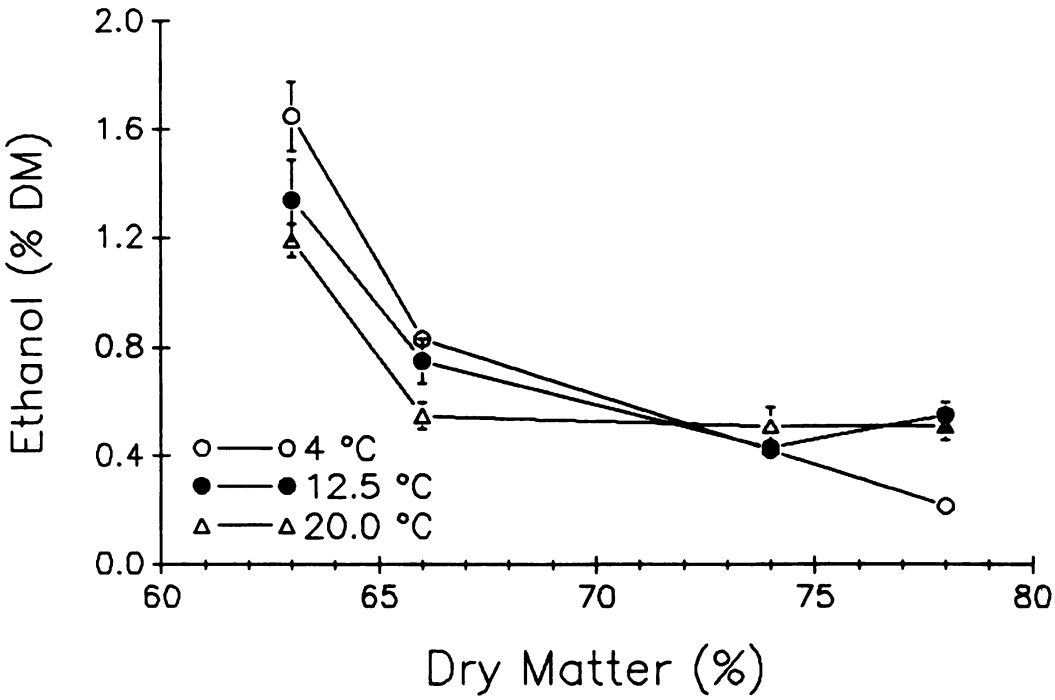

Figure 4. Effects of storage temperature and dry matter content on ethanol content. Bars represent standard deviation for each mean.

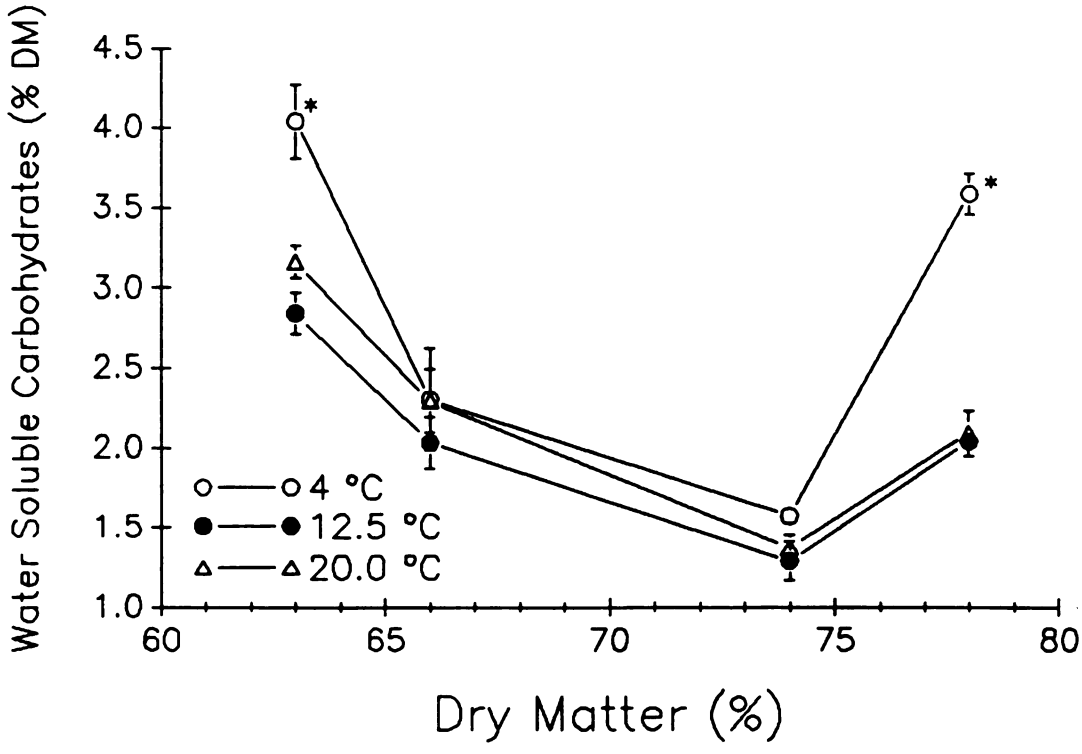

Figure 5. Effects of storage temperature and dry matter content on water soluble carbohydrate content. Bars represent standard deviation for each mean.

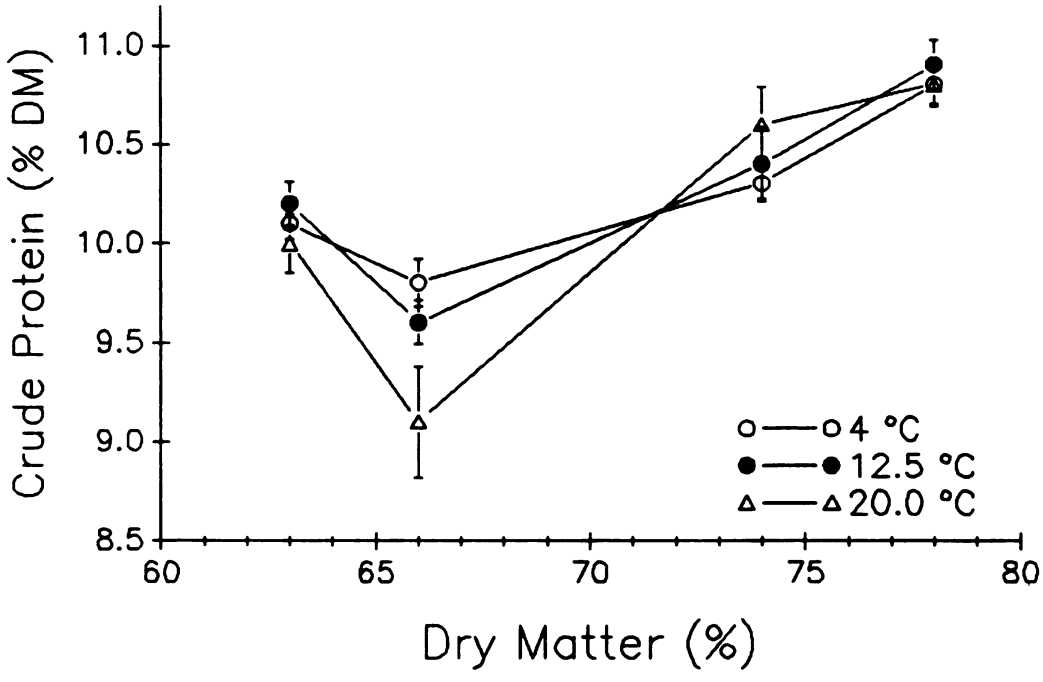

Figure 6. Effects of storage temperature and dry matter content on crude protein content. Bars represent standard deviation for each mean.

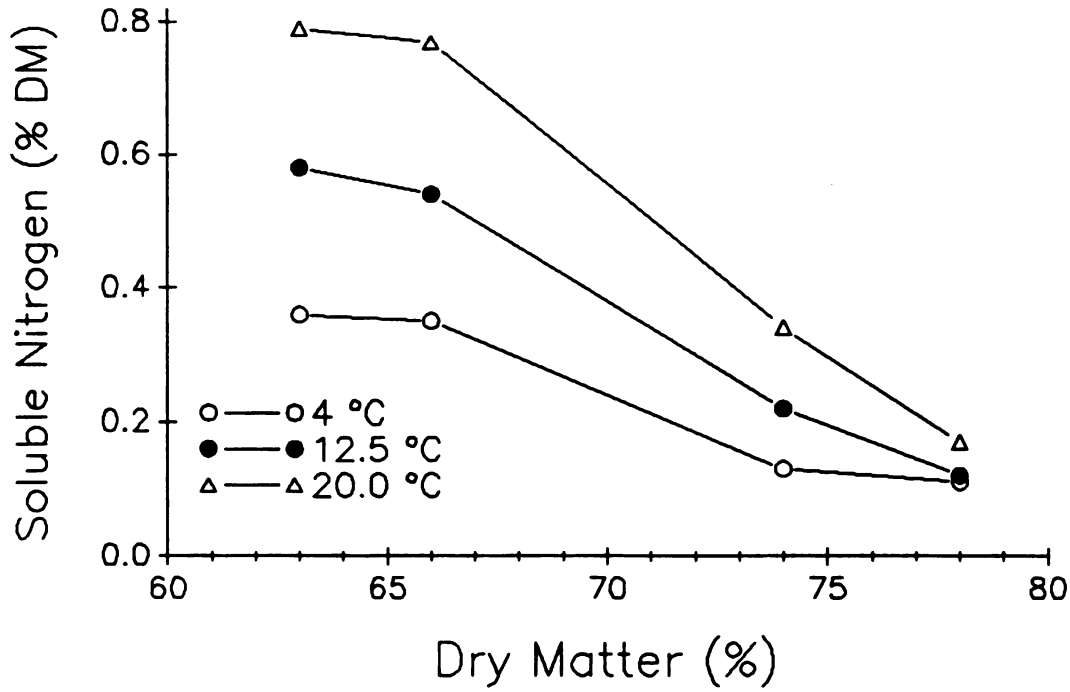

Figure 7. Effects of storage temperature and dry matter content on soluble nitrogen content. Bars represent standard deviation for each mean.

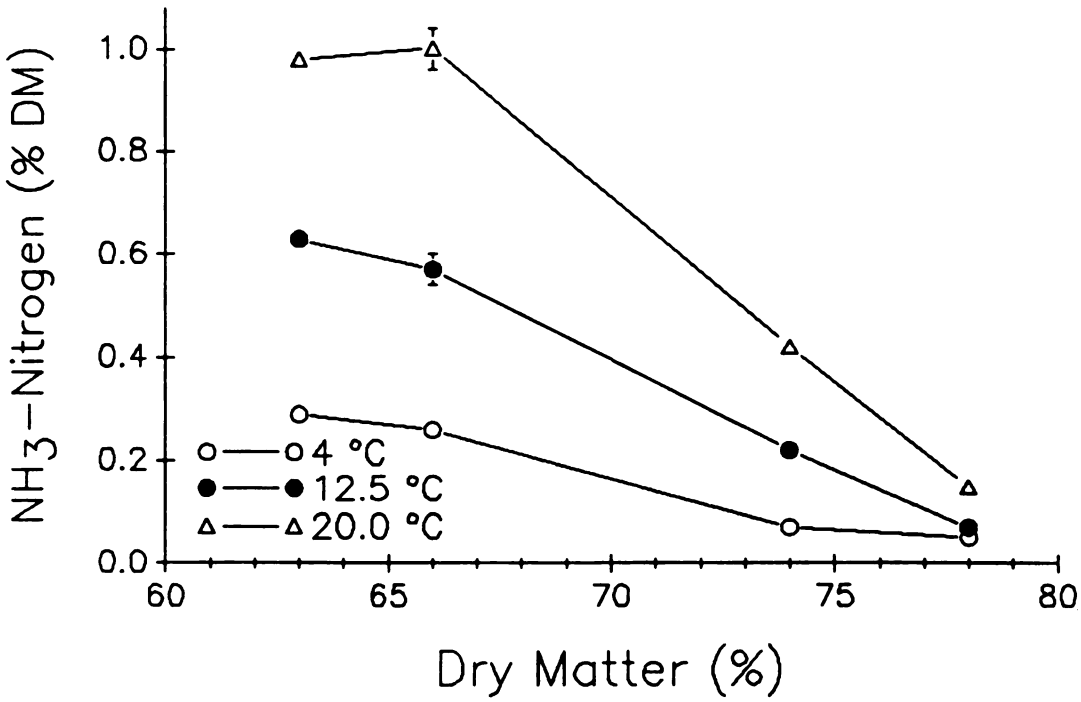

Figure 8. Effects of storage temperature and dry matter content on ammonia nitrogen content. Bars represent standard deviation for each mean.

