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FERMENTATION AND FEED VALUE OF WILTED OR UNWILTED ALFALFA HAYLAGE ENSILED WITH OR WITHOUT THE ADDITION OF BACTERIAL INOCULANTS

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By

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ABSTRACT

FERMENTATION AND FEED VALUE OF WILTED OR UNWILTED ALFALFA HAYLAGE ENSILED WITH OR WITHOUT THE ADDITION OF BACTERIAL INOCULANTS

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Laboratory quart-jar silos and farm-scale tower silos were used to evaluate the effects of inoculation of wilted (34-36% DM) and unwilted (19% DM) alfalfa (Medicago sativa) forage with commercial mixed lactic acid bacteria (LAB) cultures on silo fermentation parameters. Inoculation increased the number of LAB per gram of both wilted and unwilted forage when epiphytic populations were low, and as a result stimulated lactic acid production and rate of pH decline in the silage ($p<0.05$). However, final silage pH and dry matter recovery were not improved above those of control. In situations where water-soluble carbohydrate was limiting, inoculation did not enhance fermentation as greatly as carbohydrate addition in the form of sucrose or dextrose. When fed to Holstein dairy cows in mid-lactation, inoculated wilted alfalfa silage increased cow weight gains but not dry matter intake, milk production or milk component production as compared with cows fed untreated silage.

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Finally, the author needs to recognize her family for providing the agricultural environment and farming heritage that determined her choice of professions. The author's family has successfully operated the same dairy farm in Cayuga County, New York since 1850. Though not at the production level, the author is proud to be involved in agriculture, carrying on the family tradition.

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ADF	acid-detergent fiber
AN	ammonia nitrogen
BC	buffering capacity
cfu	colony-forming unit(s)
CP	crude protein
DM	dry matter
DMR	dry matter recovery
FCM	4% fat-corrected milk
g	gram(s)
kg	kilogram(s)
LA	lactic acid
LAB	lactic acid producing bacteria
lb	pound
m	meter(s)
mg	milligram(s)
N	nitrogen
SEM	standard error of the mean
TN	total nitrogen
VFA	volatile fatty acid(s)
WSC	water-soluble carbohydrate
WSN	water-soluble nitrogen
C	control
I	inocula
S	sucrose
I+S	inocula + sucrose
B	sodium bentonite
I+B	inocula + sodium bentonite
I+B+G	inocula + sodium bentonite + glucose

1.0 INTRODUCTION

Ensilage as a method of forage conservation has been practiced since the days of the ancient Egyptians some 3000 years ago (Woolford, 1984). A French farmer, Auguste Goffart, is credited with the practical modernization of ensiling after he published a manual on the subject in 1879. Goffart's technique of conserving green crops for winter feeding with rapid filling and sealing of the silo was soon adopted in France and in the United States. Since then, the acreage of forages preserved as silages has gradually increased throughout the temperate zones of the world (Waldo, 1977). In recent years, the practice of ensiling wilted and unwilted alfalfa in the U.S. has gained considerable popularity as the time and labor involved are considerably less as compared with hay-making. Silage production allows greater mechanization, reduced time that the crop must remain in the field, and fewer field operations than does the process of baling hay. Thus the farm operator assumes less weather associated risk and can manage a more flexible forage program (Thomas, 1980; Henderson, 1987). In addition, handling the wetter crop material (30 - 50% DM) causes less leaf loss during harvest resulting in greater conservation of original forage dry matter and digestible nutrients (Shepard et al, 1954).

Alfalfa is notoriously difficult to ensile as are other legume crops. Legumes are characteristically low in soluble carbohydrates, high in protein and have a high buffering capacity. Ensilage of wilted alfalfa often results in considerable protein degradation and occasionally protein is rendered unavailable from heat damage.

Direct-cut or unwilted silages are also associated with extensive protein breakdown and with low dry matter, energy and nitrogen recoveries. Silages produced in this manner frequently cause reduced feed intake, animal gain or production, and animal production per ton of forage when compared with hay. Some of these problems could be avoided if a more rapid pH decline and establishment of anaerobic conditions in the silo could be acheived, reducing the losses of and damage to forage nutrients.

Professor A.I. Virtanen (1933) conducted experiments practically illustrating the effectiveness of mineral acids as silage treatments for quickly reducing the pH and minimizing post-harvest losses of energy and protein in grass silage. Silages produced using this system were of good quality. Later, formic acid was introduced as mineral acids were found to cause health problems when ingested by livestock. Formic acid did not gain popularity in the U.S. as an additive for silage as it is very corrosive to machinery. However, efforts turned to developing alternative treatments to minimize ensiling losses.

Several studies have indicated that numbers of viable organisms present on fresh herbage are low and are often not the acid-tolerant, homofermentative types capable of producing a rapid and efficient lactic acid fermentation. In the 1960's and 70's, researchers began to experiment with mixed cultures of lactic acid producing organisms as silage additives. Effectiveness of microbial inoculas is thought to be related to chemical composition of the crop, moisture content, epiphytic microbial population, and crop pretreamtents such as wilting and mechanical conditioning. Many experiments have indicated that inoculation can improve chemical parameters of the silage, but unfortunately, results of nutrient recovery and animal performance trials have been inconsistent.

The following experiments were conducted in an effort to monitor the effects of a commercial microbial silage inoculant on fermentation characteristics of wilted or unwilted alfalfa forages ensiled in laboratory-scale silos. Wilted alfalfa forage was also ensiled in farm-scale silos in order to measure nutrient recovery and lactational performance of Holstein cows fed diets containing control or inoculated silage.

2.0 REVIEW OF LITERATURE

2.1 The Ensiling Processes in General

Ensiled forage follows a sequential fermentation. The type and extent of silage fermentation depends on the chemical composition of plant material, moisture content, the amount of entrapped air in the ensiled mass, and the activity of the microbial population. The first stages of this fermentation begin after the crop is cut and are mainly due to action of plant enzymes. This initial phase, referred to as respiration, is characterized by death of plant tissue and exhaustion of entrapped oxygen (O_2). Hexose sugars are converted to carbon dioxide (CO_2), water (H_2O), and energy, most of which is liberated as heat (Mo and Fyrileiv, 1979; Henderson, 1987). Some alcohol may also be produced in early ensilage (Kalac and Pivnickova, 1987). Plant respiration continues as long as O_2 and substrate are available. Respiration thus reduces the supply of carbohydrate available for later anaerobic fermentation. Recommendations to minimize this respiration phase are to fill the silo quickly, to pack the forage tightly, eliminating much of the entrapped O_2, and to seal the surface of the silo with a plastic cover to prevent O_2 penetration (McDonald, 1981; VanSoest, 1982; Woolford, 1984).

Proteolysis, the breakdown of protein, also begins immediately after the crop is cut and continues during wilting and ensiling. The rate of proteolysis and non-protein nitrogen (NPN) content of the forage are markedly increased in the ensiling phase. On the average, 50 to 60 % of the protein is broken down. Deamination of amino acids is initiated by plant enzymes but microbial activity is thought to be responsible for most ammonia production (Whittenbury et al, 1967; Ohshima and McDonald, 1978).

Subsequent stages of the silage fermentation begin several hours later, when the entrapped O_2 is eliminated and anaerobic conditions are established. This permits lactic acid producing bacteria (LAB), found initially in small numbers on the plant and added from harvesting and silo filling equipment, to increase in 2 to 4 days to 10^5 or 10^6 cfu per gram of forage (Lesins and Schultz, 1968; Henderson et al, 1972; Henderson, 1987). Lactic acid bacteria convert available carbohydrate to lactic acid (pKa=3.86) thereby reducing the pH of the ensiled mass. A low pH can then inhibit further bacterial growth and enzyme activity and stabilize or preserve the silage. Normally, silage (35% DM) with a pH of 4.0 to 4.2 is considered stable and may be stored for years if air is excluded. Wetter or drier silages stabilize at lower and higher pH respectively (McDonald, 1981; Woolford, 1984).

An aerobic deterioration or spoilage phase can occur if

the forage is exposed to air for a long period of time or if a large quantity of air is entrapped during the silo filling process. Under these conditions, much of the available carbohydrate may be utilized by aerobic microorganisms and there may not be enough left to produce the lactic and other acids necessary to decrease the pH to a point low enough to stabilize the silage. A 'secondary' fermentation can result when endospore forming anaerobes or lactic acid fermenting Clostridia become involved forming butyric acid and CO_2, degrading protein and generally causing a deterioration of the silage. This loss of carbon, as CO_2, represents dry matter loss. Similar deterioration can also occur when the silo surface is exposed to air during feedout (Henderson and McDonald, 1975; Rees, 1982).

Events in the silo can, to a certain extent, be manipulated through the choice of the silo, the crop to be ensiled, by crop pretreatments such as wilting, and through the use of additives. Many additives supply soluble carbohydrates, LAB cultures, or acids all of which can promote an initial reduction in pH and successful preservation of the ensiled feedstuff (Thomas et al, 1980; Woolford, 1984).

2.2 The Epiphytic Microflora

The microflora of silage is very different from that found on the ingoing forage crop (Cunningham and Smith, 1940;

Langston et al, 1962a; Muck and O'Connor, 1985). Epiphytic bacteria are those microorganisms found on the forage crop itself prior to ensiling. Of these microorganisms present on the growing plant, the majority are aerobic and are therefore of little importance in the anaerobic ensiling process. Lactic acid bacteria are usually detected in very low numbers prior to ensiling, often less than 10^2 cfu/g fresh forage (Langston et al, 1962a; Langston and Bouma, 1960a, b; Stirling and Whittenbury, 1963; Fenton, 1987; Muck 1989) which is approximately 0.01 to 0.1% of the entire microbial population (Daeschel et al, 1987), yet in most silages they are the dominant organisms. In the first 2 days of ensiling, they can increase 100,000-fold and determine the success or failure of the fermentation. Other types of organisms present that may play a role in the fermentation process or in the anaerobic deterioration of silage are the enterobacteria, bacilli, yeasts, molds, and clostridia. Their influence depends on numbers present, the chemical composition of the crop, and any treatment which may be applied to the crop after cutting (Henderson, 1987; Fenton, 1987).

Kroulik et al (1955) found that numbers of epiphytic organisms varied widely with a number of factors. The type of forage, season, part of the plant, and the stage of maturity of the plant had an influence on bacteria populations. Bacteria numbers increased as the plant matured

and increased during wilting and chopping. Muck and Speckhard (1984) also found that bacterial counts increased during wilting. However, other researchers (Lanigan, 1963; Pizarro and Warboys, 1979) have shown a decrease in epiphytic bacteria during wilting. Muck and Speckhard (1984) suggest that under slow drying conditions the LAB population increases while on hot, sunny days an opposite trend was observed. Harvesting machinery, such as choppers and wagons, blowers, and silos have been implicated as sources of bacterial inoculation (Wieringa, 1959; McDonald, 1976; Muck and O'Connor, 1985; Fenton, 1987). Muck and O'Connor (1985) identified the major factors affecting epiphytic LAB numbers as crop yield, wilting time, temperature, and rainfall prior to mowing. As all of these factors increased, microbial numbers also increased. Muck (1989) also developed a model which included air temperature during wilting, wilting time and the drying rate as variables and could predict LAB counts within one order of magnitude 95% of the time.

Since so many factors play a role in determining the epiphytic bacterial population, it is not surprising that numbers can vary greatly. Muck and Speckhard (1984) observed epiphytic counts ranging from 10^3 to 10^8 organisms per gram of forage. This wide variation in the epiphytic bacteria population may be the reason that inoculation trials have shown mixed results. In some cases, the natural epiphytic population of LAB may be insufficient in number or type to

ensure good silage preservation and in these cases microbial inoculation of the forage prior to ensiling may be beneficial (Ohyama et al, 1975; Carpintero et al, 1979)

2.3 Effects of Wilting on Chemical Parameters

Wilting, or the partial air-drying, of a cut crop prior to ensiling is commonly practiced to improve preservation of silage and to enhance subsequent animal performance and acceptability (Gordon, 1981). Normally, a crop would be wilted from a standing moisture content of 80 - 85% to between 55 and 70% moisture over a period of 1 to 3 days. Wilting then is associated with risks of weather and mechanical damage and DM and nutrient losses while the forage lies in the field (Mayne and Gordon, 1986; Henderson, 1987). Ensiling unwilted forage is associated with less harvesting labor as fewer field operations are required, less field loss (Waldo, 1977), more effluent losses during ensiling, and occasional spoilage problems caused by clostridia activity (Whittenbury et al, 1967). Gordon et al (1961) observed less storage losses for wilted silage than with unwilted silage. Dry matter recoveries for wilted and unwilted silages across 2 experiments averaged 92.5 and 76.5% respectively. Mayne and Gordon (1986) showed decreased field and mechanical losses for unwilted versus wilted grass silage. Field loss totaled 7.1% of available yield for wilted forage and 3.4%

for unwilted grass harvested for silage.

During wilting, biochemical transformations occur that result in a redistribution of original plant components. These chemical changes are brought about by plant enzymes and can result in DM and nutrient losses (Honig, 1979; Shukking and Overvest, 1979). Non-structural carbohydrates can be hydrolyzed into constituent hexose sugars and oxidized to CO_2 and H_2O. This loss of DM is a loss of the most fermentable fractions of the forage material. Prolonged wilting periods and a slow drying rate can cause this loss of soluble carbohydrate to be very extensive (Wylam, 1953; Gordon et al, 1961). McDonald et al (1968) have shown that although wilting reduced soluble carbohydrate content in the grass forage, there was less loss of this component during ensiling as compared with unwilted grass silage. Lesser amounts of most fermentation acids are produced during ensilage of drier forage (Gordon et al, 1965; McDonald et al, 1968).

Another consequence of plant enzymatic activity during wilting is degradation of true plant protein into smaller non-protein nitrogenous compounds. A rapid increase in soluble amino, volatile and amide nitrogen coincide with a reduction in protein nitrogen (Macpherson, 1952a; Brady, 1960). Kemble and Macpherson (1954) observed, during a wilting period of 3 days, that 20% of total protein was degraded to non-protein nitrogen. However, wilting to a greater DM content may reduce proteolysis during ensiling

(Brady, 1965; Hawkins et al, 1970). Less production of water-soluble nitrogen (Donaldson and Edwards, 1976) and ammonia-nitrogen (Gordon et al, 1965; McDonald et al 1968) has been observed in wilted versus unwilted silages. Wilted silage often takes a longer period of less active fermentation to reach stability and in the process degrades fewer of the original plant components during ensiling than unwilted silage.

2.4 Substrates for Silage Fermentation

2.4.1 Water Soluble Carbohydrate

One of the primary chemical changes in silage, as a result of plant and microbial enzyme activity, is the conversion of carbohydrate into organic acids and gases. The water soluble fraction of the carbohydrate component is readily available to the microorganisms present on the forage material. Glucose, fructose, sucrose, fructosans and starches are the main water soluble carbohydrates (WSC) in fresh forages. Fructosans are the major storage form of carbohydrate in grasses, while starches are the dominant form of storage in legumes. In general, grasses contain more WSC than legumes. Sucrose and fructosans are hydrolyzed to glucose and fructose monomers during ensilage, so glucose and fructose can be considered to be the major carbohydrate sources for microbial activity (Whittenbury et al, 1967;

McDonald et al, 1968; McDonald, 1979). Some pentoses may be produced from hemicellulase activity on plant hemicellulose and these pentoses then would also be highly available for fermentation (Dewar et al, 1963).

The WSC content of forage plants is extremely variable and dependent upon many factors. Plant species, time of day (Melvin, 1965), wilting and wilting conditions (Kung et al, 1984), weather, plant maturity (Hirst et al, 1959), field conditioning (Gibson et al, 1961), breakdown of structural carbohydrates (Dewar et al, 1963), and sugar addition (Seale et al, 1986; Thomas, 1978) have all been shown to influence WSC content of herbage. Winters et al (1987) suggest that most plant substrates are made available as the cell structure is disrupted by enzymes or physical pressure. Structural carbohydrates are normally of little importance in silage fermentation, although some researchers have reported breakdown of polysaccharides and plant cell wall constituents through acid hydrolysis or action of enzymes (Harwood, 1954; Dewar et al, 1963; Huhtanen et al, 1985; Morrison, 1988).

All WSC in the ensiled crop is not fermented. Substantial amounts may be lost through aerobic microbial oxidation and plant respiration prior to the establishment of anaerobic conditions (Henderson and McDonald, 1975). Soluble carbohydrates remaining after aerobic metabolism are fermentable by a variety of microbes. Lactic acid bacteria (facultative anaerobes) are considered to be the most

important as their growth inhibits other unwanted types of organisms. Two basic types of LAB, which differ in fermentation end products and efficiency, are found in silage (Table 2.1). Under anaerobic conditions, the homofermentative type forms 2 moles of lactic acid per mole of glucose or fructose, while the heterofermentative bacteria produce 1 mole of lactic acid, 1 mole of CO_2, and 1 mole of ethanol per mole of glucose fermented. When fructose is the substrate, heterofermentative bacteria produce lactic acid, mannitol, acetic acid (pKa=4.7), and CO_2. Both types of LAB can utilize pentoses and both produce 1 mole of lactic acid and 1 mole of acetic acid per mole of pentose fermented (Whittenbury et al, 1967; McDonald, 1979; Woolford, 1984). The efficiency of lactic acid production and the conservation of energy and dry matter by heterofermentative organisms is less than that of homofermentative microbes, therefore the homofermentative types are more desirable.

The fermentation pattern is not simple when considering different sugar substrates and different types of organisms. Predicting fermentation products is further complicated by action of some LAB on plant non-nitrogenous organic acids.

Table 2.1. Main products of anaerobic carbohydrate fermentation by lactic acid bacteria.

HOMOFERMENTATIVE	
1 Glucose ----->	2 Lactic acid
1 Fructose ---->	2 Lactic acid
1 Pentose ----->	1 Lactic acid + 1 Acetic acid
HETEROFERMENTATIVE	
1 Glucose ----->	1 Lactic acid + 1 Ethanol + 1 CO_2
3 Fructose ---->	1 Lactic acid + 2 Mannitol + 1 Acetic acid + 1 CO_2
1 Pentose ----->	1 Lactic acid + 1 Acetic acid

from Whittenbury et al, 1967

2.4.2 Organic Acids and Salts

The organic acids and their salts form the important buffering systems in plants (Playne and McDonald, 1966). Malate (pKa=3.4, 5.1) and citrate (pKa=3.1, 4.7, 5.4) are the principal organic acids in a wide range of plant species. In silage, the acids of primary interest are those which buffer against acidification within the range of pH 4 to 6. Legumes usually contain greater amounts of organic acids than grasses. In addition to the previously mentioned acids, legumes also contain large amounts of glyceric acid, protein and mineral cations. Consequently, the buffering capacity of legumes is 2 to 4 times higher than that of grasses. Plant proteins account for 10 to 20% of the buffering constituents.

