FERMENTATION AND ANIMAL PERFORMANCE WITH ALFALFA HAYLAGE TREATED WITH AMMONIA OR MICROBIAL INOCULANT

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ABSTRACT

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Plastic bag silos and large concrete stave silos were used to measure the effect of treatment of either a commercial bacterial inoculant or ammonia on fermentation parameters. Results indicate that treatment had no consistent effect on final silage pH, lactic acid, water-soluble carbohydrate, ammonia-nitrogen, soluble nitrogen as a percent of total nitrogen, or *in vitro* dry matter digestibility. Proteolysis decreased in silages treated with ammonia but not with inocula treatment.

Milk production in dairy cows and growth of steers was generally unaffected by treatment. Significant weight gain in steers fed inocula-treated haylage was followed the next year with no difference. Steers and dairy cows fed ammonia-treated haylage had lower dry matter intakes ($P<.05$).

Inocula-treated silages were less stable ($P<.05$) in aerobic storage, while ammonia was more so ($P<.05$).

A wilting trial found 40% forage dry matter to result in the greatest amount of lactic acid.

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TABLE OF ABBREVIATIONS

CRTL	Control
INOC	Inocula
AMM	Ammonia

1.0 INTRODUCTION

In recent years the practice of ensiling wilted alfalfa has gained in popularity, and the proportion of the hay crop stored in this way has increased. The reduced amount of time that the cut crop must remain in the field, when compared to haymaking, gives the operator more independence from the weather and greater flexibility in forage management. Another factor has been the ease due primarily to mechanical handling of harvesting, storing, and feeding silage compared to hay. In addition, handling the wetter forage material (30-50%) is associated with less leaf loss during harvesting. Shephard et al. 1954, compared several harvesting and storage methods of alfalfa and showed that silage had greater preservation of original forage dry matter total digestible nutrients than harvested as hay.

Legumes have long been reputed to be difficult to ensile, due to limiting amounts of fermentable carbohydrates and a high buffering capacity. In addition, several studies have shown that the numbers of viable silage organisms on fresh herbage is low and are often not comprised of acid-tolerant, homolactic types capable of driving a rapid efficient fermentation (Phillips et al. 1981). In fact, it

appears that forage handling machinery is the primary source of organisms which will later predominate the ensiling process (Woolford, 1984).

Studies done at M.S.U., utilizing experimental plastic bag silos, have shown that additions of a mixed bacterial inocula or ammonia resulted in silages with lower pH and higher lactate values than untreated alfalfa silages (unpublished data). The objectives of these experiments are to test a commercial bacterial silage inocula or ammonia and measure the fermentation effects in large field scale silos and small experimental silos. Another objective was to measure nutritive quality using growing steers and lactating cows fed haylages treated with bacterial inocula, ammonia, or left untreated.

2.0 REVIEW OF LITERATURE

2.1 Bacteria in Plant Material Before Ensiling

Most epiphytic bacteria living on forage plants are gram negative aerobes, but also occurring in lesser numbers are species of Escherichia, Klebsiella, Bacillus, Streptococcus, Leuconostoc, Lactobacillus, and Pediococcus (Edwards and McDonald, 1978). Clostridia, in endospore form, and mycelia of yeasts and molds are also present.

In practice, it is generally assumed that fresh herbage contain sufficient numbers of lactic acid bacteria of the proper type to drive a vigorous, efficient fermentation, utilizing available carbohydrates, resulting in a stable, palatable silage. However, a number of field surveys have demonstrated great variation in the number and type of microorganisms existing on forage crops (Stirling, 1956, cited by Beck, 1978; Gibson et al., 1958; Kroulik et al., 1955; Phillips et al., 1981).

Plant species (Lesins and Schultz, 1968), plant maturity (Buchner, cited by Beck, 1978), season (Watson and Nash, 1960), degree of wilting (Weisse, cited by Beck, 1978), and the amount of dead and decaying plant material

have been implicated as factors influencing the microflora on forage plants (Woolford, 1984). Forage choppers, wagons, blowers, and silos previously exposed to plant juices may also be important sources of lactic acid bacteria (Wieringa, 1960, as cited by Beck, 1978; Gibson et al., 1961, as cited by Beck, 1978; McDonald, 1976). Woolford and Wilkins (1974) compared silages made from hand-chopped and machine-chopped herbage and found that only the machine-chopped silages were of good quality.