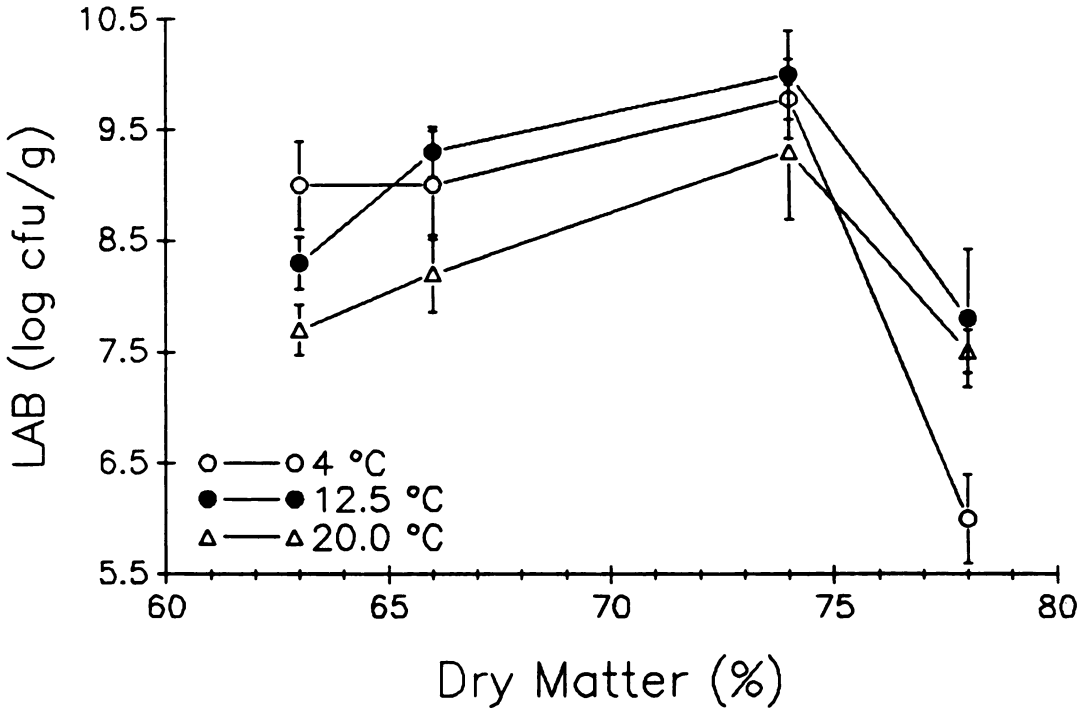

Figure 9. Effects of storage temperature and dry matter content on lactic acid producing bacteria (LAB) counts. Bars represent standard deviation for each mean.

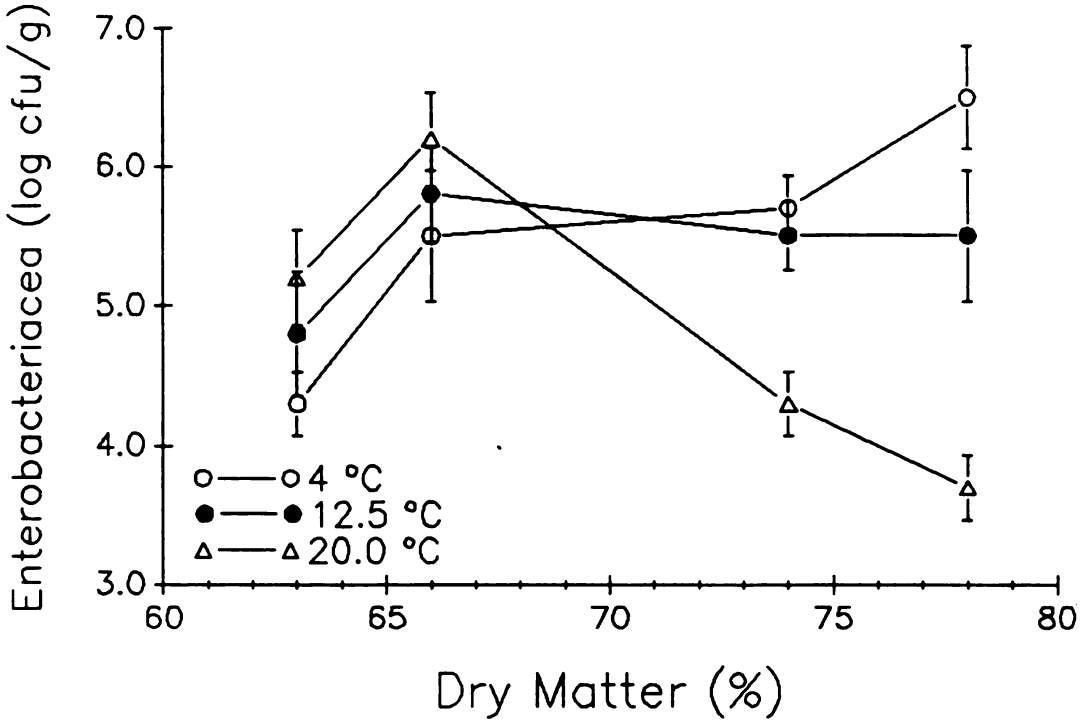

Figure 10. Effects of storage temperature and dry matter content on enterobacteriaceae counts. Bars represent standard deviation for each mean.

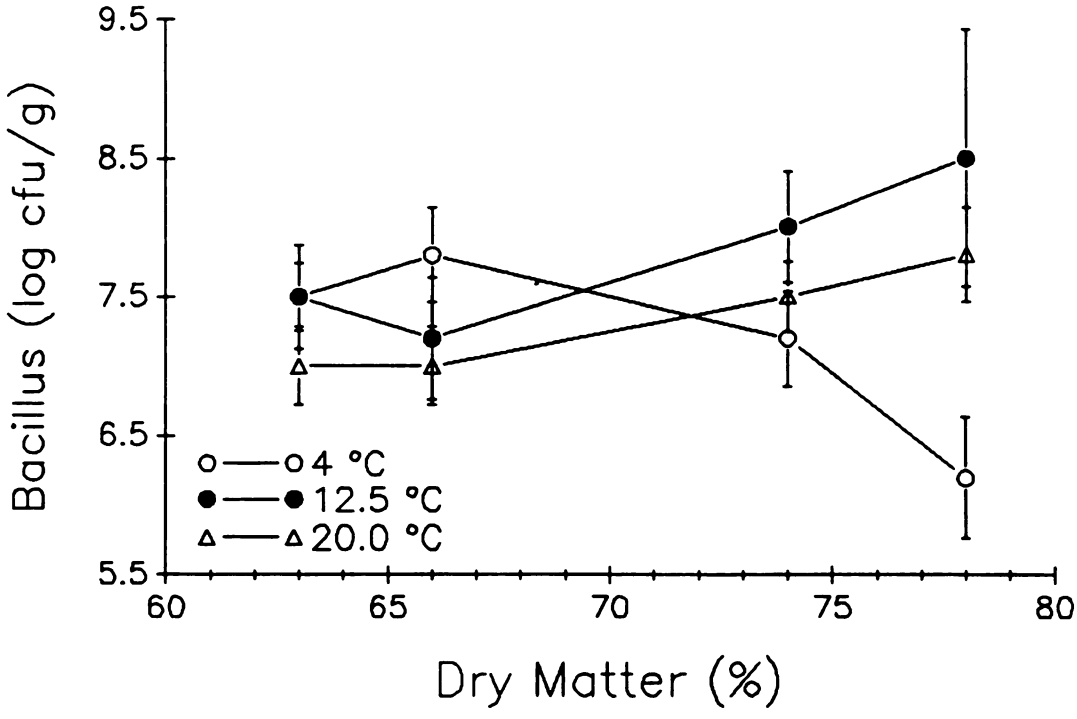

Figure 11. Effects of storage temperature and dry matter content on bacillus counts. Bars represent standard deviation for each mean.

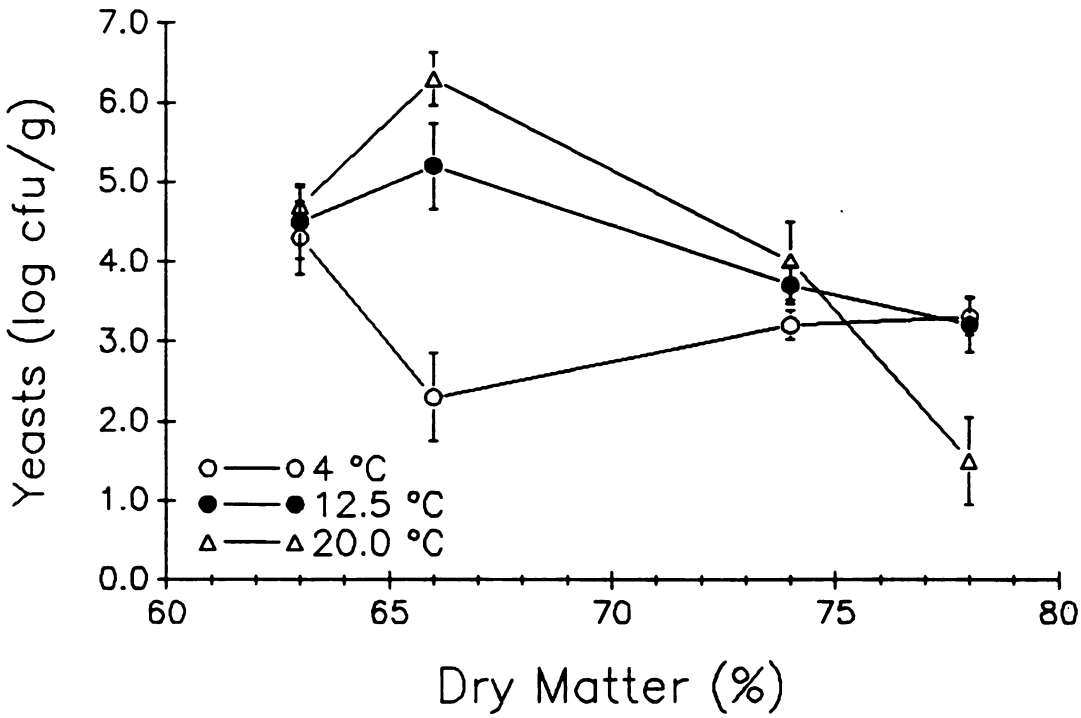

Figure 12. Effects of storage temperature and dry matter content on yeast counts. Bars represent standard deviation for each mean.

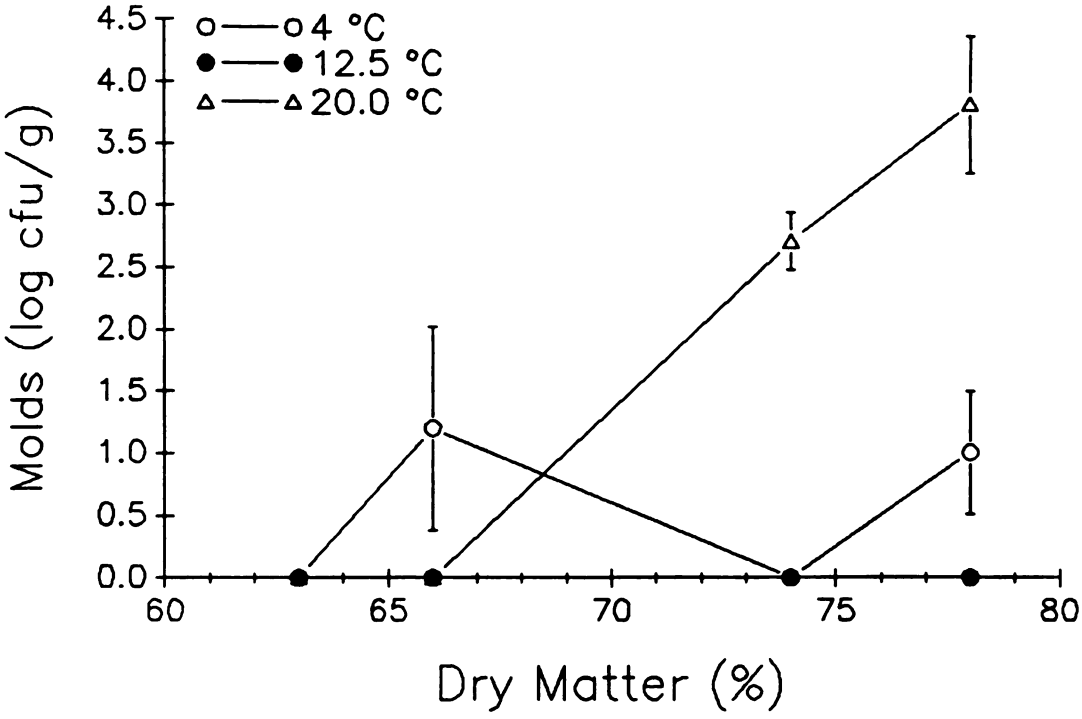

Figure 13. Effects of storage temperature and dry matter content on mold counts. Bars represent standard deviation for each mean.

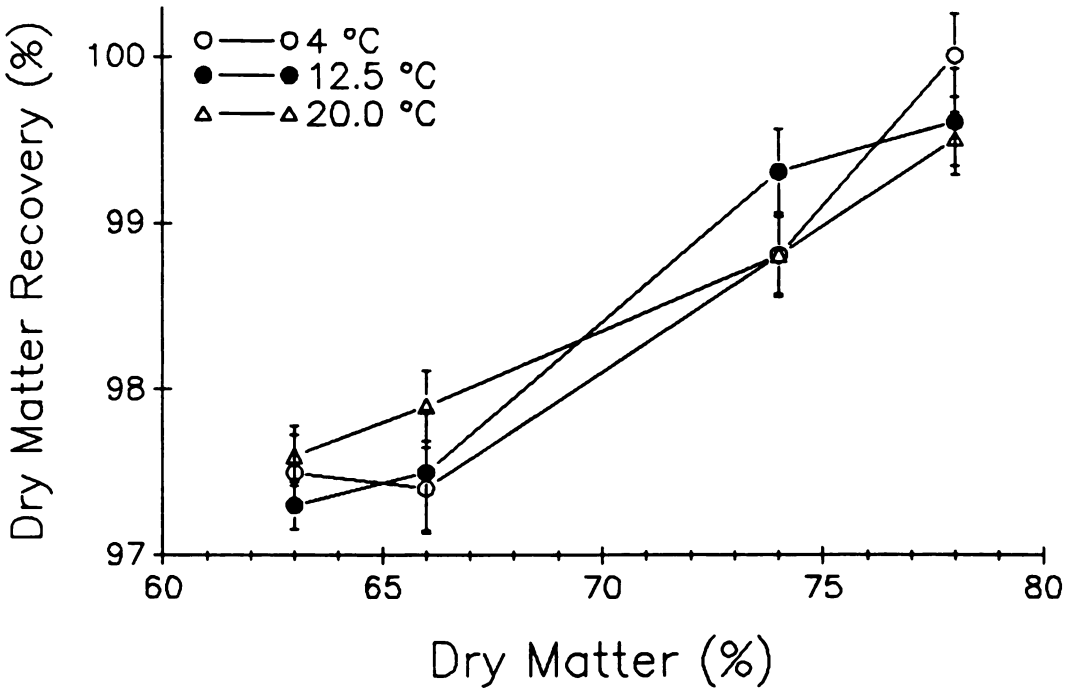

Figure 14. Effects of storage temperature and dry matter on dry matter recovery. Bars represent standard deviation for each mean.