The high protein and buffering capacity and low sugar content in legumes support observations of difficult ensiling of alfalfa and clovers (Playne and McDonald, 1966; Whittenbury et al, 1967; McDonald, 1981; Woolford, 1984)

During ensilage, a rapid and complete dissimilation of organic acids occurs, especially concerning citric and malic acids. Table 2.2 illustrates chemical changes of these two acids. The products formed by both homo- and heterofermentative LAB are acetone, lactate, acetate, or alkaline released cations. Many of the organic acids are present in plants in the salt form and their destruction by bacteria acts against silage preservation as decarboxylation results in the release of cations and CO_2. The overall effect is a replacement of organic acids with acids with stronger buffering capacity, 2 to 4 times greater, and a loss of DM in the form of CO_2 (Whittenbury et al, 1967; McDonald and Henderson, 1962; Playne and McDonald, 1966; Greenhill, 1964).

Table 2.2. Main products of anaerobic organic acid fermentation by lactic acid bacteria.

HOMO- AND HETEROFERMENTATIVE
1 Citric acid ----> 2 Acetic acid + 1 Formic acid + 1 CO_2
2 Citric acid ----> 2 Acetic acid + 1 Acetone + 4 CO_2
2 Citric acid ----> 3 Acetic acid + 1 Lactic Acid + 3 CO_2
1 Malic acid -----> 1 Lactic acid + 1 CO_2
2 Malic acid -----> 1 Acetone + 4 CO_2
1 Malic acid -----> 1 Acetic acid (or Ethanol) + 1 Formic acid + 4 CO_2

from Whittenbury et al, 1967

2.5 Changes in Plant Nitrogenous Components

Harvesting of a forage crop is followed by rapid and extensive proteolysis, both during wilting and the first few days of ensiling, which is terminated only when either a high dry matter or a low pH is reached (Ohshima and McDonald, 1978; McDonald, 1979). The fresh herbage normally contains 75 - 90% of its nitrogen in the form of true protein and 5 - 15% in the form of alpha-amino N (Mangan, 1982). The remaining fraction consists mainly of free amino acids, amides, ureides, amines, nucleotides, chlorophyll, peptides and amino acids bound in non-protein form (Hegarty and Peterson, 1973). Polyamines have also been detected (MacPherson and Violante, 1966a). The amino acid composition

of alfalfa is shown in Table 2.3. Mangan (1982) observed that up to 40% of leaf protein is in the chloroplastic enzyme ribulose-1,5-biphosphate carboxylase. Ammonia is usually present in very low concentrations, often less than 1.5% of the total nitrogen (Brady, 1960; Bergen et al, 1974).

In well-preserved silages, 50 - 60% of the true protein can be broken down (Whittenbury et al, 1967). The extent of proteolysis during ensiling depends on the rate of acidification, therefore a rapid pH decrease is important for the conservation of protein (McKersie, 1985). MacPherson (1952b) observed that initial breakdown of protein in ensiled grass sap was extremely rapid, but slowed markedly at pH 5 and essentially ceased at pH 4.3. Three to five-fold increases in water-soluble nitrogen during the first 10 days of ensiling have been shown (Brady, 1960; Ohshima et al, 1979). An increase in free amino acid content relative to that of the initial forage has been observed by several researchers (Ohshima et al, 1979; Hughes, 1970; MacPherson, 1952). Most hydrolysis of forage proteins was found to be a result of plant enzymes rather than microbial enzymes. Sterile silage protein underwent hydrolysis similar to control silage. (Kemble, 1956). Lactic acid bacteria are essentially non-proteolytic (McDonald, 1981). Forages are known to contain several different proteinases and peptidases that are pH-dependent (McKersie, 1981). The activity of plant enzymes declines rapidly within 2 to 5 days after

Table 2.3 Distribution of free amino acids in alfalfa. (% of total amino acids)

	Leaves	Stems
Asparagine	23.7	37.6
Glutamine	1.7	0.9
Aspartic acid	8.3	13.7
Glutamic acid	4.6	1.9
g-Amino butyric acid	19.1	12.4
Alanine	8.2	4.4
Glycine	1.3	0.6
Serine	7.9	4.9
Threonine	1.9	1.7
Valine	1.6	1.7
Isoleucine Leucine	8.1	8.8
Tyrosine Phenylalanine	0.8	0.2
Histidine	2.2	2.3
Lysine Arginine	1.7	1.5

from Fauconneau, 1960

ensiling, when microbial fermentation begins to predominate (Bergen et al, 1974; Ohshima and McDonald, 1978; McKersie and Buchanan-Smith, 1982).

In addition to hydrolysis of proteins, further breakdown of amino acids can occur. Some deamination and decarboxylation of amino acids occurs as a result of plant enzyme activity (Kemble, 1956; Brady, 1960). Lactic acid bacteria can also deaminate and decarboxylate some amino acids and amides (Brady, 1965; Hughes, 1970). In silages

where clostridial fermentation has dominated, catabolism is extensive. Some examples of the amino acid reactions brought about by clostridial activity are listed in Table 2.4. The extent of amino acid degradation seems to be directly related to the activity of clostridia which is related to lactic acid production and rate of pH decline (MacPherson and Violante, 1966b).

Even under ideal ensiling conditions some proteolysis will occur, but it can be minimized by the early and rapid establishment of anaerobic and acid conditions. Proteolysis will occur as a result of digestion once the protein reaches the animal. It is most important that amino acids do not undergo changes during ensilage (Woolford, 1984).

Table 2.4. Catabolism of amino acids and amides by proteolytic clostridia. Three main pathways are involved.

1. DEAMINATION

Arginine ---> Citrulline + NH_3
Citrulline \---> Ornithine + NH_3 + O_2
Aspartic Acid ---> Fumaric Acid + NH_3
Fumaric Acid \--> Acetic Acid + Pyruvic Acid
Glutamic Acid ---> Mesaconic Acid + NH_3
Mesaconic Acid \--> Acetic Acid + Pyruvic Acid
Histidine ---> Urocanic Acid + NH_3
Urocanic Acid \--> Formiminoglutamic Acid
Formiminoglutamic Acid \--> Formamide + Glutamic Acid
Lysine ---> Acetic Acid + Butyric Acid + NH_3
Methionine --> a-Ketobutyric Acid+Methylmercaptan+NH_3
Phenylalanine ---> Phenyl Propionic Acid + NH_3
Serine ---> Pyruvic Acid + NH_3
Threonine ---> a-Ketobutyric Acid + NH_3
Tryptophan ---> Indolepropionic Acid + NH_3
Tyrosine ---> p-Hydroxyphenyl Propionic Acid + NH_3
Asparagine ---> Aspartic Acid + NH_3
Glutamine ---> Glutamic Acid + NH_3

2. DECARBOXYLATION

Arginine ---> Ornithine ---> Putrescine + CO_2
Aspartic Acid ---> Alanine + CO_2
Glutamic Acid ---> g-Aminobutyric Acid + CO_2
Histidine ---> Histamine + CO_2
Lysine ---> Cadaverine + CO_2
Phenylalanine ---> b-Phenylethylamine + CO_2
Serine ---> Ethanolamine + CO_2
Tryptophan ---> Tryptamine + CO_2
Tyrosine ---> Tyramine + CO_2

3. OXIDATION / REDUCTION

A. Oxidation
Alanine + $2H_2$ (-4H) ---> Acetic Acid + NH_3 + CO_2
Leucine + $2H_2O$(-4H) ---> Isovaleric Acid+NH_3+CO_2
Isoleucine + $2H_2O$(-4H) ---> a-Methylbutyric Acid + NH_3 + CO_2
Valine + $2H_2O$(-4H) ---> Isobutyric Acid+NH_3+CO_2

B. Reduction
Glycine (+2H) ---> Acetic Acid + NH_3
Proline (+2H) ---> d-Aminovaleric Acid
Ornithine (+2H) ---> d-Aminovaleric Acid + NH_3

from Ohshima and McDonald, 1978

2.6 The Lactic Acid Fermentation

Low numbers of LAB are present on uncut grass. Most organisms on the standing crop are gram-negative aerobes (Daeschel et al, 1987). Their activity is undesirable as it contributes nothing to the preservation of the forage and consumes available sugars needed for anaerobic fermentation (Langston et al, 1962a, b; Henderson, 1987). However, the number of LAB increases during harvesting and wilting (Muck and Speckhard, 1984; Fenton, 1987). Following the sealing of the silo, when entrapped air has been used and anaerobic conditions are established, homofermentative and heterofermentative lactate producing organisms normally become dominant, inhibiting aerobic bacteria (Rook and Thomas, 1982; Woolford, 1984). Table 2.5 lists some LAB important in silage.

Henderson et al (1972) followed microbiological changes during the harvesting and fermentation period in a study with wilted perennial ryegrass (36%DM). Microbial munbers from this experiment are shown in Table 2.6. Immediately after cutting the crop, the number of LAB increased four-fold. This is consistent with other findings that harvesting equipment is an important source of organisms (Fenton, 1987). After wilting the forage for 24 hours, LAB had increased to 10^4 per gram, and 7 hours later, these bacteria numbered almost 10^6 per gram of herbage. The maximum number of LAB, about 10^9 per gram, occurred after 4 days in the silo.

Another important trend to notice in this experiment is the increase in clostridia early in the ensilage process, followed by a rapid decline as LAB became dominant. Also lactate fermenters were essentially absent. The optimum pH for clostridia development is 7.0 to 7.4 and therefore they cannot tolerate acid conditions. Also, clostridial activity is usually restricted if the forage crop has been wilted to 30% DM or more (McDonald, 1981).

Table 2.5. Classification of lactic acid bacteria important in silage.

HETEROFERMENTATIVE:
Cocci
Leuconostoc mesenteroides
Leuconostoc dextranicum
Leuconostoc cremoris
Rods
Lactobacillus brevis
Lactobacillus fermentum
Lactobacillus buchneri
Lactobacillus viridescens
HOMOFERMENTATIVE:
Cocci
Streptococcus faecalis
Streptococcus faecium
Pediococcus acidilactici
Pediococcus cerevisiae
Pediococcus pentosaceus
Rods
Lactobacillus plantarum
Lactobacillus curvatus
Lactobacillus casei
Lactobacillus coryniformis subsp. *coryniformis*

from McDonald, 1979

Table 2.6. Microbiological changes during fermentation of perennial ryegrass. Number of organisms per gram of fresh forage.

	Total Count	Lactic Acid Bacteria	Yeasts and Fungi	Proteolytic Clostridia	Lactate Fermenters	pH
Grass						
Uncut June 8, 8:00	1.2×10^3	100	<10	10^3	<10	6.2
Cut June 8, 8:05	2.2×10^6	366	30	10^3	<10	
Wilted June 9, 9:00	2.9×10^6	1.7×10^4	30	10^3	<10	
Wilted June 9, 15:00	4.5×10^6	1.1×10^5	<10	10^3	<10	
Chopped June 9, 16:00	4.6×10^6	7.2×10^5	<10	10^3	<10	6.2
Silage						
Day 1	6.5×10^8	1.0×10^7	373	10^4	<10	5.9
Day 2	2.8×10^8	1.3×10^7	26	10^4	<10	5.0
Day 3	1.7×10^9	7.3×10^6	46	10^5	<10	4.9
Day 4	5.2×10^8	8.5×10^8	4.5×10^3	10^3	<10	4.8
Day 8	3.2×10^8	3.9×10^8	4.6×10^4	10^2	<10	4.6
Day 23	2.0×10^8	0.4×10^8	1.4×10^5	<10	<10	4.3
Day 34	7.6×10^6	4.8×10^7	1.9×10^5	<10	<10	4.3
Day 71	9.0×10^7	8.0×10^7	10^2	<10	<10	4.2
Day 149	2.6×10^6	1.2×10^6	<10	<10	<10	4.2
Day 156	2.5×10^5	1.6×10^7	6.4×10^3	<10	<10	4.4

from Henderson et al, 1972

Langston and Bouma (1960a,b,c) determined the types and sequential changes of LAB in 30 orchardgrass and alfalfa silages. They studied 3,142 strains and associated them with silage quality, different cuttings and stages of fermentation. In these experiments, early bacterial flora consisted mainly of cocci. Most cocci, except for pediococci, disappeared a few days after ensiling. Among the cocci noted were Streptococcus faecalis, S. liquefaciens, Leuconostoc mesenteroides, and pediococci. In good quality silages, the dominant species of LAB were Lactobacillus brevis, L. plantarum, and pediococci. L. brevis was usually found in the later stages of fermentation. Poorer quality silages the dominant species included the three species mentioned above and L. casei. These researchers suggested that a number of interacting factors may be responsible for selecting certain organisms such as exposure of the forage mass to oxygen, increased temperature, amino acid imbalance, and carbohydrate availability.

Kroulik et al (1955) studied microbial populations during fermentation of both direct-cut and wilted alfalfa silage. They found that bacterial numbers increased more rapidly in the direct-cut (22.4% DM) forage than in the wilted (27.9% DM) alfalfa over the first few days of ensiling. Lactic acid bacteria predominated during the ensiling period in this experiment also.

Many researchers and commercial organizations have

examined the potential of selected LAB cultures that may exert some control over silage fermentation. Insufficient numbers of LAB on the fresh forage has been suggested as the reason for the inability of some silages to become stable (Wierenga, 1960). Also a more rapid acidification may serve to minimize plant respiration and consumption of WSC by competing aerobic bacteria early in the ensiling period (McDonald et al, 1968). Pitt et al (1985) and Neal and Thornley (1983) observed a direct relationship between initial bacterial concentration and the time to a rapid pH change. The rapid decrease in pH may be effected by the addition of a culture of efficient acid-producing LAB (Done, 1986).

2.7 Lactic Acid Bacterial Inoculants

In recent years a number of commercial microbial inoculants have become available as silage additives. Most of the present products are freeze-dried preparations of homofermentative LAB cultures. Homofermentative bacteria are preferred as they are more efficient in converting WSC to lactic acid than heterofermentative types (Done, 1986; Henderson, 1987). Common bacterial strains used include Lactobacillus plantarum and L. acidophilus with or without Pediococcus, and Streptococcus species to lower the pH initially.

Woolford and Sawczyc (1984a) subjected 21 strains of

homofermentative LAB to a range of tests in order to ascertain their suitability for use as inoculants for silage. Criteria they established for means of comparison were growth in the presence of competing organisms, ability to effect rapid acidification and acheive low pH in conditions simulating low and high ensiling potential. They also considered their ability to produce acids from sugars and their action against organic acids commonly found in forage crops, together with their tolerance of acid, elevated temperatures, and conditions of low moisture availability as in wilted forages. In addition, possible proteolytic and amylolytic properties were examined. None of the cultures satisfied all criteria, but three strains, Streptococcus durans, Lactobacillus acidophilus, and L. plantarum had a greater potential than the others. In a subsequent experiment, Woolford and Sawczyc (1984b) found that none of these cultures or culture mixtures had any notable influence on microbial development, the rate of acidification, or promotion of the homolactic fermentation in ensiled perennial ryegrass (20% DM) or red clover (16% DM). Inoculation in fact exacerbated the loss of insoluble nitrogen and deamination.

Results of silage inoculation experiments in a number of recent reviews (McDonald, 1981; Ehle and Goodrich, 1982; Woolford, 1984; Done, 1986; Kung et al, 1987) were highly variable, ranging from no response to significant

improvements in silage quality and animal performance. Ehle and Goodrich (1982), in a summary of silage inoculation trials using a range of microbial products and forages, found no consistent response in microbial numbers, pH, temperature, residual soluble carbohydrates, dry matter or protein recovery, dry matter or protein digestibility, feed intake, or animal weight gains due to inoculation. Inoculation appeared to improve stability upon exposure to air and enhance degradation of amino acids, peptides, and nucleic acids to ammonia. Pitt and Leibensperger (1987) developed a model of the ensilage process to study the potential benefits of silage inoculants and concluded that increased acid-tolerance of the inoculant has more effect than homofermentation. Also, these researchers suggest that inoculation levels of at least 10^5 cfu/g is necessary to induce consistent quality increases.

When some variables such as forage type and inoculation culture are controlled, as in individual experiments, benefits can often be observed. Ely et al (1981, 1982) and Moon et al (1980) have shown microbial and fermentation responses to inoculation of wheat, alfalfa, and corn silages with additions of Lactobacillus species although no improvement in dry matter recovery was evident. Kung et al (1984) observed improved fermentations in inoculated (Lactobacillus plantarum, L. brevis, and Pediococcus acidilactici mixed culture) compared with control alfalfa

silages at 50 and 60% DM. Lindgren et al (1983) showed more rapid acid production, prevention of growth of competing microbes, and decreased ensiling losses with addition of a Pediococcus acidilactici and Lactobacillus plantarum inocula to red clover (17% DM). Shockey et al (1988) showed increased LAB proliferation but no chemical advantages when poor quality alfalfa was inoculated in an attempt to improve its ensiling characteristics. Inoculation of unwilted grass has been shown to increase digestibility of dry matter, energy and nitrogen over control silage in lactating cattle (Gordon, 1989).

A factor to consider in any evaluation of inoculants for silage is the availability of substrate. An inoculant has little chance to influence the fermentation if there is insufficient substrate for it to metabolize. Several researchers have suggested that failures with inoculated silages may have been for this reason (Lesins and Schultz, 1968; Ohyama et al, 1975; Seale et al, 1986). Lesins and Schultz (1968) acheived a lower final pH and a greater titratable acidity was reached when a mixture of Lactobacillus plantarum and Pediococcus sp. was added to sedge than an uninoculated control, but the same effect with alfalfa could only be attained when the forage was also treated with 1% sugar. Possibly, a relatively low sugar content and high buffering capacity contributed to the effects shown by the addition of sugar to the alfalfa silage.

An inoculant may only assist the ensilage of crops low in sugar, where an efficient utilization of substrate is essential to ensure preservation. McDonald et al (1964) obtained a good quality silage from ryegrass containing 16% WSC (DM basis) regardless of the addition of an inoculum comprised of 8 strains of lactobacilli, whereas some benefit was observed in silage made from orchardgrass having only 4% WSC. This same group in 1965 showed that an inoculant may improve a crop which, in spite of having a relatively high content of fermentable sugars, is difficult to ensile (ie. legumes). Ohyama et al (1973, 1975) have also observed the synergistic effect of an inoculant and sugar in grass silage.