In a recent survey covering 533 corn fields in 20 states and Ontario, Phillips and co-workers (1981) found that, although epiphytic bacteria counts were high, the number of lactobacilli essential for good ensiling was less than 100 Colony Forming Units (CFU) per gram of fresh corn plant in 42% of the samples. Low numbers of silage bacteria on living forage crops have been found in previous surveys (Gibson et al., 1958; Langston and Conner, 1962; Kroulik et al., 1955). Langston and Conner (1962) demonstrated that, although the number of epiphytic lactobacilli can be low, the presence of a few species with strong acid producing ability will determine the course of fermentation. These species are at times absent on fresh plant material. The initial numbers of bacteria on alfalfa prior to ensiling has been reported by Mucker and Conner (1985).

Lactic acid bacteria are diverse, with varying abilities to multiply, utilize substrate and acidify silage.

Differences in the ability of lactic acid bacteria to utilize certain mono- and disaccharides exist (Beck, 1978). Wood (1961) classified lactic acid bacteria into homofermentative and heterofermentative types, according to differences in fermentation products and efficiency as lactate producers. Specific requirements for certain sugars and amino acids (Brady, 1966a and 1966b) have been reported. Lactic acid bacteria have also been found to differ in acid tolerance (Akuta et al., 1970) and acetate tolerance (Beck, 1969, cited by Beck, 1978).

Studies such as these provide a basis for the hypothesis that the microbial situation in forage plants may often be one in which the natural epiphytic population of lactic acid bacteria is insufficient in number or type to make good silage, and microbial inoculation of herbage might exert biological control over the natural fermentation to maximize silage preservation.

2.2 Effects of Wilting on the Epiphytic Microflora and Ensiling Qualities of Herbage

Wilting of herbage is generally associated with a decrease in all groups of microorganisms (Pizarro, 1979). The reason for this is thought to be due to the decreasing moisture content and decreased availability of plant juices to the microflora (Lanigan, 1963; Greenhill, 1963). There is evidence that the practice of wilting forage material has a qualitative effect on silage making bacteria. Stone et al. (1944) reported that while all groups of microorganisms decreased with wilting there was a relative increase in lactic acid bacteria. This apparent selection for lactic acid bacteria is most likely a result of their increased tolerance to conditions of lower moisture. Lanigan (1964) found that ensiled herbage above 33% dry matter had no growth of undesirable bacteria which could produce poor quality silage.

During the wilting period the changes in the chemical composition of cut plant material are brought about by activity of plant enzymes. The result of this activity is that non-structural carbohydrates are broken down into constituent hexose sugars and oxidized into CO_2 and H_2O. This accounts for most of the non-mechanical dry matter loss which occurs during the wilting period and represents a loss of the most fermentable fraction of the plant material.

This loss of water soluble carbohydrate can be extensive with prolonged wilting periods having a low drying rate (Wylam, 1953). Another result of plant enyzmatic activity during wilting is the progressive degradation of true plant protein into simpler non-protein nitrogenous compounds. Increases in peptides, amides, amino acids, ammonia, and redistribution of amino acid profile have been reported (Brady, 1960; Stallings et al., 1981; Brady, 1966b).

Marsh (1979) has reviewed the effects of wilting on fermentation in the silo. The general chemical changes noted with wilted silages are associated with a restricted fermentation and include: (1) greater amounts of dry matter loss (with prolonged wilting) (McDonald and Whittenbury, 1967), (2) residual water soluble carbohydrates (Gordon, 1961), and (3) lesser amounts of all fermentation acids (McDonald et al., 1968; Gordon et al., 1965), (4) silage acidity (Jackson and Forbes, 1970), (5) water soluble nitrogen (Donaldson and Edwards, 1976), and (6) ammonical nitrogen (Gordon et al., 1965; McDonald et al., 1968). A wilted silage is characterized by less fermentative activity and a longer period to reach a stability that is less dependent upon the presence of fermentation products.

2.3 Development of Lactic Acid Fermentation

Until fresh herbage is put into the silo, gram-negative aerobic bacteria are the dominant species on the crop. Their activity is undesirable, as it contributes nothing to the preservation of the crop and depletes the supply of readily available sugars required for beneficial fermentation. As the remaining oxygen is used by the aerobes, the emergence of lactic acid bacteria begins. Early in this period, rod-type lactobacilli dominate, followed by increases in the number of cocci. By the time that anaerobiosis is completed, lactic acid bacteria are the dominant species in the herbage being ensiled. This microbiological shift during the course of fermentation has been observed by Langston and Conner (1962), Beck (1978), and Miura and co-workers (1965).