Closely, with ensilement of low moisture feedstuffs as used in this study, elevated storage temperature provided for greater lactic acid concentrations.

For HMC with the two lowest dry matter levels stored at 4 °C acetate concentrations were significantly less than HMC stored at the other temperatures (Figure 3). This trend was observed with lactic acid as well, which suggests storage at lower temperatures significantly altered the extent or pattern of fermentation.

Ethanol content was less for HMC with low DM content (< 66%) stored at 20 °C as compared to storage at lower temperatures or high dry matter ensilages (Figure 4). Since ethanol has little preservative capacity (McDonald, 1981) and results in carbon loss, high levels are undesirable residual soluble carbohydrate content, a measure of substrate usage by microorganisms, was greater at lower DM content ensilages (Figure 5) and residual soluble carbohydrate was significantly greater for 78 and 63% DM HMC stored at 4 °C as compared to HMC with similar DM stored at higher temperatures. This suggests factors other than substrate availability may have limited these fermentations.

Comparison of the degree of homofermentation expressed as moles of lactate acid per mole of acetic plus ethanol suggests a more efficient fermentation occurs as moisture level increases to 34%. Efficiency of fermentation decreases at 63% DM largely because the amount of ethanol produced at all storage temperatures increased more than lactic acid. Utilization of this ratio of endproducts as predictor of homofermentation and fermentation efficiency is related to dry matter recovery (Woolford, 1984c). Calculation of stoichimetry balance for moles of substrate (Table 4) suggests less than 2% of the

Table 4. Calculation Of Substrate Conversion Efficiency

	Dry Matter Content, %			
	63.4	66.4	74.0	78.1
Soluble Carbohydrate, % DM				
Beginning	7.57	5.06	4.94	4.70
Residual	3.35	2.21	1.41	2.57
Soluble Carbohydrate, Moles				
Residual	.050	.028	.027	.026
Initial	.019	.012	.008	.014
Loss	.031	.016	.019	.012
Total Moles Of Endproducts				
Lactic Acid	2.78	2.31	.94	.30
Acetic Acid	.73	.52	.35	.05
Ethanol	3.03	1.54	.98	.88
Carbon in Endproducts				
	15.86	11.05	5.48	2.76
Carbon Lost				
From Substrate	.192	.096	.114	.072
From Amino Acid	.139	.131	.046	.005
Total	.331	.227	.160	.077

metabolized substrate could be accounted for in soluble carbohydrate in initial samples or catabolism of amino acids. Therefore, quantification of soluble carbohydrate in unensiled corn may be an inadequate predictor of the resultant fermentation. Crude protein content was highest for the lowest DM corn ensilage (Figure 6). Although total nitrogen content remained similar across storage temperatures, the form of nitrogen changed.

Soluble nitrogen and ammonia nitrogen content increased ($P < .05$) as DM content decreased and was consistently higher at elevated storage temperatures (Figures 7 and 8). Clearly, some of the carbon skeletons of the nitrogenous compounds were utilized as energy substrates. The proportion of total nitrogen in the soluble form was 7.7, 13.8, 36.4 and 35.7% for 78.1, 74.0, 66.4 and 63.4% HMC, respectively.

Populations of lactic acid producing bacteria (LAB) were significantly lower ($P < .05$) at the lowest DM content regardless of storage temperature (Figure 9). However, the effect of storage temperature on LAB growth was similar across DM content. Coliform bacteria appeared to decrease ($P < .05$) in HMC as DM content decreased with the 4 and 12.5 °C storage temperatures. Conversely, at 20 °C; coliform tended to increase as DM content decreased. Across all ensilages, the correlation between LAB and coliform is negative ($r = -.15$; $P < .20$) which supports similar trends reported by McDonald (1981). As ethanol content increased, coliform populations decreased ($r = -.32$; $P < .006$). Bacillus population remained relatively similar across storage temperature and DM contents (Figure 11). For HMC stored at 12.5 and 20 °C, yeast populations increased as DM content decreased (Figure 12). This observation

would substantiate the larger influence yeast has on fermentation of HMC as compared to other fermented feedstuffs. Yeast population was negatively related with dry matter content, ($r = -.49$; $P < .0001$) dry matter recovery ($r = -.49$; $P < .0044$), crude protein ($r = -.59$; $P < .0001$) and mold population ($r = -.46$; $P < .0001$). It would appear that coliform is a significant contributor to the fermentation of low moisture corn ensilage stored at low temperatures, whereas yeast significantly contributes to the fermentation in low moisture ensilage stored at high temperatures. Yeast populations were positively correlated with soluble nitrogen ($r = .66$; $P < .0001$), lactic acid ($r = .57$; $P < .0001$), acetic acid ($r = .46$; $P < .0001$) and ethanol ($r = .20$; $P < .084$). Ethanol concentrations are relatively high in these ensilages and yeast is the only microorganism positively correlated. This observation would suggest yeast is the originator of the ethanol. Mold organisms were not present in the initial samples (Table 1). Mold growth was not observed in ensilage with a final pH of 4.5 or less. Mold colonies were identified in HMC stored at low temperatures or in low moisture corn stored at 20 ℃ (Figure 13).

Effects of inoculation with various DM contents on pH, lactic acid, acetic acid, ethanol, soluble nitrogen and yeast are shown in Table 5. Addition of the starter culture significantly reduced pH with 74 and 78.1% DM ensilage. Populations of LAB on d-67 were increased ($P < .05$) by inoculation of the lowest moisture ensilage. Above 74% DM ensilage, LAB populations were not influenced by moisture level or inoculation. However, it is possible the strains and species of microorganisms contained in LAB fraction were altered. Yeast population decreased as DM content increased which

Table 5. Effects of Dry Matter Content and Inoculation on Fermentation Characteristics After 67d Ensilement

	INOCULATION TREATMENT								
	Control				Inoculant				
% DRY MATTER	63.4	66.4	74.0	78.1	63.4	66.4	74.0	78.1	SEM[a]
pH	4.1[e]	4.3[e]	5.1[e]	6.2[g]	4.1[e]	4.3[e]	4.7[d]	5.8[f]	.05
LAB[b], log cfu/g	8.4[de]	8.8[def]	9.4[ef]	6.4[c]	8.2[de]	8.9[def]	9.9[f]	7.8[d]	.27
Yeast, log cfu/g	3.9[de]	4.7[de]	3.6[cd]	2.7[c]	5.1[e]	4.6[de]	3.7[cd]	2.7[c]	.26
Chemical Constituents, % of DM									
Crude Protein	10.3[ef]	9.4[c]	10.6[fg]	10.8[g]	9.9[de]	9.6[cd]	10.3[ef]	10.9[g]	.10
Soluble N	.58[h]	.56[g]	.22[d]	.13[c]	.58[h]	.54[f]	.24[e]	.14[c]	.004
Ammonia N	.65[g]	.66[g]	.22[d]	.08[c]	.61[f]	.56[e]	.25[d]	.10[c]	.007
Lactic Acid	2.50[g]	1.97[f]	.65[d]	.07[c]	2.52[g]	1.82[f]	1.03[e]	.47[d]	.07
Acetic Acid	.52[g]	.36[f]	.24[e]	.02[c]	.36[f]	.25[e]	.19[d]	.03[c]	.01
Ethanol	1.48[g]	.76[f]	.53[de]	.40[cd]	1.31[g]	.66[ef]	.38[c]	.40[cd]	.03
DMR[b]	97.4[cd]	97.2[c]	98.6[ef]	99.6[g]	97.5[cd]	98.0[de]	99.3[fg]	99.8[g]	.17

[a] SEM - Standard error of the means.

[b] LAB - lactic acid producing bacteria; DMR - dry matter recovery.

[cdefgh] Means within a row with different superscripts differ ($P < .05$).

follows similar trends observed with LAB.

Lactic acid concentration was increased ($P < .05$) by inoculation of the higher DM ensilage but unchanged with the wetter ensilage. Conversely, inoculation reduced ($P < .05$) acetate levels with the 74, 66 and 63% DM HMC. This observation supports the concept that the starter culture did influence the outcome of the fermentation even though lactic acid levels or LAB enumeration estimates were similar.

Ethanol content was reduced ($P < .05$) by inoculation of the 63.4% DM ensilage as compared to control ensilage with similar DM content. Relatively small inconsistent changes due to inoculation at the different DM levels were seen in the nitrogenous fractions.

Storage temperature by inoculation interaction was significant ($P < .05$) for pH, lactic acid, acetate acid, and ethanol (Table 6). Inoculation reduced pH, ethanol, and acetate levels but increased lactic acid levels at the 12.5 and 20 °C storage temperatures. Dry matter recovery was not influenced by the addition of inoculant at the lower temperature. Consequently, at low ambient temperatures routinely exhibited during harvest of HMC, microbial starter culture may be ineffective.

Mold growth was positively correlated with DM content ($r = .42$; $P < .0002$) and DMR ($r = .28$; $P < .016$). Corn stored at high DM content has been reported to cause increased mold growth (Fox, 1976). Elevated mold growth in dry corn is often a result of poor compaction or accumulation of insufficient fermentation endproducts. The air tight storage structure ued in this study may have prevented oxygen penetration and thereby, prevented the mold growth seen with the low moisture corn ensilage under

Table 6. Effects of Storage Temperature and Inoculation on Fermentation Characteristics After 67d Ensilement

	INOCULATION TREATMENT						
	Control			Inoculation			
STORAGE TEMPERATURE, °C	4	12.5	20	4	12.5	20	SEM[a]
pH	5.2[f]	4.9[de]	4.6[c]	5.1[ef]	4.7[cd]	4.2[d]	.05
Chemical Constituents, % of DM							
Lactic Acid	.68[b]	1.33[c]	1.89[e]	.66[b]	1.60[d]	2.13[e]	.06
Acetic Acid	.18[b]	.35[d]	.33[d]	.18[b]	.22[c]	.22[c]	.009
Ethanol	.74[bc]	.88[d]	.76[cd]	.78[cd]	.66[bc]	.62[b]	.03
Dry Matter Recovery	98.2	98.2	98.3	98.7	98.6	98.7	.14

[a] Standard error of the mean.

[bcdef] Means within the same row with unlike superscripts differ ($P < .05$).

field conditions.

Dry matter recovery after aerobic exposure (DMRE) was greater ($P < .0001$) in corn which was stored at 78% DM as compared to 74, 66, or 63% DM (Table 7). The magnitude of the losses are relatively small (< 1%), however, more extended exposure may increase these losses substantially (Rust et al., 1989). Table 8 depicts the correlation coefficients between parameters measured during the aerobic stability test. Positive correlation coefficients were observed between DMRE and DM ($r = .48$; $P < .0001$), mold ($r = .40$; $P < .0005$), butyric acid ($r = .27$; $P < .02$) and dry matter recovery during the ensilement phase ($r = .22$; $P < .07$). Conversely, a negative relationship was present between DMRE and coliform ($r = -.32$; $P < .006$), yeast ($r = -.47$; P , .0001). Bacillus ($r = -.30$; $P < .01$), soluble nitrogen ($r = -.36$; $P < .002$), ammonia nitrogen ($r = -.37$; $P < .001$), lactic acid ($r = -.31$; $P < .009$) and temperature rise ($r = -.24$; $P < .04$). The factors positively correlated with DMRE were also positively related to DM content. In general, the more extensive the fermentation during the ensilement period, the less likely an ensilage with aerobic stability will be produced.

Temperature of the ensilage mass has been used to evaluate the degree of stability upon air exposure (Rust et al., 1989). The results from this trial would support that relationship since temperature rise and DMRE were negatively correlated ($r = -.24$; $P < .04$). However, the correlation coefficient is relatively low which suggests other factors may be involved. For instance, yeast population is negatively related with DMRE ($r = -47$; $P < .0001$) but is not significantly related to temperature rise ($r = .06$; $P =$

Table 7. Effects of Dry Matter Content on Dry Matter Recovery During Aerobic Exposure

	Dry Matter Content, %				
	63.4	66.4	74.0	78.1	SEM[a]
Dry Matter Recovery, %	99.1[b]	99.4[b]	99.4[b]	100.6[c]	.19

[a] Standard error of the mean.

[b,c] Means with unlike superscripts differ ($p < .001$).