The criteria which an organism should satisfy, in order to be regarded as having potential as a silage inoculant have been outlined by several researchers (McDonald, 1981; Woolford, 1984; Woolford and Sawczyc, 1984). However, the number of organisms that actually do satisfy these criteria is very small. The situation is further complicated by the constant modifications of commercial inoculation products in terms of active ingredients, inert ingredients, substrates, and application rates. Some manufacturers introduce new products nearly every season. With this rapid rate of change, proper evaluation of all new formulations is impossible (Done, 1986). McCullough (1975) described desirable characteristics of any silage additive as:

1) the cost of the additive must be less than the cost

of the silage DM that would be lost without the additive,

2) addition of the additive must result in a more efficient fermentation than occurs naturally,
3) the additive should produce a silage with a greater quantity of digestible energy and/or protein than untreated silage.

2.8 Aerobic Stability of Silage

When a silo is opened and for the duration of the feedout period, the exposed surface of the silage mass becomes an aerobic environment. Under these conditions aerobic organisms, which have remained dormant in the absence of oxygen, multiply and cause a deterioration of the silage. This deterioration of silage is undesirable as high nutrient losses are associated with it (Mo and Fyrileiv, 1979), and substantial loss of dry matter can occur as well (Honig and Woolford, 1979). The deterioration process is often referred to as a "secondary fermentation." This term is inappropriate as fermentation is always an anaerobic process and this deterioration is aerobic.

Exposure to air is the most important factor in silage preservation since it can influence the ensiling process at any point from harvesting the crop to feeding the silage (Woolford, 1984). Susceptibility to aerobic deterioration

can be increased by excessive wilting of the ensiled crop (McDonald et al, 1968; Ruxton et al, 1975), slow silo filling, poor consolidation of the forage (Lancaster and McNaughton, 1960; McDonald et al, 1965; Henderson et al, 1979), delayed or poor sealing of the silo (Henderson and McDonald, 1975) and a slow rate of silage feedout (Honig and Woolford, 1979). All of these factors promote the persistence of aerobic microorganisms.

Woolford and Cook (1978) treated corn silage with either antimycotic or antibacterial antibiotics and inhibited deterioration which suggests that bacteria rather than yeasts and molds were responsible for early silage deterioration. The principal bacteria involved seemed to be spore formers and were both saccharolytic and proteolytic. Lactic acid bacteria may also be responsible for some deterioration as some species can degrade lactic acid (Honig and Woolford, 1979; Condon, 1987). Bacteria may play a larger role in deterioration of corn silage than in grass silages (Ohyama et al, 1979).

Ohyama and Hara (1975) noted the importance of yeasts and fungi in aerobic deterioration. The yeasts involved were those belonging to the genera Pichia, Hansenula, Candida, and Saccharomyces (Ohyama and Hara, 1975; Honig and Woolford, 1979; Moon and Ely, 1979). Molds have also been found to be important in aerobic deterioration (Ohyama et al, 1977). The growth of molds often follows that of yeasts which is

reflected in the appearance of two thermal peaks during deterioration. The first rise in temperature during the first two or three days is caused by yeasts, while a second peak occurring three to four days later is a result of mold proliferation (Yamashita and Yamakazi, 1975). A large number of mold species have been detected in deteriorated silages. Some common genera observed in deteriorated corn silage were Mucor, Geotrichum, Aspergillus, and Penicillium (Britt et al, 1975).

Lactic and acetic acids, as well as soluble carbohydrates are the main substrates for most microorganisms involved in silage deterioration (Ruxton et al, 1975). In silages in which fermentation has been restricted by the addition of chemical additives or by excessive wilting, glucose, fructose, and some pentoses will be the main energy sources, whereas in untreated well preserved silage, lactic acid will likely be the primary substrate. The oxidation of these nutrients results in the production of CO_2, H_2O and heat. This loss of carbon atoms represents a loss of dry matter and energy (McDonald et al, 1973). Rees (1982) noticed a highly significant correlation (r=0.965) between DM loss and temperature increase during aerobic deterioration of grass silage under laboratory conditions and explained further that most if not all DM loss was accounted for by the loss of residual water-soluble carbohydrate. A mathematical model was developed which predicted forage dry matter losses

due to oxygen infiltration (Pitt, 1986). The model gave accurate predictions of dry matter losses dependent on forage density, dry matter content, position in the silo, and permeability of the forage mass, when compared with experiment.

In the early stages of deterioration, fiber, crude protein and ash content increase at the expense of soluble carbohydrate, while in later stages fiber and protein are often degraded (Honig and Woolford, 1979). The pH increases as a result of utilization of lactic and acetic acids and the release of ammonia from catabolism and deamination of amino acids (Ohyama and McDonald, 1975; Moon et al, 1980).

Ohyama et al (1979) attempted to identify the chemical characteristics of grass silages which are particularly susceptible to aerobic deterioration. Silages high in DM allowed greater yeast activity during a 7 day deterioration than low DM silages (DM range 14 - 73%) and silages with high butyric acid content were stable. Henderson et al (1979) also showed that the main products of clostridial activity, butyric acid and ammonia, were positively correlated with aerobic stability. Higher volatile fatty acids have been shown to inhibit the growth of microorganisms (McDonald and Henderson, 1974; Woolford, 1975). Clostridia also produce isobutyric and isovaleric acids from the catabolism of amino acids (see Table 2.5) which can have a beneficial effect in preserving silages exposed to air (Ohyama and McDonald,

1975). Ohyama et al (1979) found neither lactic acid or WSC to be correlated with rate of aerobic deterioration in grass silages. Henderson et al (1979) also observed this poor relationship.

The most obvious and effective method of preventing aerobic deterioration in silage is to feed it to animals as soon as it is exposed to oxygen. This is often not a practical solution however. Several researchers have examined the potential of addition of higher fatty acids, particularly isovaleric and caproic acids, as solutions to the deterioration problem. These acids have been shown to inhibit the organisms responsible for aerobic deterioration of silage in a number of laboratory studies (Ohyama and McDonald, 1975; Woolford, 1975; Ohyama et al, 1977). These additives probably do not completely prevent deterioration, but only delay microbial activity (McDonald, 1981).

The effect of microbial inoculation on aerobic stability of silages has shown mixed results. Moon et al (1980) observed aerobic deterioration in alfalfa, wheat and corn silages ensiled with addition of Lactobacillus acidophilus and a Candida species. The treated silages increased in pH and temperature more rapidly during the first 48 hours of exposure than control silages. Theuninck et al (1981) also showed decreased stability in corn silage treated at ensiling with LAB. Bolsen et al (1980a, b, 1981, 1982) showed contradicting results in four laboratory experiments

comparing the aerobic stability of alfalfa silage and corn silage with and without the addition of inoculant.

2.9 Feeding Value of Silages

Silage is an excellent forage and is commonly used in both dairy and beef cattle feeding systems. Many experiments have been performed to determine the voluntary intake of silages and to measure growth and production in animals given silage diets in comparison with hay diets (Gordon et al, 1961; Thomas et al, 1969; Merrill, 1971; Clark et al, 1973; Waldo, 1977; Stallings et al, 1979). These trials have indicated that proportions of hay, haylage, or corn silage in the ration can be altered without altering production when there is sufficient intake of a balanced diet. Alfalfa silage is a satisfactory alternative for a portion of hay and corn silage (Thomas, 1980).

Direct-cut silages and occasionally wilted silages have been shown to cause lowered DM intake and often lowered milk production and growth as a result of decreased DM intake (Gordon et al, 1961; Campling, 1966; Thomas et al, 1961; Savoie et al, 1986). Some research has shown that although direct-cut silage intake is lower as compared with wilted silage or hay diets, production and growth are not altered. This is usually due to an increased digestibility and more efficient use of the unwilted silage DM (Cottyn et al, 1985;

Gordon, 1981, 1986, and 1987; Haigh and Parker, 1985; Henderson, 1987).

The intake of silages has been correlated with silage pH, ammonia, lactic acid, acetic acid, and total acid concentrations suggesting that fermentation end products may be involved in intake regulation (Hutchinson and Wilkins, 1971; Hutchinson et al, 1971; Wilkins et al, 1971; Wilkinson et al, 1976; Shaver et al, 1985). Decreased intake of alfalfa silage relative to fresh alfalfa has been observed (Flores et al, 1986). Buchanan-Smith and Phillip (1986) monitored food intake in sheep following intraruminal infusions of alfalfa silage extracts with particular interest in organic acids and protein degradation products. They observed that infusions of extracts from several different silages, gamma-amino butyric acid, alpha-amino butyric acid, and mixtures of putrescine, cadaverine, histamine, and tyramine all depressed DM intake for up to 4 hours relative to iso-osmotic saline infusions. These researchers concluded that many different soluble constituents in silage can affect ingestive behavior through a post-ingestive mechanism. Studies of the effects of individual fermentation products have not allowed clear cut conclusions about their influence on appetite or their mechanisms of action (Thomas et al, 1980). Attempts to neutralize the free acids in silage with sodium bicarbonate, ammonia, or a similar agent has resulted in 10 - 20% increases in DM intake in both lactating and

growing cattle and in sheep (McLeod and Wilkins, 1970; McLeod et al, 1970; Thomas and Wilkinson, 1975; Wilkins, 1974).

The effect of addition of microbial inocula on the feed value of silage has been investigated by several researchers. Ehle and Goodrich (1982) summarized several inoculation trials and showed that feed intake or dry matter and crude protein digestibilities were not consistently affected by inoculation. Fiber digestibility and nitrogen retention were depressed in animals fed inoculated silages as compared with those fed control silages. Rate of gain in growing animals was not altered by inoculation, but feed conversion was less efficient in the animals fed diets containing inoculated silages. Kung et al (1987) found that in one experiment, inoculation did not affect intake but improved milk production in dairy cows fed inoculated low moisture alfalfa silage relative to cows fed control silage, while in a repeat experiment the following year, no differences in production or intake existed. In both experiments, silage quality was judged to be improved by inocula addition. In a growth trial with yearling beef cattle, both a liquid and a dry inoculant were found to improve both dry matter intake and daily gains on ryegrass silage diets although no differences in silage quality could be detected (Appleton and Done, 1987). Satter et al (1987) summarized 8 lactation studies designed to evaluate the effects of several different inoculants and enzyme additives on dairy cattle performance. In only one

experiment did inoculation improve dry matter intake or milk production. When measured, body weight gain of lactating cows consuming treated silage was greater than control animal gains in 3 of 5 trials. In these trials inoculation consistently improved pH decline and lactic acid production but did not affect DM recovery.

3.0 MATERIALS AND METHODS

3.1 Wilted Alfalfa Forage Ensiled With the Addition of Two Microbial Inoculants and Sucrose in Laboratory Silos.

In June and in July of 1985, two laboratory trials were performed to monitor the effects of a mixed lactic acid bacterial inoculant on fermentation of alfalfa forage ensiled in quart-jar silos over a period of 40 days. The alfalfa was cut and wilted in the field to 35 - 37% dry matter (DM), and chopped with a field chopper into a silage wagon. Subsamples (n=12) of this forage were placed in large plastic bags and transported to the laboratory.

3.1.1 Silo Filling and Sampling

The 12 subsamples, numbered 1 through 12, served as replicates for 4 treatments and were treated in numerical order. Replicate/treatment assignments are in Table 3.1. The forage was treated in such an order so as to minimize pre-treatment fermentation effects. Initial treatment means should be unaffected by time if such a procedure is used. Each forage subsample was spread out on a plastic sheet and mixed thoroughly by hand. The treatment was applied to the

forage and the forage was mixed again. Gloves were worn and plastic sheets were changed to reduce contamination of the alfalfa forage. Tared quart-jar silos were filled with 530 ± 10 grams of wet forage and were randomized as to date of opening. The jars were packed tightly and sealed with lids equipped with bunsen valves. The silos were stored at room temperature in the dark. At the designated times (0, 1, 2, 4, 8, 15, or 40 days), silos were weighed, emptied and their contents mixed and sampled. Samples were taken for dry matter determination, microbial counts, and another was frozen and stored at -20°C until later chemical analysis.

Table 3.1 Replicate/treatment assignments for experiment 3.1.

Treatment	Abbr.[1]	Replic. Nos.
Control	C	1, 5, 9
Inocula	I	2, 6, 10
Sucrose	S	3, 7, 11
Inocula + Sucrose	IS	4, 8, 12

1 Abbreviated treatment identifications

The inocula (I) was supplied by Ceva Laboratories, Inc., Overland Park, Kansas; and contained Lactobacillus plantarum, L. caseii, L. coryniformus, and amyloglucosidase in a fish meal carrier. This treatment was applied at a rate of 0.05%

of wet forage weight, recommended by the supplier, to supply > 10^5 colony forming units (cfu) per gram of fresh material (2.72 g in 30 ml distilled water per 12 lb. subsample). Sucrose was applied at a rate of 2% of wet forage in an attempt to determine if additional sucrose would stimulate microbial growth or change ensiling characteristics.

3.1.2 Microbial Numbers

Total and lactic acid bacteria were enumerated for all treatments (composite samples) at days 0, 1, 2, 4, 8, and 40 immediately after silos were opened. Two gram samples were blended in 198 ml of saline for one minute at low speed and serially diluted. A Brain Heart Infusion Agar (Difco 0418-01-5) was used for counting the total bacteria population and Rogosa SL Agar (Difco 0480-01-8) was used for enumeration of the Lactobacillus population. Triplicate plates were poured for each of 5 dilutions for each media. A set of plates was incubated aerobically at 37°C while an identical set was also incubated under 10% CO_2 at 37°C.

3.2 Fermentation and Nutritive Qualities of Alfalfa Silage Ensiled With or Without Addition of a Microbial Inocula in Large Silos.

3.2.1 Silo Filling

In July of 1985, 85 tons of second-cut alfalfa wilted to 38-40% DM was harvested with a field chopper and blown in an alternating load sequence into each of two 4.3 x 18.3 m upright cement stave silos over a period of 3 days. Forage blown into one silo was inoculated at the blower with the same microbial product from Ceva Laboratories, Inc., Overland Park, Kansas as was used in Experiment 3.1. The dry inocula was applied from a can with a perforated bottom at a rate recommended by the manufacturer to supply > 10^5 cfu per gram of wet forage. The proper amount of inocula was measured out according to the weight of each load of forage. The other silo was not treated and served as a control. Each incoming load of chopped alfalfa forage was weighed and was sampled while unloading. Load samples were combined into composites, frozen and stored at -20^{o}C until later subsampling and chemical analysis.

At three points during filling, the ensiled forage was leveled and samples of the forage in the silo were placed in numbered nylon mesh bags. The material in the bags was weighed, sampled and returned to the silo and buried. The contents of the bags, obtained as the silo was emptied, were

later used to estimate dry matter recovery.

3.2.2 Silo Temperatures

At these same three points during silo filling, thermocouples (copper/constantan) were placed in the silos through small holes drilled in the silo door to monitor the temperature of the ensiled mass during fermentation. Excess wire was left on the inside of each silo door to allow for settling of the silo contents. Three thermocouples were placed at each of the three silo levels; at the center and near the wall of the silo, and at a point midway between the center and the wall, near the buried bags. Silo temperatures were monitored daily using a potentiometer (Leeds Northrup Co., #8694-2, Philadelphia, PA) for the first thirty days of ensiling.

3.2.3 Silo Sampling

On days 1, 3, 5, and 9 post-ensiling, core samples of the silages were taken by boring with a drill and sample tube through capped ports located on door 2 of each silo. During the feedout period (Nov. 1985 through March 1986), all material removed from the silos was weighed and recorded as silage or spoilage. Silages were sampled three times per week and the samples combined by one week periods into composites, frozen, stored at -20^{o}C and later analyzed.

3.2.4 Microbial Numbers

Total and lactic acid bacteria were enumerated for both silos at days 0 and 1 immediately after sampling. Two gram samples were blended in 198 ml of saline for one minute at low speed and serially diluted. A Brain Heart Infusion Agar (Difco 0418-01-5) was used for counting the total bacteria population and Rogosa SL Agar (Difco 0480-01-8) was used for enumeration of the Lactobacillus population. Triplicate plates were poured for each of 5 dilutions for each media. A set of plates was incubated aerobically at 37°C while an identical set was also incubated under 10% CO_2 at 37°C.

3.2.5 Aerobic Stability

Aerobic stability of both treated and untreated haylage was studied during weeks 9 and 12 of the feedout period. Two kg of haylage was placed in 10 quart styrofoam containers, slightly compacted and left at room temperature. The silage in these open containers was then allowed to deteriorate at room temperature for 10 days. Temperatures of the deteriorating material were monitored twice daily and the containers were weighed and sampled at the beginning and end of the 10 day period to estimate weight and dry matter losses. Duplicate samples were taken from each silo. Temperature change served as an index of aerobic stability.

3.3 Responses of Dairy Cows to Alfalfa Silage Ensiled With and Without the Addition of Microbial Inocula.

Thirty-one Holstein cows were used to compare lactational performance when fed a diet containing control or inoculated alfalfa haylage. The 70 day trial was a split-plot design. Primiparous and multiparous cows were paired according to lactation number, days in milk, and milk production during a preliminary period and randomly assigned to a treatment group.

A 60:40 haylage:concentrate total mixed ration (TMR) was fed for 14 days prior to switching to the experimental diet. The haylage used in the preliminary diet was from a source other than the two experimental silos. After a week on this diet, cows decreased markedly in milk yield because the ration was insufficient in energy. For this reason, a 50:50 experimental diet was fed from this time on. Data collected during the preliminary period were used as covariates in the statistical analysis. Cows were fed the TMR twice daily ad libitum (allowing 10% orts). Dietary ingredients and proportions are shown in Tables 3.2 and 3.3. Feed refusals were weighed daily and were not sampled. Cows were milked twice per day and milk weights recorded. Composite (am and pm) milk samples were collected each week and analyzed for fat and protein (Michigan DHI, East Lansing, MI). Cows were weighed every 2 weeks on 2 consecutive days so that weight

changes could be estimated. Silage and TMR samples were collected 3 times per week and combined into weekly composites.

Table 3.2 Feed Ingredients and their Proportions in the Preliminary and experimental total mixed rations (TMR) fed to cows during the lactational performance trial.