Table 8. Correlation Analysis After Forty-Eight Hours of Air Exposure

	DM[a]	Temp.[b]	Inoc.[b]	pH	E.Coli	Yeast	Mold	Bacillus	DMRE[b]	CP[b]	SN[b]	NH_3[b]	Lactic Acid	Acetic Acid	Ethanol	Butyric Acid	SC[b]	Temp. Change	DMR[b]
pH	.68[a]	-.56	-.09	1.00															
	.0001	.0001	.41																
E. Coli	-.49	-.17	-.10	-.02	1.00														
	.0001	.14	.41	.84															
Yeast	-.75	.18	.03	-.58	.38	1.00													
	.0001	.12	.79	.0001	.001														
Mold	.44	.35	.03	.12	-.25	-.44	1.00												
	.0001	.02	.31	.30	.03	.0001													
Bacillus	-.63	.09	.09	-.34	.55	.45	-.18	1.00											
	.0001	.47	.44	.003	.0001	.0001	.12												
DMRE[b]	.48	.03	-.07	.19	-.32	-.47	.40	-.30	1.00										
	.0001	.77	.55	.11	.006	.0001	.0005	.01											
CP[b]	.42	-.10	.14	.43	-.07	-.36	-.13	-.10	.09	1.00									
	.0002	.41	.25	.0002	.56	.002	.26	.42	.43										
SN	-.75	.54	.03	-.88	.29	.74	-.26	.46	-.36	-.45	1.00								
	.0001	.0001	.83	.0001	.01	.0001	.03	.0001	.002	.0001									
NH3 N[b]	-.71	.59	-.04	-.84	.28	.74	-.24	.44	-.37	-.43	.98	1.00							
	.0001	.0001	.71	.0001	.02	.0001	.04	.0001	.001	.0002	.0001								
Lactate	-.68	.59	.01	-.92	.19	.69	-.18	.36	-.31	-.44	.96	.95	1.00						
	.0001	.0001	.94	.0001	.11	.0001	.13	.002	.009	.0001	.0001	.0001							
Acetate	-.61	.38	-.35	-.75	.20	.55	-.22	.24	-.15	-.40	.75	.76	.79	1.00					
	.0001	.001	.0003	.0001	.09	.0001	.06	.04	.19	.0005	.0001	.0001	.0001						
Ethanol	-.61	.31	-.36	-.65	.17	.36	-.21	.37	-.10	-.43	.67	.65	.64	.82	1.00				
	.0001	.008	.002	.0001	.17	.002	.07	.001	.40	.0002	.0001	.0001	.0001	.0001					
Butyrate	.37	.45	-.16	.003	-.47	-.34	.32	-.21	.27	.13	-.21	-.16	-.15	-.04	.04	1.00			
	.0012	.0001	.18	.98	.0001	.003	.006	.08	.02	.27	.08	.17	.21	.77	.77				
SC[b]	.08	-.10	-.12	.06	-.01	-.37	.27	-.006	.15	.13	-.07	-.09	-.06	-.04	.008	.15	1.00		
	.46	.40	.33	.64	.92	.001	.02	.95	.19	.27	.55	.47	.63	.76	.95	.20			
Temp. Ch.[b]	-.19	-.64	.16	.42	.39	.06	-.20	.23	-.24	.20	-.39	-.40	-.47	-.43	-.39	-.29	-.09	1.00	
	.10	.0001	.18	.003	.0007	.60	.09	.05	.04	.10	.0006	.0006	.0001	.0002	.0006	.015	.045		
DMR[b]	.86	.008	.20	.58	-.42	-.68	.35	-.52	.22	.36	-.60	-.59	-.54	-.61	-.61	.21	.12	-.15	1.00
	.0001	.94	.08	.0001	.0002	.0001	.002	.0001	.07	.002	.0001	.0001	.0001	.0001	.0001	.07	.30	.20	

[a] Values expressed as r with probabilities on line below.

[b] DMRE - Dry matter recovery after aerobic exposure; SN - soluble nitrogen; NH3 N - ammonia nitrogen; SC - soluble carbohydrate; Temp. Ch. - temperature change; DMR - dry matter recovery after 67d ensilement; DM - dry matter; Temp. - temperature; Inoc. - inoculant.

.60). The logical interpretation suggests yeast metabolism results in nutritive loss but does not contribute to temperature rise.

The amount of time that elapsed before a significant increase ;($P < .056$) in temperature occurred above ambient for the various treatments is shown in Table 9. As storage temperature increased, temperature rise during aerobic exposure decreased ($r = -.64$); $P < .0001$). Regardless of DM content or inoculation treatment, corn ensilage stored at 20 °C did not exhibit an increase in temperature. However, the inoculated corn appeared to deteriorate more rapidly than control corn with the other storage temperatures. With the inoculated corn stored at 12.5 °C, all DM levels within the 48 h period were unstable ($P < .05$) whereas only the 74 and 78% DM HMC were unstable at that storage temperature. Temperature stability was similar for all DM levels within inoculation treatments at the lowest storage temperature. The temperature rise during the first 48 h was positively associated with coliform ($r - .39$; $P < .0007$) and bacillus ($r : .23$; $P < .05$) bacteria.

The effects of DM and storage temperature on aerobic stability of HMC upon the storage temperature under which the ensilage was preserved are shown in Table 10. High moisture corn which contained 74 and 78% DM and stored at 20 °C had a net increase in soluble carbohydrate ($P < .0001$) after 48 h of exposure. Fermentation endproducts were greater or unchanged after exposure for the 55, 74, and 78% DM corn. A general trend was evident of bacterial populations to increase ($P < .01$) as DM content decreased while trends in fungi populations appeared more variable. It would appear the bacteria are the principle organisms in the deterioration process and the role of the fungi is less clear. However, the yeast organisms may initiate the deterioration process by metabolism of acids and subsequent increase in pH which

Table 9. Temperature Rise Above Ambient During Aerobic Exposure

		Inoculation Treatment					
		Control			Inoculant		
Storage Temp., °C		4	12.5	20	4	12.5	20
		time[a], h					
Dry Matter, %	78	>48	48	>48	>48	48	>48
	74	36	36	>48	36	36	>48
	66	36	>48	>48	24	24	>48
	63	36	>48	>48	36	36	>48

[a] Time required for a significant ($P > .05$) temperature increase above ambient.

Table 10. Effects of DM Content and Storage Temperature on Chemical and Microbial Constituents in High Moisture Corn After 48 Hours of Aerobic Exposure

	Storage Temperature, C															
	4				12.5				20					Probability		
DRY MATTER, %	63.4	66.4	74.0	78.1	63.4	66.4	74.0	78.1	63.4	66.4	74.0	78.1	SEM[b]	DM[b]	T[b]	DM*T[b]
Endproduct Disappearance, mM/100g DM[a]																
Lactic Acid	6.92[cd]	1.75[abc]	1.73[abc]	-1.62[ad]	9.30[f]	3.05[bc]	2.12[abc]	-.17[ad]	6.57[cf]	-3.63[a]	.17[ad]	1.43[abc]	1.21	.0001	.023	.022
Acetic Acid	3.67[b]	1.62[bcd]	3.18[b]	0.0[a]	3.10[bb]	1.68[acb]	3.27[b]	.08[ad]	2.72[bb]	.42[abc]	1.12[abc]	.47[abc]	.32	.0001	.0002	.0041
Ethanol	25.48[g]	9.25[bc]	9.15[bc]	2.40[ad]	17.13[f]	3.88[ad]	9.35[bc]	5.67[abc]	11.27[cf]	-1.85[a]	5.73[bc]	4.43[abc]	1.45	.0001	.0001	.0001
Butyric Acid	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	-5.0[a]	-10.0[d]	0.0	0.0	0.0	0.0
Total Residual Endproducts, Mole/100 Moles[a]																
	36.2[cd]	62.9[abcf]	6.2[a]	66.7[abf]	53.9[abc]	81.6[abcg]	33.2[cd]	58.6[abcf]	72.4[abcg]	114.8[b]	99.7[abg]	125.5[g]	12.14	.0001	.0001	.17
Residual Soluble Carbohydrate, % of Initial (d67) Values[a]																
	68.3[a]	69.6[cd]	78.3[cd]	93.3[abc]	52.8[a]	85.8[abc]	62.8[a]	80.7[cd]	69.0[a]	74.7[cd]	130.9[a]	118.3[ab]	10.23	.0001	.001	.013
Temperature Rise, °C[a]																
	46.3	22.7	30.3	12.7	28.8	8.3	28.3	13.3	4.67	-3.5	7.2	3.5	2.61	.0001	.0001	.0001
Change in Microbial Population, log cfu/g[a]																
Coliform	-2.00[cd]	-.17[b]	-.5[d]	1.00[a]	-3.00[c]	.33[a]	0.0[a]	-.67[ab]	-2.5[c]	.83[a]	.17[a]	-.67[ab]	.36	.0001	.25	.006
Yeast	1.00[cf]	-1.67[cd]	-1.67[cd]	3.33[g]	2.00[f]	-1.33[cd]	-1.00[abc]	3.17[g]	-2.00[c]	0.0[abcf]	.17[abcf]	1.50[ag]	.45	.0001	.24	.0001
Mold	0.0[cd]	1.17[ab]	0.0[cd]	.67[abc]	0.0[cd]	0.0[cd]	0.0[cd]	-1.0[c]	0.0[cd]	0.0[cd]	1.83[ab]	2.5[a]	.39	.13	.0001	.0001
Bacillus	-1.17[cd]	.67[abc]	.17[abc]	.33[abc]	-1.33[cd]	-1.17[cd]	1.00[ab]	1.00[ab]	-1.67[c]	-.83[cd]	1.67[a]	.5[abc]	.50	.0001	.94	.056

[a] Expressed as the difference between initial (day 67) and final (48 h of exposure) values.

[b] SEM - Standard error of the means; DM - dry matter; T - temperature; DM*T - dry matter by temperature.

[abcdg] Means within a row with unlike superscripts differ (P < .05).

allows the bacteria to grow. Mold organisms may become principle organisms beyond 48 h of air exposure.

As would be expected, as endproduct utilization decreased, microbial populations and temperature rise decreased as well. Storage temperature did not appear to influence DMR ($r = .0008$; $P < .94$) or DMRE ($r = .03$; $P < .77$) in this study even though many of the parameters used to monitor fermentation and deterioration were significantly altered.

Inoculated HMC containing 63% DM lost more lactic acid ($P < .02$) during the 48 h stability test than control corn, however, both control and treated 63% DM ensilages lost more lactic acid than the other DM treatments (Table 11). The major substrate for deterioration in all silages was ethanol. Inoculation of the 63 and 66% DM HMC had greater temperature rise and ethanol reduction ($P < .007$) than control silages of the same DM. Inoculation of low DM HMC appears to lessen the ability of the resultant ensilage to resist deterioration. Microbial shifts that occurred during the aerobic stability test were not influenced by inoculation treatments.

The higher the storage temperature, the smaller the temperature rise (Table 12) and the greater the mold population ($P < .007$) after 48 h of exposure. With HMC preserved at the 20 °C temperature, inoculant did increase butyric acid content ($P < .07$) as compared to the controls. This coincided with increased mold populations ($r = .32$; $P < .006$).

The magnitude of DM losses from the laboratory silos in this study were less than 5% which is similar to losses from buried bags in 225 t upright concrete stave silos

Table 11. Effects DM Content and Inoculation On Chemical and Microbial Changes In High Moisture Corn Aerobic Exposure

	Control				Inoculation					Probabilities		
Dry Matter, %	63	66	74	78	63	66	74	78	SEM[b]	DM[b]	Inoc[b]	DM*Inoc[b]
Endproduct Disappearance, mM/100g DM[a]												
Lactic Acid	5.22[d]	.42[e]	1.34[cd]	-1.11[e]	9.97[a]	.36[e]	1.33[cd]	.88[cd]	.99	.0001	.002	.06
Acetic Acid	2.60[cd]	.57[e]	2.56[d]	.78[cd]	3.72[f]	1.91[de]	2.49[d]	2.89[cd]	.26	.0001	.0012	.03
Ethanol	17.06[a]	.93[e]	7.93[d]	3.33[cd]	18.87[a]	6.59[cd]	8.22[d]	5.00[cd]	1.19	.0001	.0073	.15
Butyric Acid	0.0[d]	0.0[d]	-3.33[e]	-3.33[e]	0.0[d]	0.0[d]	-3.33[e]	0.0	0.0	0.0	0.0	0.0
Total Residual Endproducts, moles/100 moles[a]												
	66.6[cde]	93.2[de]	52.1[cd]	103.8[e]	46.7[c]	79.6[cde]	40.6[c]	63.4[cde]	9.91	.0001	.006	.43
Residual Soluble Carbohydrates, % of initial (d67) values[a]												
	63.8[cd]	80.2[cd]	87.7[cd]	96.6[d]	58.4[c]	73.2[cd]	93.6[cd]	98.2[d]	8.36	.0008	.69	.76
Temperature Rise, °C[a]												
	20.7[d]	4.8[e]	22.0[d]	10.0[e]	32.6[a]	13.6[cd]	21.9[d]	9.7[e]	2.13	.0001	.0016	.01
Changes in Microbial Populations, log cfu/g[a]												
Coliforms	-2.78[e]	.33[d]	-.44[d]	-.22[d]	-2.22[e]	.33[d]	.22[d]	0.0[d]	.29	.0001	.089	.66
Yeast	-1.44[e]	-.78[e]	-.89[e]	2.67[d]	-.56[e]	-1.22[e]	-.78[e]	2.67[d]	.37	.0001	.60	.34
Mold	0.0	.33	.89	.78	0.0	.44	.33	.67	.32	.13	.54	.74
Bacillus	-1.33[e]	-.44[cd]	.69[d]	.56[d]	-1.44[e]	-.44[cd]	1.22[d]	.67[d]	.41	.0001	.63	.86

[a] Expressed as the differental between initial (67d) and final (48 h of exposure) value.

[b] SEM - Standard error of the mean; DM - Dry matter; Inoc - Inoculant; DM*Inoc - Dry matter by inoculant.

[cde] Means within a row with unlike superscripts differ (P < .05).

Table 12. Effects of Storage Temperature and Inoculation on Chemical and Microbial constituents In High Moisture Corn After Aerobic Exposure

	INOCULATION TREATMENT									
	Control			Inoculation				Probability		
STORAGE TEMPERATURE, °C	4	12.5	20	4	12.5	20	SEM[b]	T[b]	Inoc[b]	T*Inoc[b]
Endproduct Disappearance, mM/100g DM[a]										
Lactic Acid	1.93[e]	1.58[e]	.90[e]	2.45[cd]	5.58[d]	1.37[e]	.86	.023	.022	.072
Acetic Acid	2.00[de]	1.48[ed]	.87[e]	2.23[de]	2.58[e]	1.49[ed]	.22	.0002	.0012	.19
Ethanol	11.40[de]	8.19[de]	2.35[e]	11.74[e]	9.83[de]	7.44[d]	1.03	.001	.007	.07
Butyric Acid	0.0[e]	0.0[e]	-5.00[e]	0.0[e]	0.0[e]	-2.50[d]	0.0	0.0	0.0	0.0
Total Residual Endproducts, moles/100 moles[a]										
	52.6[e]	62.5[cd]	117.9[e]	33.4[e]	51.1[e]	88.3[de]	8.58	.006	.0001	.57
Residual Soluble Carbohydrate, % of initial (d67) values[a]										
	79.1[cd]	80.1[cd]	90.4[cd]	75.7[e]	60.9[e]	106.0[d]	7.24	.69	.0011	.065
Temperature Rise, °C[a]										
	26.6[e]	15.0[d]	1.5[e]	29.4[e]	24.4[e]	4.4[e]	1.85	.0016	.0001	.13
Changes In Microbial Populations, log cfu/g[a]										
Coliform	-.33	-1.08	-.92	-.50	-.58	-.17	.25	.25	.09	.19
Yeast	.08	-.33	-.08	.42	-.25	-.08	.32	.24	.60	.86
Mold	.58[cde]	-.33[e]	1.25[e]	.33[cde]	-.17[ed]	.92[de]	.28	.0001	.004	.063
Bacillus	0.0	-.33	-.08	0.0	-.08	-.08	.36	.94	.63	.80

[a] Expressed as the difference between inital (67day) and final (48 h of exposure) values.

[b] SEM - Standard error of the mean; T - temperature; Inoc - inoculant; T*Inoc - temperature by inoculant.

[c,d,e] Means within a row with unlike superscripts differ ($P < .05$).

(Wardynski et al., 1988). Therefore, it would appear that the losses from the laboratory silos or buried bags represent fermentation losses and the remainder of the 10-15% DM losses from field scale silos represent oxidative losses from the exposed surface and air penetration. Estimates for aerobic losses in this study are much less (1%) than the 10-15%, however, the length of exposure in field scale silos is undoubtedly longer than 48 h.

Comparison of dry matter and crude protein recovery from ensilement through 48 h of air exposure is shown in Tables 13 and 14, respectively. Main effects of DM content and inoculation were the only significant variables for DM recovery estimates. Inoculation significantly increased ($P < .001$) total recovery, however, another .3% increase in DM loss during aerobic stability would negate this benefit (Table 13). Overall, crude protein recovery was not influenced by inoculation treatment. Dry matter and crude protein recovery increased ($P < .001$) as the DM content increased (Table 14). The consequence of this observation would encourage ensilement of HMC with 22% moisture.

The economic value of inoculation based on the results of this laboratory study is shown in Table 15. The economic savings from use of an inoculant is 8.3 pounds of corn (72% DM). The savings are .25, .33, .42 and $.50/ton for corn priced at 1.68, 2.24, 2.8 and $3.36/bushel. Currently, corn would need to be valued at $6.75/bushel to cover the cost of inoculation of $1.00 per ton.

Based on the results of this study producers may benefit from delayed harvest of corn until the moisture content nears 22%. The ambient temperature during the

Table 13. Effect of Inoculation on Recovery of DM and Crude Protein After 67d of Ensilement and 48 h of Aerobic Exposure.

	DM			Crude Protein		
Recovery, %	C	I	SEM[a]	C	I	SEM[a]
Silo	98.2[b]	98.7[c]	.08	99.4[b]	99.5[c]	.02
Exposure Test	99.7	99.5	.01	98.7	98.8	.009
Total	97.9[d]	98.2[e]	.001	98.1	98.3	.03

[a] Standard error of the mean.

[bc] Means within a row with unlike superscripts differ ($P < .001$).

[de] Means within a row with unlike superscripts differ ($P < .08$).

Table 14. Effects of Dry Matter Content on Recovery of DM and Crude Protein After 67d of Ensilement and 48 h of Aerobic Exposure.

	Dry Matter Content, %				
	63	66	74	78	SEM[a]
DM Recovery, %					
Silo	97.5[b]	97.6[b]	99.0[c]	99.7[d]	.12
Exposure Test	99.1[b]	99.4[b]	99.4[b]	100.6[c]	.19
Total	96.6[b]	97.0[b]	98.3[c]	110.3[d]	.17
Crude Protein Recovery, %					
Silo	99.1[b]	99.2[bc]	99.3[c]	99.9[d]	.03
Exposure Test	97.8[b]	98.8[c]	98.9[c]	99.6[d]	.13
Total	97.0[b]	98.0[c]	98.2[c]	99.5[d]	.13

[a] Standard error of the mean.

[bcd] Means within a row with unlike superscripts differ ($P < .05$).

Table 15. Calculated Savings By The Use of An Inoculant

	Corn Price $/bu. (as is)			
	1.68	2.24	2.80	3.36
Savings with an inoculant, $/ton	.25	.33	.42	.50

ensilement and fermentation period appears to have little effect on those parameters that have economic significance.

CONCLUSION

Evaluation of successful storage practices for high moisture corn (HMC) should be based on return per unit of investment. From the standpoint of microbial inoculants; nutrient density, nutrient preservation and stability are the critical parameters for consideration. Average daily gain (ADG), dry matter intake (DMI) and feed efficiency (FE) are common measures of feed quality or nutrient density. Improvements in these performance parameters could justify utilization of microbial inoculants for ensilement of HMC.

Nutrient recovery could potentially justify the use of products such as lactic acid producing bacteria (LAB) used to aid in the preservation of ensiled feeds. In this study, dry matter conserved was utilized as the parameter to evaluate preservation. Improvements in dry matter recovery (DMR) could increase profits simply by reducing the amount of feed lost during storage. Other measurements such as gross or digestible energy recovery could also be used, but are more difficult to measure.

Stability after aerobic exposure is a critical parameter to be measured. Silage additives which improve aerobic stability would allow more flexibility in feeding systems through extended bunk-life of HMC.

Fermentation characteristics such as chemical and microbial measurements are useful in explaining the observed results from feeding and storage studies; however, chemical

and microbial measurements do not dictate profitability. In this author's opinion, elevated lactic acid content alone does not always prove to be a desirable characteristic. The final decision of whether or not to use a microbial inoculant should be based on economic returns and the conditions which these returns are manifest.

In trials one and two from the first experiment, nutritive value was similar between control and inoculated corn as measured by ADG, DMI and FE. Dry matter recovery was similar for both treatments in trial one; however, DMR was lower in corn treated with inoculant as compared to control corn in trial two. Stability after aerobic exposure was measured in trial two and greater temperature rise and microbial growth were detected in corn treated with LAB than the control.

Dry matter recovery was evaluated in trials one and two of experiment two though feeding quality and aerobic stability were not measured. In trial one, lactic acid content was increased and pH decreased in both ground corn treatments. Corn stored in the whole form underwent less fermentation and thereby, incurred less DM loss. Inoculation of corn in both forms increased lactic acid concentration and reduced pH. Unfortunately, inoculation also reduced DMR. Dry matter recovery was unchanged by the addition of water, inoculants, non-protein nitrogen or acids.

Experiment three demonstrated benefits of using LAB during anaerobic storage. Microbial inoculation increased lactic acid content and decreased pH in the dry corn treatments (78 and 74% DM). Microbial inoculation also improved DMR ($P<.001$) by .5% over control corn after 67 d of fermentation. Corn stored at 78% DM had a greater DMR than corn stored at 74%, which was greater than corn stored at 66 or 63% DM.

Storage temperature did not affect DMR, but corn stored at 20 °C appeared to be more stable than corn stored at 4 or 12 °C as measured by temperature rise above ambient after 48 h of air exposure. Inoculation of corn stored at 4 or 12 °C during fermentation was less stable after air exposure than corn stored at higher temperatures. Calculation of DM recovery from harvest to consumption by the animal indicated a .3% increase in DMR with inoculation. Corn stored at 78% DM had a greater combined DMR than 74, 66 or 63% DM corn. After calculating the economic value of .3% improved DMR with LAB addition and the cost of applying a commercial microbial inoculant, use of microbial cultures is marginal.

The recommended moisture content for ensiled corn is 25-30%; however, DMR was demonstrated to be greater at the lowest (22%) moisture level studied. In addition, the dryer corn appeared to be more stable after aerobic exposure.

Small laboratory silos were utilized in experiments two and three. The validity of using laboratory silos to study fermentation as it occurs in large farm silos has been questioned. However, because of the lack of replication in large farm silos, small laboratory silos have been utilized to identify treatments which may enhance fermentation in larger silos. Experiments two and three could be used to suggest potential treatments for large silos. Even though the trials did not prove LAB inoculation to be economically beneficial, further evaluation in large farm scale silos may be warranted. Farm silos are not as air tight and DMR should be measured from the total silo. Note that DMR, in the first experiment, was measured from buried dacron bags. In experiment 2 several additives were evaluated on corn ensiled at 20% moisture with no difference in DMR.

Again, different results may occur in larger silos.

Experiment three indicates that corn stored with greater dry matter content had greater DMR. Previous research has indicated DMR can be greater with drier corn; however, the risk of heating and carmelizing the corn within the silo is also greater.

Corn with greater dry matter content was more stable after aerobic exposure, while LAB inoculation adversely affecting stability. Corn stored at 20 °C throughout fermentation was more stable than corn stored at 4 or 12.5 °C. Storing small laboratory silos at a constant, controlled temperature can be accomplished easily; however, this application on large silos is impractical. Future research is needed to evaluate the role which external or ambient temperature plays on fermentation and subsequent aerobic stability in farm-scale silos.

In summary, these studies suggest that corn stored with greater dry matter content allows for large quantities of recoverable HMC which is more stable during oxygen exposure. Corn inoculated with LAB is less stable than control corn and did not show economical benefit.

Future research should focus on improving the factors that increase output and thereby profitability. Previous research has shown that HMC fed to growing-finishing steers, as opposed to dry corn, has exhibited equal or larger daily gains, improved digestibility and feed conversion efficiency while decreasing dry matter consumption. Corn stored at elevated moisture content has increased acid and soluble nitrogen content, which can decrease DMI.

Improvements in feed conversion and ADG may be feasible. Preserving corn with

greater energy density or less proteolysis may be possible. Soluble and ammonia nitrogen have been shown to increase in HMC as moisture content increases. Elevated soluble nitrogen content has been shown to decrease intake of high moisture feeds. Therefore, strategies reduce nitrogen solubilization and proteolysis may enhance nutrient preservation and dry matter intake.

Experiment three and other trials have demonstrated that DMR can be maximized with lower moisture content by limiting the fermentation. As the fermentation process continues, more carbon is potentially lost as carbon dioxide. Consequently, the results of these trials would indicate that DMR could be maximized by limiting fermentation. However, many of the trials have been conducted using air tight laboratory silos and results from large scale silos have shown problems with preservation at moisture contents below 25% moisture. The key is to control fermentation to ensure preservation without excessive DM loss. Research in monitoring and controlling fermentation in this manner would be useful.

Aerobic stability is an area which needs more research. High moisture corn can deteriorate rapidly after oxygen exposure. Differences in deterioration rate has been shown between corn samples collected for evaluation. The explanation for these differences is not fully known. Links have been made between stability and the chemical constituents of the corn. High moisture corn which has undergone heating in the silo and contains elevated levels of butyric acid and appear ammonia have appeared to be quite stable. Corn samples which contain high acetic acid content have been associated with improved stability. Microbial characteristics post-ensiling were not identified in these

experiments and requires more testing to elucidate the characteristics associated with deterioration. The key is to produce high moisture corn with extended stability without sacrificing nutritive value or conservation efficiency.

APPENDIX

Model development to predict chemical, microbiological and dry matter recovery of ensiled and deteriorating high moisture corn.

The data from Experiment three was utilized to form the following regression tables. Corn was harvested at four dry matter contents (63.4, 66.4, 74.0 or 78.0% DM). At each moisture content, corn was rolled, inoculated with either water or a lactic acid producing microbial inoculant and 3 kg placed into polyvinyl-chloride silos (10.2 cm dia. x 45.7 cm). Each moisture by inoculation treatment combination was stored at 4, 12.5 or 20 °C. For each treatment combination, triplicate silos were evacuated 3, 7 or 67 d post-ensiling. Subsamples were collected for chemical and microbial analysis.

Tables one and two show regression coefficients, standard error of the estimate and probability for the fermentation characteristics. The tables were generated using the General Linear Model Procedure for Regression (SAS Institute, 1987). The variables used in the regression were dry matter (DM), day of evacuation (D), storage temperature (ST), inoculation (I; 0 represented control, 1 represented inoculation), DM^2, D^2, ST^2, DM*D, DM*ST, DM*I, D*ST, D*I and ST*I. Chemical and microbial characteristics were expressed as the percent of dry matter and the log of colony forming units per gram of wet corn, respectively.