Ingredient	Proportion of ingredient		
	Wet basis	Dry basis	%DM
	%	%	%
PRELIMINARY 60:40 TMR			
Alfalfa haylage	63.2	58.4	60.0
Ground high moisture shelled corn	34.2	38.0	72.0
44% Protein/Mineral Supplement	2.2	3.1	89.0
Monophosphate	0.2	0.3	99.0
Trace mineral salt	0.2	0.2	99.0
EXPERIMENTAL 50:50 TMR			
Alfalfa haylage	61.3	50.3	47.0
Ground high moisture shelled corn	35.6	44.8	72.0
44% Protein/Mineral Supplement	2.8	4.4	89.0
Monophosphate	0.2	0.3	99.0
Trace mineral salt	0.1	0.2	99.0

Table 3.3 Chemical analysis of 44% supplement fed to cows during lactational performance trial. Figures are given on a DM basis (except DM)

DM	92.40 %	Na	0.86 %
CP	48.60 %	NEl	1.70 Mcal/kg
NDF	18.60 %	NEm	1.69 Mcal/kg
P	1.67 %	NEg	1.10 Mcal/kg
K	1.40 %	Mn	253 ppm
Ca	3.46 %	Fe	1064 ppm
Mg	0.90 %	Cu	167 ppm
S	0.30 %	Zn	242 ppm

Analysis performed by Ohio Agricultural Research and Developmental Center, Wooster, Ohio

3.4 Influence of a Prefermented Microbial Inocula, Sodium Bentonite and Glucose Addition on Fermentation of Direct-cut Alfalfa Forage Ensiled in Laboratory Silos.

In September of 1986, a laboratory trial was performed to monitor the fermentation characteristics over time of direct-cut alfalfa forage ensiled in quart-jar silos and the effects of inoculation with a prefermented mixed lactic acid bacterial culture. Fourth cut alfalfa at 19% DM was field chopped into a silage wagon, subsampled (n=15) and transported to the laboratory in large plastic bags.

3.4.1 Silo Filling and Sampling

The 15 subsamples, numbered 1 through 15, were each considered a replicate of one of the 5 treatments listed in Table 3.4. Subsamples were treated in numerical order using the same procedures as in experiment 3.1. Such a procedure minimizes pretreatment fermentation effects and initial treatment means should be similar.

An inoculant was applied in an aqueous solution at a rate recommended by the supplier to add $>10^5$ cfu per gram of wet forage. The inoculant used was "H/M Plus Inoculant" (Medipharm, U.S.A. manufactured for Triple F Feeds, Des Moines, IA) containing Streptococcus faecium, Lactobacillus plantarum, and Pediococcus sp. This inoculant was

prefermented in distilled water at room temperature prior to its use to allow microbes to multiply. A 280 g package of inocula, containing $2x10^9$ cfu/g, was mixed with 10 liters of distilled water and allowed to ferment for 42 hours. This mixture was then applied at a rate of 10 ml per 20 lb. forage subsample. Dextrose was applied at a rate of 2% of wet forage by weight and Na bentonite was applied at a rate of 1%.

Seven sets of silo jars were prepared for each replicate to be opened on days 0, 1, 2, 4, 8, 16, and 40 of ensiling. Laboratory silos, equipped with bunsen valves, were filled with 750 ± 10 grams of wet forage and randomized as to date of opening. Jars were stored at room temperature in the dark. After one day of fermentation, any silos leaking fluids or foam from the valve were noted. At the designated times, silos were weighed, emptied, and their contents mixed and sampled. One sample was taken for a dry matter determination, one was taken for determination of microbial numbers, and the remainder was frozen and stored at $-20^{o}C$ for later chemical analysis.

Table 3.4 Replicate/treatment assignments for experiment 3.4.

Treatment	Abbr.[1]	Replicates
Control	C	1, 6, 11
Inocula	I	2, 7, 12
Na Bentonite	B	3, 8, 13
Inocula + Bentonite	IB	4, 9, 14
Inocula + Bentonite + Glucose	IBG	5, 10, 15

1 Abbreviated treatment identifications

3.4.2 Microbial Numbers

Lactic acid bacteria were enumerated for all treatments at days 0, 1, 2, 4, 8, 16, and 40 immediately after silos were opened. Triplicate silo samples were combined for each treatment for bacteria counts so only one count was made for each treatment. One hundred gram samples were blended in 1000 ml of distilled water for twenty seconds at low speed and serially diluted in a 0.1% peptone solution. LBS Agar (BBL Microbiology Systems #11327, Becton Dickinson and Co. Cockeysville, MD) was used for counting the Lactobacillus population. Duplicate plates were poured for each of 12 dilutions for each media. Plates were incubated aerobically at 37°C.

3.5 Preparation and Analysis of Samples

3.5.1 Dry Matter Determinations

Dry matter content was determined on aliquots of forage and silage samples by drying at 80°C in a forced air oven for 24 hours. Samples for fiber analyses were air-dried at room temperature by spreading out 200-300 g of forage on a tray for 48 to 72 hours. Trays were placed under an exhaust hood to facilitate drying. Samples dried at room temperature were ground in a Wiley mill and passed through a 1mm screen and stored in plastic at room temperature. Dry matter content of these air-dried samples was then determined by drying in an 80°C forced air oven for 24 hours.

3.5.2 Water Extract Preparations

Water-soluble components of forage and silage samples were prepared for analysis by homogenizing 15 g of wet sample with 100 ml distilled water in a Sorvall Omnimixer (Ivan Sorvall, Inc., Newton, CT) for 3 minutes. The homogenizing cup was immersed in ice to minimize heating of the homogenate. The contents of the cup were then strained through 2 layers of cheesecloth and centrifuged for 15 minutes at 20,000 x gravity. The extract was analyzed for pH (model 801 ionalyzer, Orion Research, Cambridge, MA) with a combination electrode (Corning X-EL no. 476193, Medfield,

MA). To inhibit mold growth during storage, two or more grains of thymol were added to the supernatant which was then frozen and stored until later analyses for water-soluble carbohydrate, water-soluble nitrogen, lactic acid, and buffering capacity.

3.5.3 Nitrogen Determinations

Total and water-soluble nitrogen were determined by the Kjeldahl procedure (A.O.A.C., 1975) using duplicate 3 g aliquots of fresh forage and silage or 10 ml aliquots of water extract, respectively. Aliquots were added to a 500 ml Pyrex boiling flask together with 25 ml of 98% H_2SO_4 and approximately 8-9 g $CuSO_4/K_2SO_4$ mixture. Samples were digested for approximately 1 hour. Flasks were then cooled and if necessary, left until the next day for distillation. Before distillation, 250 ml distilled water, 60 ml 50% NaOH, and 2-3 pieces of mossy zinc were added to each flask. Distillate was collected in a 4% boric acid solution containing a methylene blue/methylene red pH indicator. Titration of forage or silage samples distilates was done with 0.1 N HCl, while 0.25 N HCl was used for water extract samples distillates.

3.5.4 Water-soluble Carbohydrate Determination

Water-soluble carbohydrate was determined using a colorimetric assay by Dubois et al (1951). Duplicate aliquots of water extract were added to 16x125 mm test tubes with 0.15 ml 80% phenol and 5 ml 98% H_2SO_4 and mixed with a Vortex mixer (Scientific Industries, Queens, NY). This mixture was allowed to cool to room temperature in a water bath and the optical density was read with a spectrophotometer (Spectronic 21, Bausch and Lomb, Rochester, NY) at 470 nm. The aliquot of water extract used varied from 0.01 ml to 0.05 ml depending on the concentration of water-soluble carbohydrate expected. Water-soluble carbohydrate was then quantified by using a standard curve generated from a set of 50:50 glucose:xylose solutions varying in concentration from 0 to 150 ug/ml.

3.5.5 Lactic Acid Determination

Lactic acid content of forage and silage was determined in duplicate using a colorimetric assay by Barker and Summerson (1941). Volume of water extract used varied from 0.01 ml to 0.05 ml dependent on the concentration of lactic acid expected. The appropriate amount of extract was added to a large centrifuge tube with 9 ml distilled water, 1 ml 20% $CuSO_4/5H_2O$, and 1 g $Ca(OH)_2$. The tubes were then mixed with a Vortex mixer and allowed to stand for 30 minutes. Tubes were subsequently centrifuged at 19,000 x gravity for

10 minutes. A 1 ml aliquot of the supernatant was then added to a 16x125mm test tube with 6 ml 98% H_2SO_4 and 0.05 ml 4% $CuSO_4$ and mixed with a Vortex mixer. The tubes were then placed in a 100°C water bath for 5 minutes and cooled to room temperature with a cool water bath. At this point, 0.05 ml p-phenylphenol reagent (1.5 g p-hydroxydiphenyl in 10 ml 5% NaOH brought up to 100 ml with distilled water) was added while mixing each tube. All tubes were then placed in a 32°C water bath for approximately 45 minutes. Tubes were then boiled vigorously for 90 seconds and cooled to room temperature. Optical density of each tube was then read with a spectrophotometer (Spectronic 21, Bausch and Lomb, Rochester, NY) at 560 nm. Lactic acid was quantified by regression on a standard curve generated using a set of standard concentrations of an analytical grade lactic acid solution.

3.5.6 Volatile Fatty Acid Analyses

Volatile fatty acids (acetic, propionic, and butyric) were quantified using a gas chromatograph (Hewlett Packard model 5730A with a flame ionization detector, a model 5840A integrating terminal, and a model 7671A automatic sampler, Avondale, PA) fitted with a stainless steel column (6'x1/8" 10% SP-1200, 1% H_3PO_4, 80/100 Chromosorb WAW, Supelco MR56559, Supelco, Inc., Bellefonte, PA). Separate water

extracts were prepared for VFA analyses. Ninety grams of wet silage was homogenized in 300 ml distilled H_2O for 1 minute in a Waring blender (model CB, Waring Procucts Corp., New York, NY). Five ml aliquots of this water extract from silage samples were acidified with 1 ml 50% H_3PO_4 (w/v) and centrifuged at 20,000 x gravity for 20 minutes. Approximately 2 ml of the supernatant was placed in a small vial and frozen until analyzed. A 0.002 ml sample of this supernatant was injected into the gas chromatograph. Helium was used as the carrier gas and the flow rate was 20 ml per minute. Column temperature was 130^{o}C while the injection port and detector temperatures were 200^{o}C. Volatile fatty acid concentrations were calculated by comparing peak areas with those of analytical grade acids in a standard solution.

3.5.7 Buffering Capacity Determination

Buffering capacity was determined using the method of Playne and McDonald (1966). Fifty ml aliquots of water extracts were titrated to pH 3 with 0.1N HCl to release bicarbonate as CO_2, and then to pH 6 with 0.1N NaOH. The volume of 0.1N NaOH used to change the pH from 4 to 6 was recorded. These steps were repeated on a distilled water blank. Buffering capacity was then expressed as milliequivalents of alkali necessary to change the pH from 4 to 6 per 100 g of DM after correction for the water blank.

3.5.8 Acid Detergent Fiber and Ash Determinations

Acid detergent fiber analysis was performed according to the Goering and VanSoest (1970) procedure. Air-dried samples were ground in a Wiley mill and passed through a 1 mm screen. Approximately 1 g of ground sample was boiled in 100 ml acid detergent solution for 1 hour and filtered through a tared Gooch crucible (Pyrex glass, 50 ml capacity, coarse porosity, Fisher Scientific, Silver Springs, MD, #8-237). Residue was rinsed with boiling water and acetone to remove reagent. Crucibles were then dried at 100^{o}C overnight, hot-weighed and acid detergent fiber was calculated (ADF in this text is expresed as ADF DM on a DM basis). All crucibles were subsequently placed in a 500^{o}C muffle furnace for 5 hours to estimate residual ash. Ground samples were also placed directly in a 500^{o}C muffle furnace for 5 hours to determine total ash.

3.6 Statistical Analyses

Statistical analysis of fermentation parameters for laboratory silo experiments 3.1 and 3.4 was performed using a two factor completely random design (treatment x time) with designed orthogonal contrasts. Means within treatment across time, and means within time across treatments were compared using Tukey's Honestly Significant Difference.

Experiment 3.2 was not subjected to statistical comparison as no degrees of freedom for a treatment effect are available. The two treatments were applied to only one silo each, thus any treatment effect is confounded with silos or replicates. Animal performance data from experiment 3.3 were analyzed by repeat measurement design with blocking of subject and use of covariate measurements from a pre-trial period. The design of experiment 3.3 has the same fault as experiment 3.2 in that a treatment effect truly cannot be separated from silo variation. However, the treatments have been compared regardless of this fact.

4.0 RESULTS AND DISCUSSION

4.1 Experiment 3.1: Wilted Alfalfa Forage Ensiled With the Addition of a Microbial Inoculant and Sucrose in Laboratory Silos

The effects of inoculation of wilted alfalfa with LAB and of the addition of sucrose on fermentation characterstics are described in Tables 4.1.1 through 4.1.16. Silages in all silos were judged to be of good quality upon opening. Silage was appraised visually and olfactively and at no point were any silages characterized as spoiled or poorly preserved. Table 4.1.16 is a summary of contrasts for each of the time points silos were opened. The two trials were significantly different for most parameters on most days (Table 4.1.16). Trial 1 used first cutting alfalfa while Trial 2 used second.

Percentage dry matter (DM) content of forages for all silos are shown in Table 4.1.1. Dry matter content of fresh wilted, chopped alfalfa averaged 35.74% for all treatments. The addition of sucrose increased the DM content of silages. (See also Table 4.1.16) This effect can be noted throughout the experiment. Addition of sucrose was an addition of dry matter to the alfalfa forage. Effects of inocula on DM content was minimal and insignificant. Mean DM across treatments show a general tendency to decrease throughout the

40 day experiment. Average DM content at day 2 was 35.02%, a decrease of 0.72 percentage points from the original day 0 forage. A slight decrease in DM could arise from the production of water in the respiration of sugars. After day 2, DM remained relatively constant. This decrease could also be due to the volatilization of a different array of compounds in the forage on day 0 compared with days 2 through 40 during oven drying at 80^{o}C. Dry matter determination of fermented feeds is most accurately and precisely accomplished through the methods designed to measure only water such as toluene distillation or the Karl Fischer titration (Van Soest and Robertson, 1985).

The pH of all silages decreased rapidly from an original value of 5.86 to 4.64 on day 2 and finally to 4.30 on day 40. See Table 4.1.2. The pH values on day 0 were similar for all forages. Sucrose treated silos had reached a lower pH on days 2 through 40 than non-sucrose treated silos ($p<.001$, see Table 4.1.16). Inoculation may have caused a more rapid initial fermentation indicated by a lower pH on day 1 ($p<.025$). Average day 40 pH values were 4.29 for inoculated silos, 4.32 for non-inoculated silos, 4.23 for sucrose treated silos, and 4.38 for non-sucrose treated silos. Sucrose addition significantly reduced pH on days 2 through 40 and inoculation significantly reduced pH only on day 1. The pH of inoculated silage was lower than control on days 2, 4, 8, and 40 but differences were small and not statistically

significant. When sucrose was added, the pH was not further altered by inoculation. These results indicate that sugar content of legume forages may be very important for successful ensiling.

Lactic acid (LA) concentrations for silages are shown in Tables 4.1.3 and 4.1.16. Day 0 LA contents were low, averaging 0.06% of the DM. This component increased with time for all treatments and in both trials, reaching an average concentration across treatments of 6.57% of DM on day 40. Inoculation resulted in more LA on day 2 ($p<.05$) and less lactic acid on day 4 ($p<.10$) than non-inoculated forages. Final LA concentrations on day 40 were greatest for the inoculated silage but differences were not statistically significant ($p<.25$). Addition of sucrose did not produce a change in final LA content relative to non-sucrose treated silage.

Original water-soluble carbohydrate (WSC) concentrations as a percentage of DM were relatively high for alfalfa. Day 0 levels were 7.28% and increased to 12.65% after sucrose addition. See Tables 4.1.4 and 4.1.16. The WSC fraction in the sucrose treated silos remained significantly greater than that of non-sucrose treated silos throughout the experiment ($p<.001$). The added WSC was rapidly fermented, however LA was not the end product of this fermentation as LA levels were not affected by sucrose addition. Inoculation resulted in significantly less WSC on day 1 than non-inoculated

Table 4.1.1 Experiment 3.1. Dry matter response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

DRY MATTER (%):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	35.17^{i}	35.33^{ij}	36.47^{k}	35.97^{jk}	^{A}35.74	0.226
1	35.01	34.62	35.51	35.52	^{AB}35.17	0.315
2	34.56	34.65	35.76	35.10	^{B}35.02	0.435
4	35.04	34.67	35.53	35.41	^{AB}35.16	0.318
8	34.72^{ij}	34.57^{i}	35.63^{j}	35.38^{ij}	^{B}35.07	0.313
40	34.35	34.73	35.48	35.48	^{B}35.01	0.382
MEAN[3]	34.81^{i}	34.76^{i}	35.73^{j}	35.48^{j}	35.19	0.338
SEM	0.250	0.333	0.269	0.243	0.276	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{AB} day means within columns (treatments) with unlike superscripts differ at $p<.05$

^{ij} treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

SEM = standard error of mean

Table 4.1.2 Experiment 3.1. pH response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

pH:

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$A_{5.763}$	$A_{5.933}$	$A_{5.852}$	$A_{5.872}$	$A_{5.855}$	0.093
1	$B_{4.991}{}^{i}$	$B_{4.863}{}^{ij}$	$B_{4.890}{}^{ij}$	$B_{4.790}{}^{j}$	$B_{4.883}$	0.046
2	$C_{4.731}{}^{i}$	$C_{4.681}{}^{i}$	$C_{4.574}{}^{j}$	$C_{4.582}{}^{j}$	$C_{4.642}$	0.022
4	$CD_{4.532}{}^{i}$	$D_{4.500}{}^{ij}$	$D_{4.442}{}^{jk}$	$D_{4.454}{}^{k}$	$D_{4.482}$	0.015
8	$D_{4.471}{}^{i}$	$DE_{4.441}{}^{i}$	$DE_{4.338}{}^{j}$	$DE_{4.340}{}^{j}$	$E_{4.398}$	0.013
40	$D_{4.410}{}^{i}$	$E_{4.347}{}^{i}$	$E_{4.233}{}^{j}$	$E_{4.222}{}^{j}$	$F_{4.303}$	0.029
MEAN[3]	4.816^{i}	4.794^{i}	4.721^{j}	4.710^{j}	4.760	0.046
SEM	0.059	0.022	0.027	0.029	0.037	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

ABCDEF day means within columns (treatments) with unlike superscripts differ at $p<.05$

ijK treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

SEM = standard error of mean

Table 4.1.3 Experiment 3.1. Lactic acid response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

LACTIC ACID (%DM):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$A_{0.150}{}^{i}$	$A_{0.037}{}^{j}$	$A_{0.021}{}^{j}$	$A_{0.048}{}^{j}$	$A_{0.064}$	0.021
1	$B_{1.829}$	$AB_{1.947}$	$B_{1.766}$	$B_{2.436}$	$B_{1.995}$	0.366
2	$BC_{2.676}$	$BC_{2.941}$	$B_{2.836}$	$B_{3.495}$	$C_{2.987}$	0.285
4	$CD_{3.811}{}^{ij}$	$CD_{4.549}{}^{jk}$	$C_{5.076}{}^{k}$	$B_{3.389}{}^{i}$	$D_{4.206}$	0.287
8	$DE_{4.847}$	$D_{5.075}$	$C_{4.695}$	$C_{4.963}$	$D_{4.895}$	0.190
40	$E_{5.490}$	$E_{7.556}$	$D_{6.607}$	$D_{6.620}$	$E_{6.568}$	0.910
MEAN[3]	3.134^{i}	3.684^{j}	3.500^{ij}	3.492^{ij}	3.452	0.440
SEM	0.381	0.452	0.313	0.263	0.359	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

ABCDE day means within columns (treatments) with unlike superscripts differ at $p<.05$

ijK treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

SEM = standard error of mean

forages ($p<.025$). On days 2, 4, and 8, there tended to be less WSC in treatment I, inocula only, than in control (C) silage. Inoculation may have increased utilization of endogenous fermentable carbohydrate.