Tables three and four represent the regression coefficients, standard error of the estimate and probability of the change in chemical and microbial characteristics after aerobic exposure. After sub-sampling, corn evacuated from silos 67 d post-ensiling, were placed in plastic-lined styrofoam containers to evaluate stability during oxygen

exposure. Samples collected from silos 67 d post-ensiling also represent time zero for aerobic stability. Corn was removed from the containers after 48 h of aerobic exposure and subsamples collected for chemical and microbial analysis.

Tables three and four were generated using the same procedure as tables one and two; however, the variable of evacuation day did not apply in the aerobic stability model. The variables used in the regression model were DM, ST, I, DM^2, ST^2, DM*ST, DM*I, and ST*I. The input values for statistical analysis for the aerobic stability regression tables were calculated by subtracting the 48 h values from time zero values. Chemical and microbial characteristics were expressed as a percent of dry matter and the log of colony forming units per gram of wet corn, respectively. Temperature values were calculated by subtracting 48 h temperature from the average ambient temperature.

Table 1. Regression Coefficient For Variables Used in the Model to Predict Chemical Constituents of Silage and Dry Matter Recovery Post-ensiling

	pH			Acetic Acid[a]			Lactic Acid[a]			Butyric Acid[a]		
Variables in Model	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability
Intercept	28.78	+/-5.75	.0001	1.27	0.98	0.20	6.27	5.03	0.21	29.41	5.32	.0001
DM[b]	-0.68	0.16	.0001	-0.039	0.027	0.16	-0.22	0.14	0.13	-0.77	0.15	.0001
D[b]	-0.11	0.016	.0001	0.028	0.0032	0.0001	0.17	0.016	0.0001	-0.06	0.039	.14
ST[b]	-0.41	0.042	.0001	0.087	0.0079	0.0001	0.5	0.04	0.0001	-0.06	0.039	.14
I[b]	1.08	0.56	.055	-0.32	0.096	0.001	-0.89	0.49	0.072	—	—	—
DM[2b]	0.0052	0.0012	.0001	0.00027	0.0002	0.17	0.0017	0.001	0.087	0.0051	0.0011	.0001
D[2b]	0.0012	0.00023	.0001	-0.00013	0.000039	0.0013	-0.00082	0.0002	0.0001	0.0012	0.00021	.0001
ST[2b]	—[c]	—	—	-0.00048	0.00013	0.0003	0.0011	0.00067	0.10	—	—	—
DM*D[b]	—	—	—	-0.00022	0.000023	0.0001	-0.0015	0.00012	0.0001	0.00058	0.00012	.0001
DM*ST[b]	0.0047	0.0006	.0001	-0.00094	0.0001	0.0001	-0.0068	0.00052	0.0001	0.00078	0.00055	.16
DM*I[b]	-0.013	0.0078	.095	0.005	0.0013	0.0002	0.012	0.0068	0.092	—	—	—
D*ST[b]	0.0056	0.00012	.0001	-0.000031	0.00002	0.13	0.0005	0.0001	0.0001	—	—	—
D*I[b]	—	—	—	-0.0011	0.00027	0.0001	0.0017	0.0014	0.20	—	—	—
ST*I[b]	-0.026	0.007	.0003	-0.003	0.0012	0.012	0.007	0.0061	0.081	—	—	—

Table 1. Continued

Variables in Model	Ethanol[a] Regression Coefficient	Ethanol[a] Standard Error of the Estimate	Ethanol[a] Probability	Crude Protein[a] Regression Coefficient	Crude Protein[a] Standard Error of the Estimate	Crude Protein[a] Probability	Soluble Nitrogen[a] Regression Coefficient	Soluble Nitrogen[a] Standard Error of the Estimate	Soluble Nitrogen[a] Probability	Ammonia Nitrogen[a] Regression Coefficient	Ammonia Nitrogen[a] Standard Error of the Estimate	Ammonia Nitrogen[a] Probability
Intercept	18.85	3.01	.0001	31.97	6.60	.0001	-0.35	0.065	.0001	-0.59	0.12	.0001
DM[b]	-0.48	0.085	.0001	-0.68	0.19	.0004	0.0062	0.00091	.0001	0.0089	0.0016	.0001
D[b]	0.046	0.0098	.0001	0.01	0.022	.64	0.038	0.0019	.0001	0.043	0.0031	.0001
ST[b]	—[c]	—	—	-0.022	0.022	.31	0.065	0.0047	.0001	0.087	0.0076	.0001
I[b]	-0.39	0.29	.18	—	—	—	—	—	—	-0.11	0.092	.22
DM^{2b}	0.0029	0.0006	.0001	0.0052	0.0013	.0001	—	—	—	—	—	—
D^{2b}	-0.00037	0.00012	.0021	-0.00037	0.00026	.16	-0.00011	0.000023	.0001	-0.00011	0.000038	.0042
ST^{2b}	—	—	—	-0.001	0.00088	.24	0.00033	0.000077	.0001	0.00055	0.00013	.0001
DM*D[b]	-0.00013	0.00007	.059	0.00027	0.00015	.085	-0.00041	0.000014	.0001	-0.00049	0.000022	.0001
DM*ST[b]	0.00053	0.00031	.09	—	—	—	-0.00097	0.000061	.0001	-0.0014	0.000099	.0001
DM*I[b]	0.0059	0.0041	.15	—	—	—	—	—	—	0.0017	0.0013	.18
D*ST[b]	-0.0037	0.000062	.0001	—	—	—	0.0002	0.000012	.0001	0.00038	0.000020	.0001
D*I[b]	—	—	—	—	—	—	—	—	—	-0.00051	0.00026	.051
ST*I[b]	-0.0075	0.0037	.041	—	—	—	—	—	—	—	—	—

Table 1. Continued

Variables in Model	Soluble Carbohydrate[a] Regression Coefficient	Standard Error of the Estimate	Probability	Dry Matter Recovery[a] Regression Coefficient	Standard Error of the Estimate	Probability
Intercept	94.87	16.85	.0001	145.25	10.84	.0001
DM[b]	-2.43	0.48	.0001	-1.36	0.31	.0001
D[b]	-0.18	0.056	.0010	-0.28	0.36	.0001
ST[b]	-0.85	0.13	.0001	-0.040	0.0088	.0001
I[b]	—[c]	—	—	-1.46	1.048	.16
DM^2[b]	0.017	0.0034	.0001	0.010	0.0022	.0001
D^2[b]	0.0035	0.00066	.0001	0.0028	0.00043	.0001
ST^2[b]	0.0042	0.0022	.059	—	—	—
DM*D[b]	-0.0015	0.00039	.0001	0.00076	0.00025	.0029
DM*ST[b]	0.0086	0.0017	.0001	—	—	—
DM*I[b]	—	—	—	0.023	0.015	.11
D*ST[b]	0.0016	0.00035	.0001	0.00063	0.00023	.0061
D*I[b]	—	—	—	0.0039	0.0030	.19
ST*I[b]	—	—	—	—	—	—

[a] Recorded as percent of dry matter.

[b] DM = % dry matter content; D = length of ensilement in days; ST = storage temperature; I = inoculation treatment (0 = control; 1 = inoculation).

[c] — Indicates a non-significant variable (P > .25).

Table 2. Regression Coefficients For Variables Used in Model to Predict Microbiological Population Post-ensiling

	Yeast[a]			Mold[a]			Coliform[a]			Lactic Acid Producing Bacteria[a]		
Variables in Model	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability
Intercept	-11.79	2.85	.0001	5.49	1.28	.0001	-25.24	18.15	.17	-140.81	16.19	.0001
DM[b]	0.15	0.039	.0002	-0.070	0.018	.0001	0.79	0.52	.13	4.21	0.46	.0001
D[b]	0.73	0.086	.0001	-0.11	0.019	.0001	0.81	0.030	.0076	0.30	0.046	.0001
ST[b]	0.69	0.18	.0002	-0.51	0.093	.0001	—	—	—	0.14	0.16	.0001
I[b]	—[c]	—	—	—	—	—	—	—	—	-3.13	1.58	.050
DM^{2b}	—	—	—	—	—	—	-0.0050	0.0036	.17	-0.030	0.0033	.0001
D^{2b}	-0.0072	0.00098	.0001	—	—	—	—	—	—	-0.0033	0.0064	.0001
ST^{2b}	—	—	—	0.0052	0.0015	.0008	—	—	—	—	—	—
DM*D[b]	-0.0025	0.00056	.0001	0.0016	0.00027	.0001	-0.0013	0.00042	.0031	—	—	—
DM*ST[b]	-0.0085	0.0025	.0010	0.0053	0.0012	.0001	—	—	—	—	—	—
DM*I[b]	—	—	—	—	—	—	—	—	—	0.061	0.022	.0060
D*ST[b]	-0.00060	0.00048	.22	0.0010	0.00024	.0001	—	—	—	-0.0023	0.00034	.0001
D*I[b]	—	—	—	—	—	—	—	—	—	-0.016	0.0044	.0003
ST*I[b]	—	—	—	—	—	—	—	—	—	0.024	0.020	.23

[a] Recorded as the log of colony forming units per gram of wet corn.

[b] DM = % dry matter content; D = length of ensilement in days; ST = storage temperature; I = inoculation treatment (0 = control; 1 = inoculation).

[c] — Indicates a non-significant variable (P > .25).

Table 3. Regression Coefficient For Variables Used in Model to Predict Temperature Change and Dry Matter Recovery in Ensiled High Moisture Corn Exposed to Air

	pH			Acetic Acid[a]			Lactic Acid[a]			Butyric Acid[a]		
Variables in Model	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability
Intercept	-36.50	12.03	.0034	2.86	2.37	.23	-33.43	10.62	.0025	0.46	1.11	.68
DM[b]	1.11	0.34	.0018	-0.083	0.067	.22	0.90	0.30	.0040	-0.0074	0.016	.64
ST[b]	-0.22	0.088	.014	-0.0061	0.0076	.43	0.057	0.085	.51	-0.31	0.051	.0001
I[b]	—[c]	—	—	-0.40	0.23	.082	—	—	—	1.072	0.60	.081
DM^{2b}	-0.0082	0.0024	.0011	0.00058	0.00047	.22	-0.0060	0.0021	.0066	—	—	—
ST^{2b}	—	—	—	0.00040	0.00031	.21	0.0028	0.0014	.055	0.0032	0.00082	.0002
DM*ST[b]	0.0027	0.0013	.033	—	—	—	-0.0017	0.0011	.14	0.0041	0.00065	.0001
DM*I[b]	—	—	—	0.0051	0.0032	.11	—	—	—	-0.013	0.0085	.12
ST*I[b]	—	—	—	—	—	—	—	—	—	-0.021	0.0076	.0070

Table 3. Continued

	Ethanol[a]			Crude Protein[a]			Soluble Nitrogen[a]			Ammonia Nitrogen[a]		
Variables in Model	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability
Intercept	-31.27	7.37	.0001	-23.89	18.12	.19	-0.52	0.095	.0001	-3.25	1.28	.014
DM[b]	0.81	0.21	.0003	0.71	0.52	.17	0.0065	0.0013	.0001	0.090	0.036	.016
ST[b]	0.26	0.056	.0001	—	—	—	0.030	0.0069	.0001	0.010	0.0047	.034
I[b]	0.055	0.12	.66	—	—	—	—	—	—	0.025	0.022	.26
DM^{2b}	-0.0052	0.0015	.0008	-0.005	0.0036	.16	—	—	—	-0.00063	0.00026	.017
ST^{2b}	—[c]	—	—	—	—	—	—	—	—	-0.00031	0.00017	.070
DM*ST[b]	-0.0031	0.00077	.0001	—	—	—	-0.00039	0.000097	.0001	—	—	—
DM*I[b]	—	—	—	—	—	—	—	—	—	—	—	—
ST*I[b]	-0.014	0.0090	.14	—	—	—	—	—	—	-0.0024	0.0016	.13

Table 3. Continued

Variables in Model	Soluble Carbohydrate[a] Regression Coefficient	Standard Error of the Estimate	Probability	Dry Matter Recovery[a] Regression Coefficient	Standard Error of the Estimate	Probability	Temperature[a] Regression Coefficient	Standard Error of the Estimate	Probability
Intercept	-46.37	18.22	.013	153.82	24.42	.0001	48.32	35.59	.18
DM[b]	1.24	0.52	.020	-1.62	0.69	.022	-0.42	0.50	.40
ST[b]	-0.053	0.059	.37	—	—	—	-5.95	1.61	.0005
I[b]	—[c]	—	—	—	—	—	37.74	19.34	.055
DM^2[b]	-0.0082	0.0037	.028	0.012	0.0049	.017	—	—	—
ST^2[b]	0.0033	0.0024	.18	—	—	—	-0.062	0.027	.024
DM*ST[b]	—	—	—	—	—	—	0.087	0.021	.0001
DM*I[b]	—	—	—	—	—	—	-0.49	0.27	.077
T*I[b]	—	—	—	—	—	—	—	—	—

[a] Dry matter recovery recorded as percent of recoverable dry matter; temperature was recorded as the difference from ambient; soluble carbohydrate was recorded as percent of dry matter.

[b] DM = % dry matter content; D = length of ensilement in days; ST = storage temperature; I = inoculation treatment (0 = control; 1 = inoculation).

[c] — Indicates a non-significant variable (P > .25).

Table 4. Regression coefficient for variables used in model to predict microbiological population in ensiled HMC exposed to air

	Bacillus[a]			Coliforms[a]			Yeast[a]			Mold[a]		
Variables in Model	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability	Regression Coefficient	Standard Error of the Estimate	Probability
Intercept	96.35	35.12	.0078	177.29	34.77	.0001	-206.21	44.06	.0001	-7.98	3.197	.015
DM[b]	-2.69	1.00	.0087	-4.89	0.99	.0001	6.08	1.25	.0001	0.09	0.045	.048
ST[b]	0.54	0.26	.042	-0.12	0.29	.67	—	—	—	1.13	0.25	.0001
I[b]	—[c]	—	—	0.34	0.58	.56	—	—	—	—	—	—
DM[2b]	0.019	0.0070	.0099	0.033	0.0070	.0001	-0.044	0.0089	.0001	—	—	—
ST[2b]	—	—	—	-0.0055	0.0046	.24	—	—	—	-0.016	0.0041	.0002
DM*ST[b]	-0.0075	0.0036	.043	0.0050	0.0036	.17	—	—	—	-0.011	0.0032	.0011
DM*I[b]	—	—	—	—	—	—	—	—	—	—	—	—
ST*I[b]	—	—	—	-0.058	0.042	.18	—	—	—	—	—	—

[a] recorded as the log of colony forming units per gram of wet corn.

[b] DM = % dry matter content; D = length of ensilement in days; ST = storage temperature; I = inoculation treatment (0 = control; 1 = inoculation).

[c] — indicates a non-significant variable (P > .25).

LITERATURE CITED

AOAC. 1984. Official Methods of Analysis (14th Ed.) Association of Official Analytical Chemists. Washington, DC.

Aguirre, E.O., A.L. Goetsch and F.N. Owens. 1984. Fermented corn grain - site and extent of nutrient digestion in steers. Oklahoma State Univ. and USDA Res. Rep. MP-116. p 194.

Aguirre, E.O., F.N. Owens, D.R. Gill and J.H. Thornton. 1983. Effect of moisture addition on fermented high moisture corn. Oklahoma State Univ. and USDA Res. Rep. MP-114. p 90.

Alaspaa, M. 1986. Effect of treatment with urea or a urea and phosphate mixture on the nutritive value of whole crop silage. Ann. Agric. Finnial 25:99.

Alhadhrami, G. J.T. Huber, G.E. Hegginbatham and J.M. Harper. 1989. Nutritive value of high moisture alfalfa hay preserved with urea. J. Dairy Sci. 72:972.

Ashbell, G. and N. Lisker. 1988. Aerobic deterioration in maize silage stored in a bunker silo under farm conditions in a subtropical climte. J. Sci. Food Agri. 45:307.

Aumaitre, A. and S.Z. Zelter. 1975. The effect of high moisture corn on animal nutrition and health. Some aspects of its chemical composition and nutritional value. In: L.D. Hill,(ed.) Corn Quality In World Markets. Interstate Printers and Publishers, Inc. Danville, IL. p 31.

Baertsche, S.R., M.T. Yokoyama and J.W. Hanover. 1986. Short rotation, hardwood tree biomass as potential ruminent feed - chemical composition nylon bag ruminal degradation and ensilement of selected species. J. Anim. Sci. 63:2028.

Baker, F.S. 1973. Feeding Value of High Moisture Corn and Sorghum Grain For Beef Cattle, Symp. on Effects of Processing on the Nutritional Value of Feeds. National Academy of Sciences, Washington, D.C.

Barker, S.D. and W.H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138:535.

Baron, V.S., K.R. Stevenson and J.G. Buchanan-Smith. 1986. Proteolysis and fermentation of grain-corn ensiled at several moisture levels and under several simulated storage methods. Can. J. Anim. Sci. 66:451.

Barnett, A.J.G. 1954. Silage Fermentation Academic Press, London.

Beck, T. 1978. The microbiology of silage. In: M.E. McCullough (Ed.) Fermentation of Silage-A Review. Nat. Feed Ing. Ass. DesMoines, IA. p 61.

Bergen, W.G. 1971. The role of nitrogenous constituents of silage in animal performance. Mich. Agric. Exp. Sta., Res. Rep., 143:71.

Bergen, W.G., E.H. Cash and H.E. Henderson. 1974. Changes in nitrogenous compounds of the whole corn plant during ensiling and subsequent effects on dry matter intake by sheep. J. Anim. Sci. 39:629.

Bolsen, K., M. Hinds and H. Ilg. 1984. CULBAC and ADD-F (formic acid) additives for sudangrass and high moisture shelled corn silages. Kansas Ag. Exp. Sta., Report of Progress 448, p 64.

Brady, C.J. 1965. Nitrogen redistribution, during ensilage at low moisture level. J. Sci. Food Agric. 16:508.

Brown, W.O. and J.A.M. Kerr. 1965. Losses in the conservation of heavily wilted herbage sealed in polyethelene film in lined trench silos. J. Br. Grassland Soc. 20:227.

Bryan-Jones, D.G. 1969. PhD. Thesis, University of Ediburgh.

Buchanan, R.E. and N.E. Gibbons. 1974. Bergey's Manual of Determinative Bacteriology (8th ed.). Williams and Wilkens Co., Baltimore.

Buchanan-Smith, J.G. 1982. Preservation and feeding value for yearling steers of whole-plant corn ensiled at 28 and 42% dry matter with and without cold flow ammonia treatment. Can. J. Anim. Sci. 62:173.

Buchanan-Smith, J. 1976. Fermentation and starch availability and digestion. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 61.

Burroughs, W., D.F. Pitgen, A.H. Trenkle, R.L. Vetter and C.C. Cooper. 1970. Artificially dried shelled corn fed whole vs. rolled daily before feeding vs. high-moisture shelled corn rolled daily before feeding. Iowa Agric. Exp. Sta. Res. Bull. ASL R131.

Cook, A.R. 1976. Urease activity in the rumen of sheep and the isolation of ureolytic bacteria. J. Gen. Microbiol. 92:32.

Copeland, L.O. and M.B. McDonald. 1985. Principles of Seed Science and Technology. Macmillan Publishing Co.0

Corah, L.R., 1976. Nutritional value of high moisture corn and milo. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 179.

Daeschel, M.A., R.E. Anderson and H.P. Fleming. 1987. Microbial ecology of fermenting plant materials. Federation of FEMS Microbiology Reviews Wuroplan Microbiol. Soc., 46:357.

Daley, M.M. and R.L. Vetter. 1974. Artificially altered corn grain harvested at three moisture levels. III. In vitro utilization of the carbohydrate and nitrogen fraction. J. Anim. Sci. 38:430.

Daynard, T.B. and W.G. Duncan. 1969. The black layer and grain maturity in corn. Crop Sci. 9:473.

Dewar, W.A., P. McDonald and R. Whittenbury. 1963. The hydrolysis of grass hemicelluloses during ensilage. J. Sci. Food Agric. 14:411.

Dexheimer, C.E. 1973. Fermentation Characteristics and Net Energy Value of Dry and Ensiled High Moisture Corn Grain. Ph.D. Thesis, Univ. of Minnesota, St. Paul.

Driedger, A. 1976. Chemical preservation of high moisture grains. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 42.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

Edwards, R.A. and P. McDonald. 1978. The chemistry of silage. In: M.E. McCullough (Ed.) Fermentaion of Silage-A Review. National Feed Ingredients Assoc. Des Moines, IA. p 27.

Ely, L.O. 1978. The use of added feedstuffs in silage production. In: M.E. McCullough (Ed.) Fermentaion of silage-A review. Nat. Feed Ing. Ass. Des Moines, IA. p233.

Ely, L.O., E.M. Sudweeks and N. J. Moon. 1981. Inoculation with Lactobacillus plantarium on wheat silages. J. Dairy Sci. 64:2378.

Fenton, M.P. 1987. An investigation into sources of lactic acid bacteria in grass silage. J. of Appl. Bact. 62:181.

Florance, H.D. Jr., J.K. Riggs and G.D. Potter. 1968. Physical characteristics of reconstituted sorghum grain. J. Anim. Sci. 21:1161 (Abstr.).

Fox, D.G. 1976. Systems for storing and handling high moisture corn. High Moisture Grains Sump. Oklahoma State Univ., Stillwater. p 15.

Garcia, A.D., W.D. Olson, D.E. Otterby, J.G. Linn and W.P. Hansen. 1989. Effects of temperature, moisture, and aeration on fermentation of alfalfa silage. J. Dairy Sci. 72:93.

Ghate, S.R. and W.K. Bilanski. 1979. Treating high moisture alfalfa with urea. Trans. Am. Soc. Agric. Eng. 23:504.

Ghate, S.R., W.K. Bilanski and J.E. Winch. 1981. Urea as a forage preservative. Trans. Am. Soc. Agric. Eng. 24:564.

Gibson, T., A.C. Stirling, R.M. Keddie and R.F. Roseschberger. 1958. Bacteriological changes in silage made at control temperatures. J. of Gen. Micro. 19:112.

Gill, D.R., F.N. Owens, J.J. Martin, R.A. Zinn, D.E. Williams and R.J. Hillier. 1982. Corn Moisture and processing. Oklahoma State Univ. and USDA Res. Rep. MP-112. p 207.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. The Iowa State Univ. Press. Ames, Iowa.

Goodrich, R.D., F.M. Byers and J.C. Meiske. 1975. Influence of moisture content, processing and reconstitution on the fermentation of corn grain. J. Anim. Sci. 41:876.

Goodrich, R.D., C.E. Dexheimer and J.C. Meiske. 1974. Nutritive values of dry corn, ensiled high moisture corn and acid treated corn grain. Animal Science Department Mimeo, University of Minnesota, St. Paul.

Goodrich, R.D. and J.C. Meiske. 1976. Influence of maturity and moisture content on the fermentation of high moisture corn. High Moisture Grains Sump., Oklahoma State Univ., Stillwater. p 51.

Gordon, C.H., H.G. Wiseman, J.C. Derbishire, W.C. Jacobson and D.T. Black. 1959. Effect on silage of chopping and bruising the forage. J. Dairy Sci. 42:1394.

Grazia, L. and G. Suzzi. 1984. A survey of lactic acid bateria in Italian silage. J. of Appl. Bact. 56:373.

Guyer, P.Q. and S. Farlin. 1976. Effects of prestorage processing on the feeding value of high moisture harvested grains. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 23.

Haigh, P.M. 1987. The effect of dry matter content and silage additives on the fermentation of grass silage on commercial farms. Grass and Forage Sci. 42:1.

Haigh, P.M., M. Appleton and S.F. Clench. 1987. Effect of commercial inoculant and formic acid + or - formalin silage additives on silage fermentation and intake and on liveweight change of young cattle. Grass and Forage Sci. 42:405.

Henderson, H.E. and W.G. Bergen. 1970. Dry corn vs. high moisture corn vs. reconstituted corn for finishing yearling steers on an 80% concentrate ration. Mich. Agric. Exp. Sta. Res. Rep. AH-BC-692.

Henderson, A.R., J.W. Ewart and G.M. Robertson. 1979. Studies on the aerobic stability of commercial silages. J. Sci. Food Agric. 30:223.

Henderson, A.R., P. McDonald and D.H. Anderson. 1982. The effect of silage additives containing formaldehyde on the fermentation of ryegrass ensiled at different dry matter levels and on the nutritive value of direct-cut silage. Anim. Feed Sci. and Technol. 7:303.

Hinders, R.G. 1976. Reconstitution of grains. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 93.

Hoffman, M.P. and H.L. Self. 1975. Comparison of artificially dried corn with high moisture corn stored in two silo types. J. Anim. Sci. 41:500.

Huhtanen, P., K. Hissa, S. Jaakkola and E. Poutiainen. 1985. Enzymes as silage additive effect fermentation quality, digestion in sheep, degradability in sacco and performance in growing cattle. J. Agric. Sci. Finland 57:284.

Hungate, R.E. and D.W. Fletcher. 1962. Laboratory Manual of General Bacteriology (2nd Ed.) Pub Scholar's Library. p 23.

Johnson, R.R. 1969. Techniques and procedures for in vitro and in vivo rumen studies. J. Anim. Sci. 25:855.

Johnson, R.R., K.E. McClure, E.W. Klosterman and L.J. Johnson. 1967. Corn plant maturity. 3. Distribution of nitrogen in corn silage treated with limestone, urea and diammonium phosphate. J. Anim. Sci. 26:394.

Jones, D.I.H. 1988. The effect of cereal incorportaion on the fermentation of spring and autumn cut silages in laboratory silos. Grass and Forage Sci. 43:167.

Jones, R.J., B.G. Gemgembach and V.B. Cardwell. 1981. Temperature effects on in vitro kernel development of maize. Crop Sci. 21:761.

Klosterman, E.W., R.R. Johnson, H.W. Scott, A.L. Moxon and J.V. Stavern. 1960. Whole plant and ground ear silages, their acid content, feeding value and digestibility. J. Anim. Sci. 19:522.

Kofoid, K.D., J.W. Maranville and M.W. Ross. 1982. Relationship of the test to agronomic and nutritional traits in sorghum. Crop Sci. 22:352.

Kroulik, J.T., L.A. Burkey, C.H. Gordon, H.G. Wiseman and C.G. Melin. 1955. Microbial activities in alfalfa and orchard grass ensiled under certain conditions in experimental silos. J. Dairy Sci. 38:263.

Kung, L., Jr., W.M. Craig, L.D. Satter and G.A. Broderick. 1986. Effect of adding formaldehyde, glutaraldehyde or dimetholurea to alfalfa before ensiling. J. Dairy Sci. 69:2846.

Kung, L., Jr. L.D. Satter, B.A. Jones, K.W. Genin, A.L. Sudoma, G.L. Enders, Jr. and H.S. Kim. 1987. Microbial inoculation of low moisture alfalfa silage. J. Dairy Sci. 70:2069.

Largen, H.J. 1980. Ensiled grains for dairy cattle feeding. J. Dairy Sci. 63:148(Abstr.).