Total nitrogen (TN) as a fraction of DM was similar across treatments at the onset of the experiment and increased over the 40 day ensiling period. See Tables 4.1.5 and 4.1.16. Original TN on day 0 averaged 2.70% (or approximately 16.9% crude protein) across treatments. This increase with time is most likely due to a gradual loss of DM. Sucrose treatment lowered the TN concentration slightly throughout the experiment. This effect could be the result of a dilution of the forage TN by the added sucrose DM.

Original ammonia nitrogen (AN) concentrations in sucrose treated and non-sucrose treated forages were 0.009% and 0.007% on a DM basis respectively, and were different at $p<.10$. See Tables 4.1.6 and 4.1.16. These concentration are extremely low and the difference may again be due a dilution of the AN by the sucrose DM. Fermentation of sucrose treated material resulted in significantly less AN on day 40 than non-sucrose treated silos (0.0489% vs. 0.0621%, $p<.001$), which indicates less deamination of nitrogenous components of the forage. These final AN contents are still very low and are similar to values obtained in other wilted alfalfa silage experiments in the U.S. and are probably not of any real significance in terms of nutritional value of the silages.

Table 4.1.4 Experiment 3.1. Water-soluble carbohydrate response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

WATER-SOLUBLE CARBOHYDRATE (%DM):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$A_{7.280}^{i}$	$A_{7.292}^{i}$	$A_{12.540}^{j}$	$A_{12.900}^{j}$	$A_{10.003}$	0.463
1	$B_{3.518}^{i}$	$B_{2.706}^{i}$	$B_{5.648}^{j}$	$B_{5.034}^{j}$	$B_{4.227}$	0.285
2	$C_{1.850}^{i}$	$C_{1.546}^{i}$	$C_{3.246}^{j}$	$C_{3.240}^{j}$	$C_{2.470}$	0.226
4	$CD_{1.474}^{i}$	$C_{1.254}^{i}$	$CD_{2.275}^{j}$	$CD_{2.505}^{j}$	$D_{1.877}$	0.216
8	$CD_{1.123}^{i}$	$C_{0.986}^{i}$	$D_{1.873}^{j}$	$DE_{2.006}^{j}$	$D_{1.497}$	0.103
40	$D_{0.755}^{i}$	$C_{0.832}^{i}$	$D_{1.234}^{j}$	$E_{1.241}^{j}$	$E_{1.015}$	0.083
MEAN[3]	2.667^{i}	2.436^{i}	4.469^{j}	4.488^{j}	3.515	0.262
SEM	0.179	0.236	0.252	0.178	0.214	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

ABCDE day means within columns (treatments) with unlike superscripts differ at $p<.05$

ij treatment means within rows (times) with unlike superscripts differ at $p<.05$

1 indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

2 day mean

3 treatment mean

SEM = standard error of mean

Table 4.1.5 Experiment 3.1. Nitrogen response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

TOTAL NITROGEN (%DM):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$A_{2.713}$	$AB_{2.748}$	$AB_{2.662}$	$AB_{2.679}$	$A_{2.701}$	0.044
2	$B_{2.913}$	$B_{2.907}$	$B_{2.797}$	$B_{2.803}$	$B_{2.855}$	0.060
40	$A_{2.709}^{i}$	$A_{2.594}^{ij}$	$A_{2.532}^{j}$	$A_{2.543}^{j}$	$C_{2.594}$	0.049
MEAN[3]	2.778^{i}	2.750^{ij}	2.664^{j}	2.675^{j}	2.717	0.051
SEM	0.052	0.061	0.066	0.057	0.059	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

ABC day means within columns (treatments) with unlike superscripts differ at $p<.05$

ij treatment means within rows (times) with unlike superscripts differ at $p<.05$

1 indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

2 day mean

3 treatment mean

SEM = standard error of mean

Water-soluble nitrogen (WSN) on a DM basis averaged 0.80% across treatments on day 0, and 1.39% on day 40. This component increased for all treatments over the 40 day ensiling period. See Tables 4.1.7, 4.1.8 and 4.1.16. The inocula/sucrose combination treatment (I+S) resulted in the lowest average WSN content across days. No treatment effects existed within a day. The %WSN / %TN ratio increased from a mean of 0.30 on day 0 to 0.42 on day 2 to 0.53 at day 40. This parameter also remained generally unaffected by treatment except on day 40 where inoculation tended to increase the WSN/TN ratio when sugar was added ($p<.10$ for interaction).

Though extensively used in silage experimentation, the WSN determination could easily be improved. The pH of an extraction can affect protein solubility (Smith et al, 1959; Jancarik and Proksova, 1970). If day 0 soluble N is to be compared to day 40 soluble N, then the same extraction method should be used on both sets of samples. On day 0, the water used is buffered at pH 7 by the forage acids, on day 40 it is buffered at 4. How much of the difference in nitrogen solubility is due to the difference in acidity or to actual microbial degradation of protein is not known. A typical conclusion is that it is a result of microbial degradation. Since the silage will be fed to cattle whose rumens are buffered at pH 6 to 7, a pH 7 buffer should be used at both points to standardize the extraction. In addition, this

buffer should be iso-osmolar with rumen fluid to make the soluble N measurement somewhat meaningful.

Acid detergent fiber (ADF) contents of silages are shown in Table 4.1.9. Mean ADF concentration on a DM basis of original forage was 38.18% across treatments. No time effects were noted except for a slight fluctuation for sucrose treated silages (S). The ADF content increased for this treatment from day 0 to day 8, though not significantly, and then decreases from day 8 to day 40 ($p<.05$). No explanation for this trend is known outside the possibility of error of ADF determination. An effect of sucrose addition can be noted on day 40 and among treatment means across days. Sucrose treated silages contained less ADF on day 40 than did the non-sucrose treated silages ($p<.05$). This difference on day 40 is large enough to cause the treatment means across days to reflect the same effect. Control (C) and inocula only (I) increased slightly in ADF concentration over the course of the experiment, though this effect is not significant, while this trend is not evident in treatments S and I+S. Silos treated with sucrose in fact tend to decrease, also not a significant trend. Constant ADF concentration over time can indicate good recovery of DM. This improved conservation of DM is not evident in any of the estimates of dry matter recovery that will be discussed. This slight decrease in ADF in sucrose treated silos is possibly related to the residual sucrose remaining in the

Table 4.1.6 Experiment 3.1. Ammonia response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

AMMONIA NITROGEN (%DM):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$^{A}0.009$	$^{A}0.008$	$^{A}0.007$	$^{A}0.007$	$^{A}0.008$	0.001
2	$^{B}0.416$	$^{B}0.439$	$^{B}0.422$	$^{B}0.421$	$^{B}0.424$	0.010
40	$^{C}0.062^{i}$	$^{C}0.062^{i}$	$^{C}0.049^{j}$	$^{C}0.049^{j}$	$^{C}0.055$	0.001
MEAN[3]	0.162^{ij}	0.170^{i}	0.159^{j}	0.159^{j}	0.163	0.006
SEM	0.005	0.009	0.004	0.007	0.007	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{ABC} day means within columns (treatments) with unlike superscripts differ at $p<.05$

^{ij} treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

SEM = standard error of mean

Table 4.1.7 Experiment 3.1. Water-soluble nitrogen response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

WATER-SOLUBLE NITROGEN (%DM):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$A_{0.833}$	$A_{0.801}$	$A_{0.786}$	$A_{0.789}$	$A_{0.802}$	0.018
2	$B_{1.198}$	$B_{1.273}$	$B_{1.168}$	$B_{1.179}$	$B_{1.204}$	0.038
40	$C_{1.424}$	$C_{1.415}$	$C_{1.391}$	$B_{1.317}$	$C_{1.387}$	0.037
MEAN[3]	1.152^{ij}	1.163^{i}	1.115^{ij}	1.095^{j}	1.131	0.032
SEM	0.024	0.042	0.021	0.053	0.037	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{ABC} day means within columns (treatments) with unlike superscripts differ at $p<.05$

^{ij} treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

SEM = standard error of mean

Table 4.1.8 Experiment 3.1. Response of water-soluble nitrogen, in relation to total nitrogen, to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

WATER-SOLUBLE NITROGEN (%DM) / TOTAL NITROGEN (%DM):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	^{A}0.310	^{A}0.295	^{A}0.297	^{A}0.297	^{A}0.300	0.005
2	^{B}0.416	^{B}0.439	^{B}0.422	^{B}0.421	^{B}0.424	0.010
40	^{C}0.527	^{C}0.546	^{C}0.549	^{C}0.518	^{C}0.535	0.015
MEAN[3]	0.418	0.427	0.423	0.412	0.420	0.011
SEM	0.012	0.011	0.007	0.017	0.012	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{ABC} day means within columns (treatments) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

SEM = standard error of mean

Table 4.1.9 Experiment 3.1. Acid detergent fiber response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

ACID DETERGENT FIBER (%DM):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	39.11	38.53	^{AB}37.25	37.83	38.18	0.671
8	38.40	39.08	^{B}38.56	37.27	38.33	0.729
40	39.44^{i}	39.62^{i}	^{A}36.63^{j}	36.79^{j}	38.12	0.537
MEAN[3]	38.98^{i}	39.08^{i}	37.48^{j}	37.30^{j}	38.21	0.651
SEM	0.721	1.016	0.569	0.618	0.531	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{AB} day means within columns (treatments) with unlike superscripts differ at $p<.05$

^{ij} treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

SEM = standard error of mean

silage at day 40. See Table 4.1.4. This effect, if present, should also be reflected in a slight dilution of ADF on day 0. In fact a slight but insignificant difference in ADF content can be noticed on day 0 between non-sucrose treated and sucrose treated silos. Fiber determinations have been shown to be affected by length of storage period at a wide range of temperatures before drying (O'Neil and Allen, 1990). Respiration of harvested forages continues during drying or wilting until a DM of 65 to 75% is reached (Greenhill, 1961; Wood and Parker, 1971). To minimize the effects of respiration during storage and drying, samples shoud be dried as quickly as possible at a temperature as high as possible (in this case, 55^{o}C). Air drying of samples takes twice as long as oven drying at 55^{o}C and as a consequence, respiration can cause significant losses of available carbohydrate. A loss of soluble carbohydrate concentrates fiber fractions and these analyses no longer reflect the original forage as sampled.

Table 4.1.10 lists mean ash concentrations on a DM basis. Average ash concentration as a percentage of DM across treatments was 7.2% on day 0 and 8.2% on day 40. Increased ash contents over the 40 day ensiling period are evident for all treatments. These increases reflect losses of DM. Dilution of ash by added sucrose DM can also be noted both on day 0 means and overall treatment means. The absolute amount of ash present in the original forage should

still remain at the end of the ensiling period as it is not directly involved in fermentation chemical reactions. A situation in which great volumes of effluent is lost from the silo would not follow this rule of ash conservation. This theory supports the use of ash, as a fraction of DM, in the later calculations of DM recovery.

Dry matter recovery (DMR) values as a percentage of original DM ensiled are listed in Table 4.1.11. Listed DMR values were calculated according to the following equation: ((final weight x %DM of final silage) / (initial weight x %DM of initial forage) x 100). Using this equation, a value of 100% was assigned to day 0 as these weights were used as a standard. Silo variation was too large to detect many significant decreases in DMR. Recoveries for non-sucrose treated silos tended to decrease, but not significantly. No treatment effects were evident throughout the experiment. Sucrose treated silos elicited a significant decrease in DMR. The S group DMR values did not differ significantly from day 0 until day 40, while the variation among I+S silos allowed significant differences between each of the first 3 time points. A general tendency for DMR to decrease to day 2 and afterward remain fairly constant can be noted for all treatments. This is probably related to DM content eliciting the same trend. A possible cause for this pattern could be the volatilization of a different group of compounds during these two phases in the course of the 80^{o}C oven DM

Table 4.1.10 Experiment 3.1. Ash response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

ASH (%DM):

	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$A_{7.248}^{ij}$	$A_{7.493}^{j}$	$A_{7.000}^{i}$	$A_{6.935}^{i}$	$A_{7.169}$	0.001
8	$B_{7.997}$	$B_{8.103}$	$B_{7.728}$	$B_{7.847}$	$B_{7.919}$	0.002
40	$B_{8.280}$	$B_{8.458}$	$B_{8.140}$	$B_{7.890}$	$C_{8.192}$	0.002
MEAN[3]	7.842^{ij}	8.018^{i}	7.623^{j}	7.557^{j}	7.760	0.002
SEM	0.002	0.002	0.003	0.002	0.002	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

[AB] day means within columns (treatments) with unlike superscripts differ at $p<.05$

[ij] treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

SEM = standard error of mean

determination.

Table 4.1.12 lists another set of DMR values calculated with the following equation: ((initial ash, % of DM) / (final ash, % of DM) x 100). This equation again sets all day 0 values at 100%. Values calculated in this manner are much lower than those in Table 4.1.11 calculated by a simple weight loss equation. Each treatment shows a significant decrease in DMR over the 40 day ensiling period when using the ash calculation. Mean DMR on day 40 is 87.84% compared with 97.09% using the weight equation. No treatment effects are evident when the ash DMR calculation is used. Variation is great among these estimates as well.

Lactobacilli counts are listed in Table 4.1.13. No statistical comparisons were made among treatments as there are no degrees of freedom available. Each value listed in the table is a single count performed on a composited sample from 3 replicate silos. Day 0 counts averaged 10^7 in the first trial and about 10^6 in the second. In both trials, inoculation did not increase the number of lactobacillus since the harvested forage already contained 10^7 cfu/gram and the inoculant was added to supply 10^5 cfu/gram. In the first trial, inoculated silage contained 10 times more LAB and in trial 2, inoculation did not affect lactobacilli counts. First cut alfalfa appears to have had a greater concentration of epiphytic LAB than second cut alfalfa. In trial 1, peak lactobacillus numbers were reached on day 2 except for the

Table 4.1.11 Experiment 3.1. Dry matter recovery in response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

DRY MATTER RECOVERY (%)[1]:

	C	I	S	I+S	MEAN[3]	SEM
Day[2] 0	100.00	100.00	^{A}100.00	^{A}100.00	^{A}100.00	---
1	99.46	97.61	^{AB}96.94	^{B}98.31	^{B}98.08	0.841
2	98.63	97.49	^{AB}97.50	^{C}97.01	^{B}97.66	1.050
4	99.10	98.45	^{AB}96.93	^{BC}97.88	^{B}98.09	1.020
8	98.09	97.25	^{AB}97.08	^{BC}97.73	^{B}97.54	0.878
40	96.48	97.53	^{B}96.53	^{BC}97.81	^{B}97.09	1.189
MEAN[4]	98.63	98.05	97.50	98.12	98.08	0.916
SEM	1.059	0.730	0.723	0.248	0.748	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{ABC} day means within columns (treatments) with unlike superscripts differ at $p<.05$

[1] calculated as (final wt. x %DM) / (initial wt. x %DM) x 100

[2] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[3] day mean

[4] treatment mean

SEM = standard error of mean

Table 4.1.12 Experiment 3.1. Dry matter recovery (estimated using ash content) in response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

DRY MATTER RECOVERY - ASH (%)[1]:

	C	I	S	I+S	MEAN[3]	SEM
Day[2] 0	$^{A}100.00$	$^{A}100.00$	$^{A}100.00$	$^{A}100.00$	$^{A}100.00$	---
8	$^{B}90.74$	$^{AB}93.00$	$^{B}91.29$	$^{B}89.21$	$^{B}91.06$	3.412
40	$^{B}87.62$	$^{B}88.71$	$^{B}86.38$	$^{B}88.63$	$^{B}87.84$	2.170
MEAN[4]	92.79	93.90	92.56	92.61	92.97	2.335
SEM	2.400	2.671	3.144	2.508	2.696	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{AB} day means within columns (treatments) with unlike superscripts differ at $p<.05$

[1] calculated as (initial ash % of DM) / (final ash % of DM) x 100

[2] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[3] day mean

[4] treatment mean

SEM = standard error of mean

inoculated silage which reached a maximum on day 4. In trial 2, lactobacilli counts reached a maximum on day 1 and remained at a plateau until day 4 before decreasing again. Inoculation seems to have had little impact on lactobacillus numbers however some inoculation effects were observed among the chemical parameters. Since colonies were never positively identified as being lactobacillus species, either microscopically or otherwise, these counts may not be accurate. Also it is necessary to keep in mind that the handling of volatiles in this experiment was not optimal and it is unknown how this may have affected DM estimates which are used to standardize the expression of all chemical results.

Buffering capacity means of initial forage are listed in Table 4.1.14. Day 0 buffering capacities were lower ($p>.05$) for second cut alfalfa than first cut, 549 mequiv./kg vs. 520 mequiv./kg. No treatment effects were observed in either trial or across both trials.

Acetic acid, propionic acid and butyric acid were quantified and results are given in Table 4.1.15. No butyric acid was detected in any sample, reinforcing the fact that to human senses, none of the silages appeared to be poorly preserved upon silo emptying. Acetic acid content increased over the ensiling period for all treatments. The only significant treatment effect is noted on day 2 when C silage contained 0.004%, I contained 0.004%, S contained 0.005%, and

IS contained 0.006%. This difference, though significant, is very small and probably of no consequence. These acetic acid concentrations are all fairly low and indicate a highly homofermentative fermentation. All samples were also low in propionic acid. Treatment did not affect propionic acid production either as all silages increased to about the same concentration over 40 days.