Lee, S., M.A. Hanna and L.B. Bullerman. 1986. Carbon dioxide and alfatoxin production in high-moisture corn treated with potassium sorbate. Cereal Chem. 63:82.

Leibensperger, R.Y. and R.E. Pitt. 1987. A model of clostridial dominance in ensilage. Grass and Forage Sci. 42:297.

Lindgren, S., P. Lingvall, A. Kaspersson, A. Kartzow and E. Rydberg. 1983. Effect of inoculants, grain and formic acid on silage fermentation. Swedish J. Agric. Res. 13:91.

Lindgren, S., K. Pettersson, A. Jonsson, P. Lingvall and A. Kaspersson, 1985a. Silage inoculation-selected strains, temperature, wilting and practical application. Swedish J. Agric. Res. 15:9.

Lindgren, S., K. Petterson, A. Kasperson, A. Johnson and P. Lingvall. 1985b. Microbial dynamics during aerobic deterioration of silages. J. of Sci. Food Agric. 36:765.

Lindgren, S., and O. Refai. 1984. Amylolytic lactic acid bacteria in fish silage. J. of App. Bact. 57:221.

Luther, R.M. 1986. Effect of microbial inoculation of whole-plant corn silage on chemical characteristics, preservation and utilization by steers. J. Anim. Sci. 63:1329.

Mader, T.L., R.A. Britton, V.E. Krause and D.E. Pankaskie. 1985. Effect of additive on alfalfa silage fermentation characteristics and feedlot performance of steers. J. Dairy Sci. 68:1744.

Mann, E.M. and P. McDonald. 1976. The effect of formalin and lower volatile fatty acids on silage fermentation. J. of Sci. Food Agric. 27:612.

Martin, J.J., F.N. Owens, D.R. Gill, D.E. Williams, R.J. Hillier and R.A. Zinn. 1980. Protein sources for steers fed steam flaked, high moisture or whole shelled corn. Oklahoma State Univ. and USDA Res. Rep. MP-107. p 114.

Mayne, C.S. and F.J. Gordon. 1986. Effect of harvesting system on nutrient losses during silage making. 2. In-silo losses. Grass and Forage Sci. 41:341.

McDonald, P. 1981. The Biochemistry of Silage. John Wiley and Sons, Chichester.

McDonald, P., A.R. Henderson and R. Whitterburg. 1966. The effect of temperature on ensilage. J. Sci. Food Agric. 17:475.

McDonald, L.C., R.F. McFeeters, M.A. Daeschel and H.P. Fleming. 1987. A differential medium for the enumeration of homofermentative and heterofermentative lactic acid bacteria. App. and Env. Microbiol. p 1382.

McHan, F. 1986. Cellulase-treated coastal bermudagrass silage and production of soluble carbohydrates, silage acids, and digestibility. J. Dairy Sci. 69:431.

McKnight, D.R., G.K. MacLeod, J. Buchanan-Smith and D.N. Mowat. 1973. Utilization of ensiled acid treated high moisture shelled corn by cattle. Can. J. Anim. Sci. 53:491.

Middelhoven, W.J. and M.M. Franzen. 1986. The yeast flora of ensiled whole-crop maize. J. Sci. Food Agric. 37:855.

Moon, N.J. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. J. of App. Bact. 55:453.

Muck, R.E. and P.L. O'Connor. 1985. Initial bacterial numbers on alfalfa prior to ensiling. Trans. Am. Soc. Agric. Eng. (Abstr.) No. 85.

Muck, R.E. and M.W. Speckhard. 1984. Moisture level effects on alfalfa silage quality. Trans. Am. Soc. Agric. Eng. 84-1532.

Murphy, J.J. 1986. A comparative evaluation of the feeding value for dairy cows of silage treated with formic and sulphuric acid. Ir. J. Agric. Res. 25:1.

Ohyama, Y., S. Hara and S. Masski. 1980. Analysis of the factors affecting aerobic deterioration of grass silages. Br. Grassland Soc. Occas. Symp. 11:257.

Ohyama, Y., S. Masaki and S. Hara. 1975. Factors influencing aerobic deterioration of silages and changes in chemical composition after opening silo. Sci. Food Agric. 26:1137.

Oshima, M. and P. McDonald. 1978. A review of the changes in nitrogenous compounds in herbage during ensiling. J. Sci. Food Agric. 29:497.

Owens, F.N., W.M. Sharp and G. Davis. 1981. Corn silage ammoniation time and protein solubility. Oklahoma State Univ. and USDA Res. Rep. MP-108. p 139.

Owens, F.N. and J.H. Thornton. 1976. Moisture content versus intake and energy value of high moisture corn. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 193.

Palmquist, D.L. and H.R. Conrad. 1970. Effects of feeding high moisture corn to dairy cows. J. Dairy Sci. 53:649.

Perry, T.W. 1976. Feed intakes, gains, and efficiency. High Moisture Grains Symp. Oklahoma State Univ., Stillwater. p 113.

Pitt, R.E. 1986. Dry matter losses due to oxygen infiltration in silos. J. Agric. Eng. Res. 35:193.

Pitt, R.E. and J.Y. Parlange. 1987. Effluent production from silage with application to tower silos. Trans. Am. Soc. Ag. Eng. 30:1198.

Prigge, E.C. 1976. Ensiling conditions and soluble nitrogen and high moisture corn utilization. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 74.

Prigge, E.C., R.R. Johnson, F.N. Owens and D. Williams. 1976. Soluble nitrogen and acid production of high moisture corn. J. Anim. Sci. 42:490.

Rode, L.M., K.J. Cheng and J.W. Costerton. 1986. Digestion by cattle of urea-treated ammonia-treated or rolled high moisture barley. Can. J. Anim. Sci. 66:711.

Russell, R.W., J.C.M. Lin, E.E. Thomas and E.C. Mora. 1988. Preservation of high moisture milo with urea grain properties and animal acceptability. J. Anim. Sci. 66:2131.

Rust, S.R., H.S. Kim and G.L. Enders. 1989. Effects of microbial inoculant on fermentation characteristics and nutritional value of corn silage. J. of Prod. Agric. 2:235.

Rust, S.R., M.T. Yokoyama and D.G. Main. 1987. Effect of a dry and liquid microbial inoculant on nutritive value and fermentation characteristics of high moisture corn. J. Anim. Sci. 65:116 (Suppl. 1).

Ruxton, I.B., B.J. Clark and P. McDonald. 1975. A review of the effects of oxygen on ensilage. J. Br. Grassland Soc. 30:23.

SAS Institute. 1987. SAS/STAT Guide for Personal Comuters (6th Ed.) SAS Institute, Cary, NC.

Salisbury, F.B. and C. Ross. 1969. Plant Physiology. Wadsworth Co. Inc. Belmont, CA.

Seale, D.R. 1986. Bacterial inoculants as silage additives. J. of Appl. Bact. Symp. (Supplement) p 9.

Seale, D.R., A.R. Henderson, K.O. Petersson and J.F. Lowe. 1986. The effect of addition of sugar and inoculation with two commercial inoculants on the fermentation of lucerne silage in laboratory silos. Grass and Forage Sci. 41:61.

Shaver, R.D., R.A. Erdman and J.H. Vadersale. 1984. Effects of silage pH on voluntary intake of corn silage. J. Dairy Sci. 67:20245.

Siddons, R.C., C. Arricastres, D.L. Gale and D.E. Beever. 1984. The effect of formaldehyde or glutaraldehyde application to lucerne before ensiling on silage fermentation and silage N digestion in sheep. Br. J. Nutr. 52:391.

Siegfried, B.R., H. Ruckemann and G. Stumpf. 1984. Method for the determination of organic acids in silage by high performance liquid chromatography. Landwersch Foschung 37:298.

Singh, R., D.N. Kamra and R.C. Jakhmola. 1985. Ensiling of leguminous green forages in combination with different dry roughages and molasses. Anim. Feed Sci. Technol. 12:133.

Spargue, J.I. 1976. Digestive disorders and feeding problems of high moisture corn and a soluble protein concept for evaluating rations. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 161.

Sprague, J.L. and G.W. Breniman. 1969. High moisture corn and cattle performance. Feedstuffs 41:46.

Steen, R.W.J. 1985. The effect of field wilting and mechanical treatment on the feeding value of grass silage for beef cattle and on beef output per hectare. Anim. Prod. 41:281.

Stevenson, K.R. 1976. Stability and bunk life of high moisture corn. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 105.

Stock, R.A., D.R. Brink, R.T. Brandt, J.K. Merrell and K.K. Smith. 1987. Feeding combinations of high moisture corn and dry corn to finishing cattle. J. Anim. Sci. 65:282.

Svensson, L. and M. Treit. 1964. Effect of different supplements on the fermentation process in silage. J. Sci. Food Agric. 15:78.

Thomas, J.W. 1978. Preservatives for conserved forage crops. J. Anim. Sci. 47:721.

Thomas, J.W., Y. Yu, T. Middleton and C. Stallings. 1980. Estimations of protein damage. In: Protein Requirements For Cattle: Symp., Okla. Agric. Exp. Sta. Rep. M-109. p 81.

Thornton, J.H. 1976. Chemical indices of quality ensiled high moistue corn grain. High Moisture Grains Symp., Oklahoma State Univ., Stillwater. p 150.

Thornton, J.H., F.N. Owens, R.W. Fent and K. Poling. 1978. Buffers and high moisture corn digestion. Oklahoma State Univ. and USDA Res. Rep. MP-114. p 72.

Thornton, J.H., F.N. Owens, D.E. Williams, M. Arnold. 1977a. Chemical characterization of ensiled ground high moisture corn grain. Oklahoma State Univ. and USDA Res. Rep. MP-101. p 56.

Thornton, J.H., F.N. Owens, D.E. Williams and E.C. Prigge. 1977b. Fermentation and digestion of formaldehyde treated ensiled high moisture corn grain. Oklahoma State Univ. and USDA Res. Rep. MP-101. p 62.

Tonroy, B.R., T.W. Perry and W.M. Beeson. 1974. Dry ensiled high moisture, ensiled reconstituted high moisture corn for growing and finishing beef cattle. J. Anim. Sci. 39:931.

Tyrrell, H.F. 1984. Energy value of ear vs. shelled corn stored either dry or ensiled at high moisture. J. Dairy Sci. 67:131 (Suppl. 1).

Voelker, H.H., D.P. Casper, F.C. Ludens and D.J. Schengoethe. 1989. High moisture corn preserved with esters of propionic acid for lactating cows. J. Dairy Sci. 72:89.

Voelker, H.H., D.J. Schengoethe, J.K. Drackleys and A.K. Clark. 1985. High moisture corn preserved by different methods for lactating cows. J. Dairy Sci. 68:2602.

Waldo, D.R. 1978. The use of direct acidification in silage production. In: M.E. McCullough (Ed.) Fermentation of Silage-A Review. Nat. Feed Ing. Ass. Des Moines, IA. p 117.

Wardynski, F.A., S.R. Rust and M.T. Yokoyama. 1988. Effects of microbial inoculation on nutritive value, fermentation characteristics and aerobic stability of high moisture corn. J. Anim. Sci. 66:473 (Suppl. 1).

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

Whittenburg, R. 1961. An Investigation of the Lactic Acid Bacteria, Ph.D. thesis, Univ. of Edinburgh.

Wilkinson, J.M, J.T. Huber and H.E. Henderson. 1976. Acidity and proteolysis as factors affecting the nutritive value of corn silage. J. Anim. Sci. 42:208.

Williams, J.H., D.E. Baldridge, C.D. Jones, O.O. Thomas and D.J. Tronrud. 1979. Effects of storage structure (oxygen limiting vs. concrete bunker) upon nutritive value and storage losses of corn silage. Montana Agric. Exp. Sta. Res. Rep. 158.

Woolford, M.K. 1978. The aerobic deterioration of silage, ARC Res. Rev. 4:8.

Woolford, M.K. 1984a. The antimicrobial spectra of some salts of organic acids and glutaraldehyde in respect of their potential as silage additives. Grass and Forage Sci. 39:53.

Woolford, M.K. 1984b. Managing aerobic deterioration in silage. In: M.E. McCullough and K.K. Bolsen (Eds.) Silage Management. Silage Technology Division National Feed Ingredients Ass., p 42.

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

Woolford, M.K. and J.E. Cook. 1978. A note on the effects on the aerobic deterioration of maize silage of the manipulation of the microflora by means of antibiotics. Anim. Feed Sci. and Technol. 3:89.

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

Woolford, M.K. and M.K. Sawezyc. 1984b. An investigation into the effect of cultures of lactic acid bacteria on fermentation in silage. 2. Use of selected strains in laboratory scale silages. Grass and Forage Sci. 39:149.

Woolford, M.K. and A.C. Wilkie. 1984. Investigations into the role of specific micro-organisms in the aerobic deterioration of maize silage. J. Agric. Sci. Camb. 102:97.

Woolford, M.K. and R.J. Wilkins. 1975. The evaluation of formaldyhyde, bronopol, tylosin and pimaricin as additives in simulated silage. J. Sci. Food Agr. 26:869.

Woolford, M.K., R.J. Wilkins and C. Wall. 1975. A note on the laboratory evaluation of 2-bromo-2-nitropropane-1, 3-diol as a potential silage additive. J. Sci. Food Agri. 26:1699.

Young, B., R. Smith, K. Bolsen and H. Ilg. 1984. High moisture corn ensiled with urea for cattle finishing rations. Kansas Agric. Exp. Sta. Rep. Progress 448. p 58.

Zimmer, E. 1967. Nahrstofferluste beider Vergarung van Futterpflanzen. Wirtschaftlegene Futter. 13:271.