Table 4.1.13 Experiment 3.1. Lactobacilli numbers in response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

LACTOBACILLI NUMBERS (LOG):

		C	I	S	I+S	MEAN[2]
Trial 1:						
Day[1]	0	6.301	7.255			7.000
	1	TNC	TNC	TNC	TNC	
	2	9.663	9.663	9.653	9.362	9.594
	4	9.491	9.929	10.146	10.000	9.949
	8	9.079	10.000	9.000	9.204	9.538
	40	7.079	7.041	6.301	6.602	6.860
Trial 2:						
Day1	0	5.778	5.301			5.602
	1	9.041	9.176	9.146	9.204	9.146
	2	8.845	9.041	8.845	8.699	8.875
	4	9.000	9.000	8.845	8.903	8.942
	8	8.699	8.301	8.301	8.602	8.512
	40	6.594	7.301	6.477	7.000	7.021

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

[1] indicates days post-ensiling. Tabular entries represent logs of counts performed on a composite sample of triplicate laboratory silos.

[2] day mean

TNC = too numerous to count at 10^{-7} dilution

Table 4.1.14 Experiment 3.1. Buffering capacity values of fresh wilted alfalfa after treatment and before ensiling in laboratory silos.

BUFFERING CAPACITY (meq/kg DM):

	C	I	S	I+S	MEAN[1]	SEM
Trial 1	545	555	542	555	^{A}549	6.47
Trial 2	519	518	521	520	^{B}520	1.25
MEAN[2]	532	537	532	537	535	3.99

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{AB} day means within columns (treatments) with unlike superscripts differ at $p<.05$

[1] trial mean

[2] treatment mean

SEM = standard error of mean

Table 4.1.15 Experiment 3.1. Volatile fatty acid production in response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.[4]

ACETIC ACID (%DM):	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$A_{0.0014}$	$A_{0.0011}$	$A_{0.0010}$	$A_{0.0015}$	$A_{0.0013}$	0.0002
2	$AB_{0.0041}^{ij}$	$B_{0.0036}^{i}$	$B_{0.0048}^{jk}$	$B_{0.0058}^{k}$	$B_{0.0046}$	0.0003
8	$B_{0.0094}$	$C_{0.0089}$	$C_{0.0093}$	$C_{0.0097}$	$C_{0.0093}$	0.0011
40	$C_{0.0155}$	$D_{0.0147}$	$D_{0.0128}$	$D_{0.0146}$	$D_{0.0144}$	0.0018
MEAN[3]	0.0076	0.0071	0.0070	0.0079	0.0074	0.0009
SEM	0.0017	0.0004	0.0004	0.0005	0.0009	

PROPIONIC ACID (%DM):	C	I	S	I+S	MEAN[2]	SEM
Day[1] 0	$A_{0.0001}^{i}$	$A_{0.0000}^{ij}$	$A_{0.0000}^{j}$	$A_{0.0000}^{j}$	$A_{0.0000}$	0.0000
2	$A_{0.0000}$	$A_{0.0000}$	$A_{0.0000}$	$A_{0.0000}$	$A_{0.0000}$	0.0000
8	$A_{0.0002}$	$A_{0.0004}$	$A_{0.0004}$	$A_{0.0002}$	$A_{0.0003}$	0.0001
40	$B_{0.0013}$	$B_{0.0020}$	$B_{0.0017}$	$B_{0.0009}$	$B_{0.0015}$	0.0005
MEAN[3]	0.0004	0.0006	0.0005	0.0003	0.0004	0.0002
SEM	0.0002	0.0002	0.0007	0.0001	0.0002	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

^{ABCD} day means within columns (treatments) with unlike superscripts differ at $p<.05$

^{ijk} treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

[4] butyric acid was not detected in any silage samples

Table 4.1.16 Experiment 3.1. Orthogonal contrasts among treatments within days and over all days.

	DM	pH	LA	WSC	TN	WSN/TN	AN
DAY 0 - INITIAL FORAGE:							
C	35.62	5.76	0.15	7.28	2.71	0.31	0.01
I	35.33	5.93	0.04	7.29	2.75	0.30	0.01
S	36.47	5.85	0.02	12.54	2.66	0.30	0.01
I+S	35.93	5.92	0.05	12.76	2.70	0.30	0.01
p,trtmt	<.001	NS	<.001	<.001	NS	<.25	<.25
C,S vs. I,I+S	NS	<.25	<.05	NS	NS	<.25	NS
C,I vs. S,I+S	<.001	NS	<.005	<.001	<.25	NS	<.10
Interaction	<.10	NS	<.005	NS	NS	<.10	NS
Trial 1	34.87	5.89	0.13	8.71	2.47	0.33	0.01
Trial 2	36.58	5.85	0.00	11.23	2.93	0.27	0.01
p,trial	<.001	NS	<.001	<.001	<.001	<.001	<.25
DAY 1:							
C	35.01	4.99	1.83	3.52			
I	34.62	4.86	1.95	2.71			
S	35.51	4.89	1.77	5.65			
I+S	35.75	4.79	1.92	5.03			
p,trtmt	<.025	<.025	NS	<.001			
C,S vs. I,I+S	NS	<.025	NS	<.025			
C,I vs. S,I+S	<.005	<.05	NS	<.001			
Interaction	<.25	NS	NS	NS			
Trial 1	34.32	4.78	2.73	4.13			
Trial 2	36.13	4.99	1.00	4.33			
p,trial	<.001	<.001	<.001	NS			
DAY 2:							
C	34.56	4.73	2.68	1.85	2.91	0.42	
I	34.65	4.68	2.94	1.55	2.91	0.44	
S	35.76	4.57	2.84	3.25	2.80	0.42	
I+S	35.10	4.58	3.50	3.24	2.80	0.42	
p,trtmt	<.10	<.001	<.25	<.001	<.25	=.25	
C,S vs. I,I+S	NS	=.25	<.05	NS	NS	<.25	
C,I vs. S,I+S	<.05	<.001	<.25	<.001	<.05	NS	
Interaction	NS	<.25	NS	NS	NS	<.25	
Trial 1	34.27	4.57	4.14	2.21	2.60	0.45	
Trial 2	35.76	4.72	1.83	2.73	3.11	0.40	
p,trial	<.001	<.001	<.001	<.025	<.001	<.001	

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

Table 4.1.16 Experiment 3.1 continued.

	DM	pH	LA	WSC	TN	WSN/TN	AN
DAY 4:							
C	35.04	4.53	3.81	1.47			
I	34.67	4.50	4.55	1.25			
S	35.53	4.44	5.08	2.28			
I+S	35.41	4.45	3.39	2.51			
p,trtmt	<.25	<.001	<.001	<.001			
C,S vs. I,I+S	NS	NS	<.10	NS			
C,I vs. S,I+S	<.001	<.001	NS	<.001			
Interaction	<.10	<.10	<.001	<.25			
Trial 1	34.49	4.43	5.52	1.64			
Trial 2	35.84	4.54	2.90	2.12			
p,trial	<.001	<.001	<.001	<.025			
DAY 8:							
C	34.71	4.47	4.86	1.12			
I	34.57	4.44	5.08	0.99			
S	35.63	4.34	4.70	1.87			
I+S	35.57	4.34	4.96	2.01			
p,trtmt	<.025	<.001	NS	<.001			
C,S vs. I,I+S	NS	NS	<.25	NS			
C,I vs. S,I+S	<.005	<.001	NS	<.001			
Interaction	NS	<.25	NS	<.25			
Trial 1	34.30	4.38	5.74	1.65			
Trial 2	35.94	4.42	4.06	1.34			
p,trial	<.001	<.025	<.001	<.005			
DAY 40:							
C	34.35	4.41	5.49	0.76	2.71	0.53	0.06
I	34.73	4.35	7.56	0.83	2.59	0.55	0.06
S	35.48	4.23	6.61	1.23	2.53	0.55	0.05
I+S	35.48	4.22	6.62	1.24	2.54	0.52	0.05
p,trtmt	<.10	<.001	NS	<.001	<.05	<.25	<.001
C,S vs. I,I+S	NS	<.25	<.25	NS	<.025	NS	NS
C,I vs. S,I+S	<.025	<.001	NS	<.001	<.025	NS	<.001
Interaction	NS	NS	<.25	NS	<.25	<.10	NS
Trial 1	34.09	4.29	7.71	1.27	2.56	0.52	0.05
Trial 2	35.93	4.31	5.43	0.76	2.63	0.55	0.06
p,trial	<.001	<.001	<.001	<.001	<.001	<.001	<.001

C, I, S, I+S in column headings indicate control, inocula, sucrose and inocula+sucrose treatments respectively.

In summary, in this trial inoculation or glucose addition appeared not to have any important effects on the preservation of alfalfa haylage. The sugar disappeared but did not reappear in the measurement of any fermentation acids. It was either respired or was converted to an acid or other component that was not measured. No improvements in dry matter recovery were detected. Treating the forage prior to ensiling served to change some chemical parameters of the fermentation, many would call this an improvement. However, translating the changes observed in lactic acid, water-soluble nitrogen, and pH into significant improvements in actual feed value or aerobic stability of the resultant silage is difficult, especially when the cost of the applications is considered. Inoculation costs approximately $1 per ton of forage as ensiled. This is not a large cost, but it is an unnecessary expense if nothing is returned. Adding 2% sucrose is simply not a practical idea. This would mean treating a 200 ton silo with 4 tons of sugar.

The methods employed in this experiment need modification. Samples to be dried should be dried immediately as researchers have shown that storage at any temperature can affect fiber and nitrogen fractions (O'Neil and Allen, 1990; Kohn and Allen, 1990). Samples to be extracted should be extracted as soon as possible as sugars and acids can be respired. In this trial, subsequent WSC analyses on the same samples were noticed to decline with

each thawing and refreezing. Thymol was added to each water extract before freezing, but did not entirely solve the problem of disappearing WSC. Acidification of these extracts to be frozen may help to prevent the decrease in respirable components during storage.

A more precise and accurate technique to measure water content of silage samples should be used. Dry matter determination is central to most feed experiments as most all other variables are expressed on a DM basis. In this trial, since DM is overestimated due to the volatilization of non-water compounds, all other parameters given on a DM basis are underestimated. While Karl Fischer titration requires special equipment and chemicals and is slow, toluene distillation is fast, easy and affordable. Oven drying at 80^{o}C is further complicated by not removing as much water as possible (Van Soest, unpublished). So the end result is simply a DM estimate we cannot be confident in, which causes us to question the accuracy of all other figures based upon DM.

4.2 Experiment 3.2: Fermentation and Nutritive Qualities of Alfalfa Silage Ensiled With or Without the Addition of a Microbial Inocula in Large Scale Silos

The effects of ensiling wilted alfalfa in large scale silos with and without microbial inocula are described in Tables 4.2.1 through 4.2.9. No statistical comparisons

between the treatments are possible in this experiment as silo and treatment are confounded. All statements made in this section about differences or similarities between silos are based solely on the author's judgement.

The forage blown into the two silos was similar in content; about 42% DM, 10% WSC, 0.1% LA, 2.9% N, 0.9% WSN, and 29% ADF with a pH of 5.8. Silages were fed out approximately 4 months after ensiling. Composition of the silages as they were unloaded from the silos was not markedly different. See Table 4.2.1. DM decreased slightly to about 40% and pH had decreased to 4.6 in the control silo and 4.5 in the inoculated silo. WSC decreased from 10% to 1.7% in both silos. LA content was 5.4% in the control silo and 6.3% in the inoculated silo, a very small difference at best. The WSN as a fraction of TN increased similarly, from 0.3 to 0.6 in both silos. ADF content increased from 29% to 31% in control silage and to 30% in treated silage, possibly a very small difference.

Silage, as it is fed out from a silo, has been influenced by two forces which can alter the original forage as ensiled - fermentation and aerobic exposure. It may be possible for a silage treatment to affect fermentation but then this effect may be cancelled by reactions taking place during aerobic exposure at feedout. In this experiment, accurately attributing changes to either phase individually is impossible.

Total bacteria and LAB counts are listed in Table 4.2.2. Wilted alfalfa contained about 5 x 10^6 epiphytic LAB per gram of wet forage. Inoculation of alfalfa did not appear to increase total or LAB above control levels. The number of bacteria in the treated silage remained equal or less than those in the control silage through the first 24 hours of fermentation.

Table 4.2.3 lists the compositions of samples removed from ports in door 2 (second door from the bottom) of each silo over the first 9 days of ensiling. Due to variation in forage loads blown into the silos some compositions are differ slightly from the composite means in Table 4.2.1. pH values may have decreased more rapidly in the inoculated silo than the control. By day 9, silage at door 2 of the control silo had produced a pH of 4.6 while the inoculated silage had produced enough acid to depress the pH to 4.2. A more dramatic drop in WSC and a rise in LA in the treated silage as compared with control are supportive to the premise that perhaps inoculation stimulated fermentation at least over the first 9 days of ensiling in the bottom portion of the silo.

Weight and DM losses for both silos are described in Table 4.2.4. On a wet basis, weight losses were similar for the two silos, 7.2% in the control silo and 6.2% in the treated silo. If top spoilage is included in these calculations, total weight loss becomes 8.3% for control and 7.8% for inoculated silage. If this effect was repeatable,

Table 4.2.1 Experiment 3.2. Forage composition into and out of silos. All components are expressed on a DM basis except DM and pH.

		DM	pH	WSC	LA	TN	WSN	WSN /TN	ASH	ADF
Control	In[1]	41.20	5.81	10.29	0.11	2.97	0.90	0.30	6.79	29.52
	Out[2]	39.88	4.63	1.78	5.39	3.27	1.86	0.57	8.33	31.17
Inocula	In[1]	42.30	5.85	9.78	0.09	2.82	0.89	0.32	6.70	29.04
	Out[2]	40.49	4.50	1.76	6.29	3.19	1.97	0.62	8.23	29.98

[1] weighted mean of 6 composite samples

[2] weighted mean of 20 weekly composite samples

Table 4.2.2 Experiment 3.2. Bacterial numbers in silage ensiled in large-scale upright silos.

	Silo	Day 0	Day 1
Total bacteria[1]:	Control	5.5×10^6	2.0×10^8
	Inoculated	8.3×10^6	1.9×10^8
Lactobacillus[2]:	Control	7.3×10^6	2.2×10^8
	Inoculated	1.8×10^6	2.4×10^7

[1] Brain Heart Infusion agar, incubated aerobically

[2] Rogosa SL agar, incubated anaerobically

Table 4.2.3 Experiment 3.2. Composition of samples removed from ports in door 2. All components are expressed on a DM basis except DM and pH.

	DAY	DM	pH	WSC	LA	TN	WSN	WSN /TN	ASH
Control	1	40.44	5.64	9.44	0.10	2.50	1.32	0.43	
	3	47.87	5.28	7.28	1.04	2.90	1.33	0.46	
	5	46.26	4.93	4.90	1.30	2.97	1.60	0.54	
	9	47.39	4.61	4.52	2.29	2.86	1.63	0.57	7.19
Inocula	1	48.54	5.73	8.33	0.07	2.78	1.23	0.44	
	3	47.73	5.18	8.95	0.89	2.99	1.44	0.48	
	5	47.58	4.63	5.27	3.05	2.89	1.45	0.50	
	9	47.25	4.22	2.63	5.87	2.94	1.54	0.52	7.70

Table 4.2.4 Experiment 3.2. Weight and DM loss calculations based on weight of forage put into and removed from each silo.

WEIGHT LOSS

Control:	IN	166,760 lb.		Inocula:	IN	175,140 lb.	
	OUT	154,760			OUT	164,280	
	LOSS	12,000	(7.20%)			10,860	(6.20%)
TOP SPOILAGE		1,900	(1.14%)			2,800	(1.60%)
TOTAL LOSS		13,900	(8.33%)			13,660	(7.80%)

DRY MATTER LOSS

Control:	IN	166,760 x 0.4120 =	68,705.12 lb.	
	OUT	154,760 x 0.3988 =	61,718.29	
	LOSS		6,986.83	(10.17%)
Inocula:	IN	175,140 x 0.4230 =	74,084.22 lb.	
	OUT	164,280 x 0.4049 =	66,516.97	
	LOSS		7,567.25	(10.21%)

inoculation could preserve an additional 750 lbs. in a 150 ton silo. On a DM basis however, both silos lost 10.2%.

Silo temperatures as monitored over the first 35 days of ensiling are given in Table 4.2.5. Potentiometer readings were taken from all thermocouples each morning. Temperature values obtained were at times somewhat sporadic. For example, ambient temperature on day 3 was not 118 ^{o}F. The accuracy of these readings is questionable. Both silos averaged about 13 ^{o}F above ambient temperature over the 35 days.

Weight and DM recoveries for the nylon mesh bags buried in the silo are listed in Table 4.2.6. Seven of the 12 buried bags were wholly recovered. Recoveries of weight and DM are not very informative. The nylon mesh must be permeable to small particles of DM and that the moisture component is influenced by the water content of that forage loaded above the mesh bag. Dry matter recovery calculated from ash content in the control silo is 91.2% and in the inoculated silo is 93.3%. This must be considered at most a very small difference.

Chemical analyses of the forage in the mesh bags are given in Table 4.2.7. Forages put in the bags were similar in each silo and also similar to the mean values given in Table 4.2.1. Contradictory to the whole silo means and the composition of the silage samples removed through door 2, buried bags indicate a greater mean production of lactic acid

than those in the inoculated silo.

In an attempt to quantitate aerobic stability upon exposure to air, temperature and weight of samples exposed to air at room temperature were monitored and are listed in Tables 4.2.8 and 4.2.9. Replicate (n=2) stability trials were performed. Samples for the first trial were collected and exposed after half of the silage had been fed out. The second trial was initiated near the end of the feedout period. No differences in aerobic stability between the two silos could be detected either through temperature changes or weight recovery.

In summary, there was no apparent effect of inoculation in this experiment. Small differences in chemical parameters were contradicted by other similar measurements. Additionally, even if some differences existed, there is no way to test their validity with statistics since between silo variation cannot be estimated. Differences in this experiment do not appear to be greater than normal silo variation in other experiments (Kung et al, 1987; Gordon, 1989).

The two silos used in this trial should have been selected more carefully. Silo 5, the control silo is on the north side of the east-west oriented feed building and silo 6, the treated silo is on the south side. This arrangement allows silo 6 more exposure to sun and wind than silo 5 which could influence the dissipation of heat by the silage mass.

Table 4.2.5 Experiment 3.2. Buried bag weight and dry matter recoveries. C=control, I=inoculated

	% Weight Recovery		% DM recovery	
Silo:	C	I	C	I
Buried at Door 2:	100.3	98.6	102.5	105.0
	99.2	99.9	103.7	103.6
Door 9:	93.6	100.3	99.9	100.8
		104.4		105.0
Mean:	97.7	100.8	102.3	103.6

Table 4.2.6 Experiment 3.2. Composition of forage in buried bags. C=control, I=inoculated

	DM		WSC		LA		ADF		Ash	
Silo:	C	I	C	I	C	I	C	I	C	I
ORIGINAL										
Door 2	45.6	48.8	6.3	9.2	.05	.07	29.4	29.6	7.5	7.5
Door 9	38.6	36.7	9.4	10.4	.07	.07	27.1	27.1	7.2	7.3
Mean:	42.1	42.8	7.9	9.8	.06	.07	28.2	28.4	7.3	7.4
AS RECOVERED										
Door 2	44.4	47.2	2.0	1.7	8.0	6.4	31.6	32.1	7.7	7.5
Door 9	36.2	36.5	1.4	1.6	14.5	9.1	29.6	28.9	8.4	8.3
Mean:	40.3	41.8	1.7	1.7	11.3	7.7	30.6	30.5	8.0	7.9

Table 4.2.7 Experiment 3.2. Temperature profile of control and inoculated silos monitored using copper / constantan thermocouples. Temperatures are given in ^{o}F.

Control Silo:

		Thermocouple no.							
Day	Ambient[1]	1	2	3	4	5	6	Average[2]	Difference[3]
0	72	73	76	70	67	63	65	69.0	-3.0
1	84	90	90	92	96	96	102	94.3	10.3
2	79	92	93	96	96	97	99	95.5	16.5
3	118	135	134	139	139	141	141	138.2	20.2
5	85	95	98	101	98	102	105	99.8	14.8
8	86	90	99	101	94	104	106	99.0	13.0
12	91	89	96	100	90	100	104	96.5	5.5
16	89	92	99	101	96	102	108	99.7	10.7
21	70	90	98	103	100	108	115	102.3	32.3
26	79	85	92	99	88	96	100	93.3	14.3
35	68	82	90	96	84	84	68	84.0	16.0
									13.7 avg

Inoculated Silo:

		Thermocouple no.							
Day	Ambient[1]	1	2	3	4	5	6	Average[2]	Difference[3]
0	72	72	73	77	70	70	70	72.0	0.0
1	84	92	90	85	90	90	91	89.7	5.7
2	79	92	92	85	92	94	93	91.3	12.3
3	118	134	135	126	137	139	140	135.2	17.2
5	85	98	97	84	102	104	75	93.3	8.3
8	86	100	99	85	104	98	105	98.5	12.5
12	91	97	95	77	101	90	100	93.3	2.3
16	89	98	98	81	103	97	103	96.7	7.7
21	70	106	102	84	112	100	109	102.2	32.2
26	79	102	95	76	107	91	98	94.8	15.8
35	68	97	90	72	105	88	98	91.7	23.7
									12.5 avg

[1] Ambient temperature measured with potentiometer in water allowed to equilibrate for several hours with ambient temperature.

[2] Average reading of 6 thermocouples

[3] Difference between ambient temperature and average thermocouple reading.

Table 4.2.8. Experiment 3.2. Temperature profile of silage samples exposed to air at room temperature for 10 days.

Aerobic Stability Trial I:

		CONTROL				INOCULA			
Day	Ambient	C1[1]	C2	Avg.[2]	Diff.[3]	I1	I2	Avg.	Diff.
0	55	57	54	55.5	0.5	57	55	56.0	1.0
1	74	70	69	69.5	-4.5	71	71	71.0	-3.0
2	75	70	70	70.0	-5.0	72	73	72.5	-2.5
3	74	71	71	71.0	-3.0	72	74	73.0	-1.0
4	77	74	73	73.5	-3.5	75	76	75.5	-1.5
5	75	72	72	72.0	-3.0	74	74	74.0	-1.0
6	74	71	70	70.5	-3.5	71	72	71.5	-2.5
7	75	69	69	69.0	-6.0	71	72	71.5	-3.5
8	76	70	70	70.0	-6.0	72	74	73.0	-3.0
10	74	69	68	68.5	-5.5	70	70	70.0	-4.0
Mean:					-3.6				-1.7

Aerobic Stability Trial II:

		CONTROL				INOCULA			
Day	Ambient	C3	C4	Avg.	Diff	I3	I4	Avg.	Diff.
0	68	67	68	67.5	-0.5	68	68	68.0	0.0
1	77	70	72	71.0	-6.0	70	72	71.0	-6.0
2	73	70	71	70.5	-2.5	70	71	70.5	-2.5
3	71	70	70	70.0	-1.0	69	70	69.5	-1.5
4	71	70	72	71.0	0.0	70	71	70.5	-0.5
5	74	70	72	71.0	-3.0	70	72	71.0	-3.0
6	75	73	74	73.5	-1.5	72	74	73.0	-2.0
7	77	74	75	74.5	-2.5	73	74	73.5	-3.5
8	80	76	78	77.0	-3.0	77	78	77.5	-2.5
10	84	78	80	79.0	-5.0	80	80	80.0	-4.0
Mean:					-2.3				-2.3

[1] indicates replicates 1 through 4 for control (C) and inoculated (I) silages.

[2] average of two replicates

[3] difference between average temperature and ambient temperature

Table 4.2.9. Experiment 3.2. Weight loss of silage samples exposed to air at room temperature for 10 days.

Aerobic Stability Trials I and II.

		% Weight Loss		
	Rep.	Trial I	Trial II	Mean
Control	1	9.46	9.10	
	2	9.65	9.21	
	Mean	9.56	9.15	9.35

		% Weight Loss		
	Rep.	Trial I	Trial II	Mean
Inocula	1	9.12	9.13	
	2	9.43	9.20	
	Mean	9.27	9.16	9.22

4.3 Experiment 3.3: Responses of Dairy Cows to Alfalfa Silage Ensiled With and Without the Addition of Microbial Inocula

The effects of feeding control or inoculated alfalfa haylage to lactating Holstein cows are listed in Table 4.3.1. The values are given by week of the experiment. In the first week cows fed inoculated silage produced more milk than the cows fed control silage. During weeks 4 and 5, control cows produced more 4% fat-corrected milk (FCM) than those on the inoculated silage diet ($p=.07$, $p=.001$ respectively). Average milk and FCM production over the 10 weeks was not different ($p<.05$). Milk production persistency was 89.9% for control cows and 85.6% for treatment cows (calculated by dividing week 10 milk production per day by pre-trial production). For FCM production, persistencies were 86.1% and 80.4% for control and inoculated groups.

Average milk fat and protein contents were nearly identical; 3.47 vs. 3.43% milk fat and 3.31 vs. 3.36% milk protein for the control and inoculated groups respectively. During weeks 3 and 4, milk from cows fed inoculated haylage contained more protein than that from cows fed control haylage ($p<.03$, $p<.07$).

Average dry matter intake was not different between the two groups (42.65 vs. 43.43 lb/cow/day). Cows fed inoculated haylage appeared to consistently gain weight more rapidly

than control cows however this difference was only significant in week 8 of the trial.

The results of this trial do not indicate any advantage to feeding inoculated alfalfa haylage to lactating dairy cows as dry matter intake, production and milk composition were unaffected.

The data from the first 2 weeks of this trial are not useful. Cows were not given an opportunity to adjust to ration changes before data collection began. It would have been advantageous to observe cows during the pre-trial covariate period, switch diets and allow 2 weeks adjustment and then begin actual data collection as is common for feeding and production trials of this nature. Some cows went completely off feed and most cows reduced intake somewhat for several days after the ration was changed to the experimental diet. These events may have clouded the ability to detect any diet effects for the first 2 or 3 weeks. A switchback design would also have allowed greater power to detect any treatment effects while using the same number of cows.

Table 4.3.1 Milk and milk component production in response to an inoculated silage diet.

	WEEK											10wk
	P	1	2	3	4	5	6	7	8	9	10	MEAN
Milk, lb.												
Control	60.77	57.24[a]	58.44	58.60	58.48	57.96	55.89	56.97	55.82	56.55	54.64	57.06
Inocula	60.94	59.63[b]	58.98	59.51	58.24	57.13	56.03	54.53	53.13	53.25	52.15	56.26
SEM		0.599	0.644	0.874	1.195	1.127	1.310	1.338	1.752	1.841	1.659	
p		0.03										
4% Fat Corrected Milk, lb.												
Control	57.22	51.65	51.67	56.27	54.37	53.84[a]	50.63	51.50	50.34	51.70	49.28	52.13
Inocula	58.48	54.43	52.97	55.50	51.78	50.66[b]	49.91	50.08	47.62	48.97	47.04	50.90
SEM		2.586	1.222	1.429	1.050	0.630	1.291	1.824	1.736	1.718	1.558	
p					0.07	0.001						
Milk Fat, %												
Control	3.68	3.38	3.22	3.83	3.61	3.56	3.42	3.44	3.36	3.47	3.37	3.47
Inocula	3.85	3.48	3.34	3.60	3.31	3.34	3.35	3.50	3.41	3.54	3.38	3.43
SEM		0.230	0.097	0.176	0.165	0.149	0.133	0.120	0.157	0.135	0.146	
p												
Milk Protein, %												
Control	3.10	3.20	3.54	3.20[a]	3.32	3.24	3.29	3.25	3.33	3.35	3.34	3.31
Inocula	3.07	3.25	3.21	3.30[b]	3.41	3.51	3.36	3.33	3.38	3.44	3.41	3.36
SEM		0.048	0.260	0.045	0.038	0.154	0.052	0.059	0.054	0.063	0.055	
p				0.03	0.07							
Dry Matter Intake, lb.												
Control	39.19	37.10	39.76	40.44	41.00	43.65	42.65	41.07	42.43	41.78	41.96	42.65
Inocula	40.32	37.29	40.04	40.93	42.06	46.12	47.86	47.33	47.80	45.82	46.25	43.43
SEM		2.111	2.491	2.860	2.822	2.970	2.316	2.231	2.390	2.677	2.375	
p												
Body Weight Change, lb.												
Control			3.53		12.33		47.47		47.07[a]		71.29	
Inocula			10.88		21.88		59.50		67.63[b]		97.06	
SEM			3.220		9.754		13.376		6.740		9.743	
p									0.05			

a, b means with unlike superscripts differ at $p < 0.05$

4.4 Experiment 3.4 Influence of a Prefermented Microbial Inocula, Sodium Bentonite and Glucose Addition on Fermentation of Direct-cut Alfalfa Forage Ensiled in Laboratory Silos.

The effects of inoculation (I) of unwilted alfalfa with LAB and of the addition of glucose (G) and sodium bentonite (B) on fermentation characteristics as compared with control (C) silage are listed in tables 4.4.1 through 4.4.11. DM of the harvested forage was approximately 19%. No silages were visibly molded or poorly preserved at any point during the 40 day ensiling period.

Dry matter concentrations for silages throughout the experiment are given in Table 4.4.1. DM of the forages before ensiling were about 18.8% for the non-bentonite treated forages and 20.0% for the bentonite treated forages. Glucose treatment increased DM content significantly above other treatments to 22.2%. Average DM concentration decreased by over 1 unit during the 40 day ensiling period from 20% to 18.7% DM.

Table 4.4.2 lists the number of laboratory silos leaking effluent after the first 24 hours of fermentation. The addition of B significantly decreased the loss of liquid from the B, IB, and IBG treated silos as compared with C and I silos. This effect of sodium bentonite has been observed in the ensiling of high-moisture grass silages by European

workers (Cook et al, 1980; Woolford et al, 1983).

The pH response to ensiling is illustrated in Table 4.4.3. Forage at ensiling averaged a pH of 6.0. Small differences in pH on day 0 are most likely due to the time spent processing the forage subsamples prior to ensiling (3 hours) and to sampling error. The pH decreased to a minimum in day 1 for inocula (I) and inocula+bentonite (IB) treatments. Inoculation appears to have enhanced pH decline on days 1, 2, and 4 while bentonite was ineffective. Bentonite treatment did not appear to have any associative effects with inocula. The only treatment to remain at a low pH past day 8 to day 40 was the inocula+bentonite+glucose (IBG) silage, indicating that WSC may have been a limiting factor in this fermentation. Non-glucose treated silages were not stable beyond day 4 or 8 as is evident from an inability to maintain a low pH.

Changes in WSC concentration of silages during ensiling are given in Table 4.4.4. Alfalfa forage used in this trial was a September fourth cutting. As a result, WSC resources are low, averaging 2.5% of DM. Glucose addition significantly increased WSC available for fermentation. Concentrations were 2.5% for C, I, B, and IB treatments and 6.0% for the IBG treated forage. WSC analyses were highly variable for the C, I, B, and IB treated silages throughout the experiment as is indicated by the high SEM relative to the treatment means. WSC resources were only 50% exhausted

Table 4.4.1 Experiment 3.4. Dry matter response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.

DRY MATTER (%):

	C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1] 0	18.76^{i}	18.92^{i}	20.16^{i}	19.68^{i}	22.21^{j}	^{A}19.95	0.256
1	18.77^{i}	19.26^{i}	19.92^{ij}	19.67^{i}	21.44^{j}	^{A}19.81	0.282
2	18.86^{i}	18.84^{i}	19.57^{i}	19.16^{i}	21.74^{j}	^{A}19.63	0.141
4	18.83^{i}	19.04^{ij}	19.92^{ij}	19.58^{ij}	21.24^{j}	^{A}19.72	0.369
8	18.02^{i}	18.48^{ij}	19.81^{jk}	19.01^{ij}	21.34^{k}	^{AB}19.33	0.274
16	17.97^{i}	18.76^{i}	19.31^{ij}	19.23^{ij}	20.84^{j}	^{AB}19.22	0.280
40	17.98^{i}	17.94^{i}	18.59^{ij}	18.46^{ij}	20.60^{j}	^{B}18.71	0.404
MEAN[3]	18.46^{i}	18.75^{ij}	19.61^{k}	19.26^{jk}	21.34^{l}	19.48	0.297
SEM	0.301	0.196	0.291	0.177	0.105	0.251	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

^{AB} day means within columns (treatments) with unlike superscripts differ at $p<.05$

^{ijkl} treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

SEM = standard error of mean

Table 4.4.2 Experiment 3.4. Effect of sodium bentonite addition to unwilted alfalfa forage before ensiling on laboratory silo seepage after 24 hours of fermentation.

Number of silos:	C	I	B	I+B	I+B+G
Seepage	7	11	4	3	3
No seepage	11	7	14	15	15

Bonferroni Chi-square: $p<0.01$ C, I vs. B, IB, and IBG
$p<0.05$ I vs. IB

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

Table 4.4.3 Experiment 3.4. pH response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.[4]

pH:

	C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1] 0	$A_{5.903}^{i}$	$A_{5.928}^{ij}$	$A_{5.964}^{jk}$	$A_{6.003}^{kl}$	$A_{6.038}^{l}$	$A_{5.967}$	0.009
1	$B_{5.196}^{k}$	$C_{4.649}^{j}$	$B_{5.106}^{k}$	$C_{4.715}^{j}$	$B_{4.402}^{i}$	$CD_{4.814}$	0.034
2	$D_{4.848}^{jk}$	$C_{4.655}^{j}$	$B_{5.031}^{k}$	$C_{4.753}^{j}$	$C_{4.225}^{i}$	$D_{4.702}$	0.038
4	$CD_{4.959}^{k}$	$C_{4.714}^{j}$	$B_{5.013}^{k}$	$C_{4.784}^{j}$	$D_{4.113}^{i}$	$D_{4.717}$	0.028
8	$BC_{5.064}^{k}$	$C_{4.700}^{j}$	$B_{5.025}^{k}$	$C_{4.830}^{jk}$	$D_{4.035}^{i}$	$D_{4.731}$	0.053
16	$BC_{5.143}^{i}$	$C_{4.901}^{i}$	$B_{5.209}^{i}$	$BC_{5.017}^{i}$	$D_{4.030}^{j}$	$C_{4.860}$	0.055
40	$B_{5.217}^{i}$	$B_{5.235}^{i}$	$B_{5.273}^{i}$	$B_{5.233}^{i}$	$D_{4.086}^{j}$	$B_{5.009}$	0.063
MEAN[3]	5.190^{i}	4.969^{j}	5.232^{i}	5.048^{j}	4.418^{k}	4.971	0.043
SEM	0.026	0.045	0.040	0.047	0.014	0.037	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

ABCD day means within columns (treatments) with unlike superscripts differ at $p<.05$

ijkl treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

[4] treatment x day interaction $p < 0.0001$

SEM = standard error of mean

in the non-glucose treated forages by day 40. Perhaps the WSC milieu was inappropriate or unavailable for silage fermentation. Inocula+B+G treated silage still contained more WSC than other treatments on day 40.

Lactic acid profiles of ensiled forages are listed in Table 4.4.5. Concentration of lactic acid in alfalfa forage at the time of ensiling was 0.2% on a DM basis. Day 0 levels differ slightly between treatments simply due to the time spent ensiling the forage and to sampling variation. Lactic acid levels in all treatments increased through day 4 but then considerable fluctuation occurred through day 40. Variation in this component is more evident in this trial than in experiment 3.1. This variation could be due to a general instability is direct-cut silage or to a sample preservation problem. Inocula+B+G treated silage contained a higher mean concentration of lactic acid across days.

Total nitrogen content of fresh and ensiled alfalfa forages are listed in Table 4.4.6. Fresh alfalfa contained 3.9% N, or 24.4% crude protein (CP). Fourth cut alfalfa is of much higher quality than that normally used for silage. Day 0 variation is most likely caused by sampling error and to a dilution of N by bentonite and glucose DM. This dilution effect is still evident at day 40. In the I, IB and IBG treatments, N concentration increased over the 40 day ensiling period to a mean of 4.3% on a DM basis. If N can be used as a marker, DM recovery is 94.8%, 87.7%, 93.5%, 84.9%,

Table 4.4.4 Experiment 3.4. Water-soluble carbohydrate response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos

WATER-SOLUBLE CARBOHYRATE (%DM):

	C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1] 0	$A_{2.576}^{i}$	$AB_{2.491}^{i}$	$AB_{2.026}^{i}$	$A_{3.449}^{i}$	$A_{6.039}^{j}$	$A_{3.316}$	0.340
1	$C_{0.614}^{i}$	$B_{2.793}^{i}$	$AB_{2.149}^{i}$	$B_{1.081}^{i}$	$A_{5.782}^{j}$	$BC_{2.484}$	0.146
2	$C_{0.666}^{i}$	$AB_{2.488}^{i}$	$AB_{2.347}^{i}$	$AB_{1.951}^{i}$	$AB_{4.648}^{j}$	$BC_{2.420}$	0.315
4[4]	$ABC_{1.498}^{i}$	$B_{2.710}^{i}$	$AB_{2.387}^{i}$	$AB_{2.422}^{i}$	$AB_{4.497}^{j}$	$AB_{2.703}$	0.209
8	$AB_{2.323}^{i}$	$B_{2.781}^{i}$	$B_{2.472}^{i}$	$AB_{2.654}^{i}$	$AB_{4.133}^{j}$	$AB_{2.873}$	0.202
16	$BC_{1.049}^{i}$	$AB_{2.460}^{j}$	$AB_{2.032}^{ij}$	$B_{1.088}^{i}$	$B_{2.835}^{j}$	$CD_{1.893}$	0.171
40	$C_{0.824}^{i}$	$A_{1.200}^{i}$	$A_{1.250}^{i}$	$AB_{1.552}^{i}$	$B_{2.767}^{j}$	$D_{1.519}$	0.169
MEAN[3]	1.364^{i}	2.418^{j}	2.095^{j}	2.028^{j}	4.386^{k}	2.458	0.275
SEM	0.188	0.181	0.155	0.259	0.333	0.233	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

^{ABCD} day means within columns (treatments) with unlike superscripts differ at $p<.05$

^{ijk} treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

[4] treatment x day interaction $p < 0.0001$

SEM = standard error of mean

Table 4.4.5 Experiment 3.4. Lactic acid response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.[4]

LACTIC ACID (%DM):

	C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1] 0	$A_{0.000}^{i}$	$A_{0.072}^{i}$	$A_{0.527}^{k}$	$A_{0.334}^{j}$	$A_{0.115}^{i}$	$A_{0.210}$	0.029
1	$A_{0.747}^{i}$	$B_{1.901}^{ij}$	$AB_{2.141}^{ij}$	$BC_{3.036}^{j}$	$B_{7.215}^{k}$	$BC_{3.008}$	0.315
2	$B_{2.616}^{i}$	$BC_{3.021}^{i}$	$BC_{3.880}^{i}$	$C_{4.736}^{ij}$	$B_{6.346}^{j}$	$D_{4.120}$	0.379
4	$B_{2.264}^{i}$	$C_{3.609}^{j}$	$C_{5.135}^{k}$	$C_{4.576}^{jk}$	$D_{10.050}^{l}$	$E_{5.127}$	0.173
8	$B_{2.968}^{i}$	$C_{4.409}^{j}$	$B_{2.729}^{i}$	$AB_{1.790}^{i}$	$B_{6.377}^{k}$	$CD_{3.655}$	0.228
16	$C_{10.430}^{ij}$	$D_{9.897}^{ij}$	$D_{11.510}^{j}$	$D_{8.324}^{i}$	$C_{12.480}^{j}$	$F_{10.528}$	0.496
40	$B_{2.975}$	$B_{2.003}$	$AB_{2.148}$	$AB_{1.958}$	$A_{2.267}$	$B_{2.270}$	0.282
MEAN[3]	3.143^{i}	3.559^{ij}	4.010^{j}	3.536^{ij}	6.407^{k}	4.131	0.305
SEM	0.187	0.198	0.265	0.245	0.389	0.258	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

ABCDEF day means within columns (treatments) with unlike superscripts differ at $p<.05$

ijkl treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

[4] treatment x day interaction $p < 0.0001$

SEM = standard error of mean

Table 4.4.6 Experiment 3.4. Total nitrogen response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.

TOTAL NITROGEN (%DM):

		C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1]	0	4.237^{i}	$A_{3.893}{}^{ij}$	4.283^{i}	$A_{3.630}{}^{j}$	$A_{3.650}{}^{j}$	$A_{3.939}$	0.078
	40	4.470^{j}	$B_{4.437}{}^{j}$	4.580^{j}	$B_{4.277}{}^{ij}$	$B_{3.990}{}^{i}$	$B_{4.351}$	0.061
MEAN[3]		4.354^{k}	4.165^{jk}	4.432^{k}	3.954^{ij}	3.820^{i}	4.145	0.070
SEM		0.102	0.038	0.138	0.151	0.091	0.111	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

[AB] day means within columns (treatments) with unlike superscripts differ at $p<.05$

[ijk] treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

SEM = standard error of mean

Table 4.4.7 Experiment 3.4. Water-soluble nitrogen response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.

WATER-SOLUBLE NITROGEN (%DM):

	C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1] 0	1.230	^{A}1.123	^{A}0.973	^{A}0.980	^{A}1.030	^{A}1.067	0.051
40	2.913	^{B}3.090	^{B}2.763	^{B}2.670	^{B}1.933	^{B}2.674	0.231
MEAN[3]	2.072	2.107	1.868	1.825	1.482	1.871	0.168
SEM	0.571	0.060	0.108	0.111	0.103	0.265	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

[AB] day means within columns (treatments) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

SEM = standard error of mean

and 91.5% for C, I, B, IB, and IBG treatments respectively.

Water soluble N as a percentage of DM for all forages are in Table 4.4.7. Concentrations of WSN in fresh forage averaged about 1.1% or 6.7% CP. This component increased over the 40 days to a mean concentration of 2.7% or 16.7% CP. No treatment effects were noted at $p>.05$ though WSN appears to have been slightly diluted by the addition of bentonite and glucose DM.

Table 4.4.8 lists WSN concentrations as a proportion of total N. Again no treatment effects were found. Mean day 0 ratio of WSN/TN was 0.27 and increased to 0.61 over 40 days of ensiling; a significant increase at $p<.05$. On day 40, IBG treated silage appeared to contain less WSN as a fraction of TN (0.49 vs. 0.65 for all other treatments) however this difference is not significant.

As was mentioned in the discussion of Experiment 3.1, the WSN measurement may be more meaningful if the extraction was standardized. A buffered extraction was proposed as it would extract the same fraction both before and after ensiling since the pH is controlled. This buffer should have a pK between 6 and 7 and be iso-osmolar with rumen fluid.

ADF contents of silages are shown in Table 4.4.9. Mean ADF concentration on a DM basis of original forage was 27.1% across treatments. IBG treated silage was low in ADF throughout the experiment due to a dilution effect of the glucose DM. In general, ADF content increased over the 40

Table 4.4.8 Experiment 3.4. Water-soluble nitrogen in relation to total nitrogen in response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.

WATER-SOLUBLE NITROGEN / TOTAL NITROGEN:

	C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1] 0	0.291	^{A}0.288	^{A}0.228	^{A}0.270	^{A}0.281	^{A}0.272	0.012
40	0.647	^{B}0.697	^{B}0.603	^{B}0.632	^{B}0.485	^{B}0.613	0.048
MEAN[3]	0.469	0.493	0.416	0.451	0.383	0.442	0.035
SEM	0.117	0.017	0.012	0.027	0.024	0.055	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

^{AB} day means within columns (treatments) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

SEM = standard error of mean

days as carbohydrates were utilized by microorganisms, though there was some fluctuation. Degradation of ADF and cellulose during ensiling have been reported (Morrison, 1988).

Ash response to ensiling control and treated direct-cut alfalfa is listed in Table 4.4.10. The main treatment effect in day 0 ash values was due to bentonite addition. Bentonite is largely ash so this response was expected. Some dilution of this effect was caused by the glucose addition in the IBG treatment. Across treatments, ash concentrations appeared to decrease to day 16 and then rise to day 40. Perhaps a seepage of effluent caused the first loss then fermentation of carbohydrate caused the subsequent concentration of ash through day 40. Another possibility is DM determination problems. Perhaps due to the oven temperature a volatilization of a different array of compounds on days 0, 16 and 40 the DM value, which the ash value is based on, is actually what is changing. The second possibility is not very likely, if this were true other components may have responded in the same fashion.

Table 4.4.11 lists the lactobacillus counts for each treatment throughout the experiment. Average LAB counts on fresh forage were 2.5×10^3, greater than counts observed in previous research (Langston and Bouma, 1960a, b; Fenton, 1987; Muck, 1989) though this may be due to the fact that the forage was not wilted. After inoculation, counts were increased to 6.3×10^5. Silages receiving inocula reached

peak lactobacillus populations on day 1 (7.9×10^{13}) where C silage reached its peak on day 2 (5×10^{12}) and B silage reached its peak on day 4 (5×10^{9}). No treatment comparisons within day are possible as there are no degrees of freedom for treatment. Across days, no treatment differences are significant at $p<.05$.

At no time during this experiment were the microbial colonies on the plates being counted accurately identified as being lactobacillus. Silage researchers do not commonly identify the colonies, either microscopically or by other means, on the count plates to make certain the organism of interest is in fact the organism being counted, though it would be a logical step to add to the procedure. The media used is meant to be selective for lactobacillus. The possibility of contamination cannot be ruled out however. Clean but not sterile equipment was used to blend the silage material. It is possible, though not likely, that some addition of LAB to the silage extracts could have occurred.

Acetic, propionic, and butyric acid content of silages are given in Table 4.4.12. Silages through the entire experiment contained very low levels of acetic, propionic, and butyric acids. No butyric acid was detected in any samples until day 40 when the mean level was 0.0002% of DM. No treatment effects were noted. Acetic acid concentrations increased over the 40 day experiment from a mean level of 0.0034% in fresh forage to 0.032% across day 40 silages. IBG

Table 4.4.9 Experiment 3.4. Acid detergent fiber response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.

ACID DETERGENT FIBER (%DM):

	C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1] 0	$A_{26.19}^{ij}$	26.62^{ij}	$A_{28.56}^{ij}$	$A_{28.96}^{j}$	25.06^{i}	$A_{27.08}$	0.622
16	$B_{29.66}^{ij}$	29.03^{i}	$AB_{29.21}^{i}$	$B_{31.95}^{j}$	27.45^{i}	$B_{29.46}$	0.412
40	$B_{28.87}^{ij}$	30.49^{ij}	$B_{29.81}^{ij}$	$B_{31.65}^{j}$	26.64^{i}	$B_{29.49}$	0.672
MEAN[3]	28.24^{j}	28.71^{j}	29.19^{jk}	30.85^{k}	26.39^{i}	28.68	0.580
SEM	0.401	1.164	0.214	0.560	0.962	0.783	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

AB day means within columns (treatments) with unlike superscripts differ at $p<.05$

ijk treatment means within rows (times) with unlike superscripts differ at $p<.05$

1 indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

2 day mean

3 treatment mean

SEM = standard error of mean

Table 4.4.10 Experiment 3.4. Ash response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.

ASH (%DM):

		C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1]	0	9.90^{i}	$A_{9.73^{i}}$	$A_{12.43^{jk}}$	$A_{13.10^{k}}$	$A_{11.67^{j}}$	$A_{11.37}$	0.167
	16	9.30	$B_{8.83}$	$B_{11.37}$	$B_{11.60}$	$B_{9.77}$	$C_{10.17}$	0.113
	40	9.90^{i}	$A_{9.40^{ij}}$	$B_{11.60^{k}}$	$B_{11.77^{k}}$	$B_{10.27^{j}}$	$B_{10.59}$	0.124
MEAN[3]		9.70^{i}	9.32^{i}	11.80^{k}	12.16^{k}	10.57^{j}	10.71	0.139
SEM		0.170	0.128	0.104	0.177	0.159	0.180	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

[ABC] day means within columns (treatments) with unlike superscripts differ at $p<.05$

[ijk] treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

SEM = standard error of mean

Table 4.4.11 Experiment 3.4. Lactobacilli numbers in response to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.

LACTOBACILLI NUMBERS (LOG):

		C	I	B	I+B	I+B+G	MEAN[2]	SEM
Day[1]	0	3.398	5.699	3.398	6.000	5.699	^{A}4.839	
	1	9.602	13.699	9.602	14.000	14.000	^{C}12.180	
	2	12.699	10.699	9.653	9.699	9.176	^{BC}10.390	
	4	8.699	9.362	9.699	10.699	11.176	^{BC}9.909	
	8	10.398	8.922	9.336	9.176	10.669	^{BC}9.700	
	16	9.146	8.826	9.079	10.230	7.813	^{B}9.019	
	40	9.079	8.919	8.914	8.342	8.813	^{B}8.813	
MEAN[3]		8.999	9.447	8.522	9.731	9.621	9.264	1.128
SEM							0.489	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

^{ABC} day means within columns (treatments) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos.

[2] day mean

[3] treatment mean

SEM = standard error of mean

treated silage consistently contained less acetic acid than other treatments throughout the experiment. Fresh forage contained an average of 0.0002% propionic acid on a DM basis. Day 0 concentrations differed due to a dilution of propionate by bentonite and glucose. By day 40, propionic acid concentration had risen to an average of 0.0046% of DM. On day 2, silages treated with sodium bentonite contained more propionate than C and I silages at $p<.05$. Inocula+B+G treated silage contained the least propionate on day 40, 0.0014%, and I treated silage contained the most, 0.0071%. This difference perhaps indicates a more homolactic fermentation as a result of glucose addition.

Table 4.4.12 Experiment 3.4. Volatile fatty acid production in reponse to addition of bacterial inocula and glucose to unwilted alfalfa ensiled in laboratory silos.

ACETIC ACID (%DM):

	C	I	B	IB	IBG	MEAN[2]	SEM
Day[1] 0	$A_{0.0035}$	$A_{0.0035}$	$A_{0.0042}$	$A_{0.0035}$	$A_{0.0022}$	$A_{0.0034}$	0.0012
2	$B_{0.0139}^{ij}$	$AB_{0.0098}^{i}$	$B_{0.0155}^{ij}$	$B_{0.0187}^{j}$	$AB_{0.0096}^{i}$	$B_{0.0135}$	0.0013
8	$B_{0.0203}^{j}$	$B_{0.0138}^{ij}$	$B_{0.0208}^{j}$	$AB_{0.0171}^{j}$	$AB_{0.0086}^{i}$	$B_{0.0161}$	0.0012
40	$C_{0.0324}^{i}$	$C_{0.0424}^{i}$	$C_{0.0384}^{i}$	$C_{0.0350}^{i}$	$B_{0.0112}^{j}$	$C_{0.0319}$	0.0025
MEAN[3]	0.0175^{i}	0.0174^{i}	0.0197^{i}	0.0186^{i}	0.0079^{j}	0.0162	0.0016
SEM	0.0012	0.0018	0.0015	0.0027	0.0016	0.0018	

PROPIONIC ACID (%DM):

	C	I	B	IB	IBG	MEAN[2]	SEM
Day[1] 0	$A_{0.0006}^{i}$	$A_{0.0006}^{i}$	$A_{0.0000}^{j}$	$A_{0.0000}^{j}$	0.0000^{j}	$A_{0.0002}$	0.0000
2	$A_{0.0000}^{i}$	$A_{0.0000}^{i}$	$A_{0.0013}^{j}$	$A_{0.0015}^{j}$	0.0010^{j}	$A_{0.0008}$	0.0001
8	$B_{0.0015}$	$A_{0.0011}$	$A_{0.0019}$	$A_{0.0022}$	0.0011	$B_{0.0015}$	0.0002
40	$C_{0.0032}^{ij}$	$B_{0.0071}^{k}$	$B_{0.0048}^{jk}$	$B_{0.0064}^{jk}$	0.0014^{i}	$C_{0.0046}$	0.0018
MEAN[3]	0.0013^{ij}	0.0022^{k}	0.0020^{jk}	0.0025^{k}	0.0009^{i}	0.0018	0.0003
SEM	0.0001	0.0004	0.0004	0.0004	0.0004	0.0004	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

[ABC] day means within columns (treatments) with unlike superscripts differ at $p<.05$

[ijk] treatment means within rows (times) with unlike superscripts differ at $p<.05$

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

Table 4.4.12 Experiment 3.4. Volatile fatty acid production in response to addition of bacterial inocula and sucrose to alfalfa ensiled in laboratory silos.

BUTYRIC ACID (%DM):

	C	I	B	IB	IBG	MEAN[2]	SEM
Day[1] 0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
40	0.0001	0.0001	0	0.0001	0.0006	0.0002	0.0002
MEAN[3]	0.0000	0.0000	0	0.0000	0.0001	0.0000	0.0001
SEM	0.0000	0.0000	0	0.0000	0.0002	0.0001	

C, I, B, G in column headings indicate control, inocula, sodium bentonite, and glucose treatment respectively.

[1] indicates days post-ensiling. Tabular entries represent means of triplicate laboratory silos in each of two trials.

[2] day mean

[3] treatment mean

0 = none detected

SEM = standard error of mean

5.0 CONCLUSIONS

Conclusions to be drawn from these experiments are:

1. Addition of microbial inocula to wilted alfalfa silage can increase some silage fermentation end products such as lactic acid, and can hasten the decline in silage pH.
2. Sugar addition can have associative effects with microbial inocula when added to wilted alfalfa silage.
3. Inoculation of wilted alfalfa haylage did not improve milk or fat-corrected milk production but did affect weight gain in lactating Holstein cows.
4. Addition of microbial inocula to direct-cut fourth cut alfalfa silage raised lactic acid bacteria numbers and enhanced fermentation.
5. Sodium bentonite addition to unwilted alfalfa forage did not appear to improve ensiling characteristics.
6. Addition of glucose to fourth-cut unwilted alfalfa haylage enhanced silage fermentation and reduced solubilization of nitrogen.

Also more generally,

7. Addition of microbial inocula does not necessarily result

in a significant increase in numbers of viable lactic acid bacteria in the silage mass.

8. Increased number of lactic acid bacteria through addition of a microbial inocula can but does not always result in an improved silage fermentation as measured through chemical analyses.
9. Enhanced silage fermentation as measured by chemical analysis is difficult to interpret in terms of feed quality for ruminants.

Some improvements in silage analysis methods are needed. As mentioned in earlier text, WSN determination, lactic acid bacteria enumeration and sample handling and processing can easily affect data used as a criteria to evaluate silage pretreatments. Some difficulty is involved in the evaluation of chemical analyses performed in silage trials in terms of feed value for ruminants without performing a feeding or production trial. In a recent publication by Moiso and Heikonen (1989), researchers attempted to approach silage evaluation from a broader, though not completely objective, angle. Silage extracts were titrated from pH 2 to pH 12. The resultant titration curve, stored on computer disk, includes information regarding lactic acid, acetic acid, reducing sugars, amino acid, and protein degradation product levels. The amount of saliva required to raise the pH to 6.5 is also calculated. This information can be then entered into an equation, designed by the researcher according to how

he or she wishes to weight each fraction, and a more rapid and less subjective evaluation of silage is produced. This approach indicates an innovative and logical understanding of the field of forage preservation and is perhaps the direction more researchers should follow.

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