Beck (1978) studied the qualitative and quantitative changes of lactobacilli in grass and red clover silages of low and high levels of dry matter. In all silages, the homofermentative lactic acid bacteria, with the species of L. curvatus and L. plantarum arabinosus were most numerous. Homofermentative bacteria accounted for 84% of all bacteria at day four of fermentation. As fermentation progressed, the heterofermentative organisms emerged as the dominant forms of lactic acid bacteria. Dry matter content

had an effect on the final proportion of homo- and heterofermentative types. Low dry matter silage was 75% heterofermentative bacteria and the high dry matter silage 95.5%. This microbial shift during fermentation may be a succession from acid-producing bacteria that cannot tolerate the acid they produce to more acid-tolerant types. Beck (1969, cited by Beck, 1978), stated that acetate tolerance may also be a determinant of the shift in bacterial populations, though not all silages have high amounts of acetate.

The microbial shift during fermentation has been experimentally examined in test tube silos by Whittenbury and co-workers (1967), who used, with good success, Streptococcus faecalis and Lactobacillus plantarum as a mixed inoculum in low dry matter Italian ryegrass and cocksfoot silages. The fast-growing S. faecalis, which are homofermentative bacteria, were able to live aerobically as well as anaerobically, and were able to produce large amounts of lactic acid, but were not very acid tolerant. They functioned in the inoculum to rapidly lower the silage pH to a level at which the more acid tolerant L. plantarum could continue acidification.

That the inoculation of silage with lactic acid bacteria can shift the microbiological population to favor the inoculated species is clear. However, very few workers have studied the specific changes in bacterial populations

after inoculation with a particular inoculum. McDonald and co-workers (1964) found that inoculation with eight strains of homofermentative lactobacilli (3.4×10^7 organisms/gram fresh forage) resulted in silages in which homofermentative lactobacilli were the dominant organisms. In uninoculated silages, a mixture of heterofermentative and homofermentative pediococci and lactobacilli were isolated. Nishiyama and co-workers (1972) inoculated ladino clover at ensiling with Lactobacillus plantarum, L. brevis, and Leuconostoc mesenteroides and found that the principal homofermentative organisms were L. plantarum and L. casei, and the principal heterofermentative organisms were L. brevis, L. buchneri, and Leuconostoc mesenteroides. This experiment was repeated with Italian ryegrass silage (Nishiyama et al., 1973) and after a four month fermentation period, L. plantarum, L. arabinosus, L. brevis, and L. fermenti dominated the silage microflora, with small numbers of L. acidophilus and Leuconostoc dextranicum still present.

In 1909, Bouillant and Crolbois first applied the principle of microbial inoculation to improve the fermentation of a feedstuff, by applying lactic acid inoculants to beet pulp (Watson and Nash, 1960). Silage made from beet pulp residues is frequently unpalatable because of the large amounts of butyric acid formed during the natural fermentation. Inoculation with beet juice that had been seeded with pure cultures of lactic acid bacteria

resulted in a silage that was pleasant smelling and did not produce diarrhea in cows, as did the ordinary silage. The technique of inoculating beet pulp, called "lacto-pulp", became common practice in France. Bacterial additions to improve silage quality rapidly expanded in subsequent years to other crops such as potatoes, done in 1915; corn, 1911; Italian ryegrass, 1918; sunflowers, 1920; lupines, 1931; and alfalfa, 1934.

Results of this early work with silage inoculation, as reviewed by Watson and Nash (1960) and more recent reviews (Owens, 1977; Woolford, 1984; Ehle and Goodrich, 1982) were variable, ranging from no response to significant improvements in silage quality. Interpretation of these studies was complicated by uncontrolled factors such as unknown bacterial species, widely varying numbers of bacteria, unexplored chemical composition of fresh herbage, and simultaneous additions of easily fermentable materials such as molasses and whey.

Commercially available silage bacterial inoculi currently on the market are numerous and differ in the species of bacteria (monoculture versus mixed culture), dose rate, type of preparation (dried, liquid, freeze-dried), storage form (bottles, vacuum packs, feed sacks). Whittenbury (as cited by Beck, 1978) described the attributes of a good silage microorganism as follows:

1. It must be fast growing and able to compete with and dominate other microorganisms present in silage;
2. It must be homofermentative;
3. It must be acid tolerant down to a silage pH of 4.0;
4. It must possess the ability to ferment glucose, fructose, sucrose, and preferably fructosans and pentosans;
5. It should have no action on organic acids.

McCullough (1975) described qualities of a good biological silage additive. They are:

1. The cost of the additive must be less than the value of the silage 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 in untreated silage.

2.4 Water Soluble Carbohydrate as a Substrate for Silage Fermentation

The major water-soluble carbohydrates in fresh herbage are glucose, fructose, fructosans, and starches. Fructosan is the major storage carbohydrate in grasses grown in temperate areas, whereas starches fulfill this function in legumes and subtropical and tropical grasses. Grasses are generally higher in water-soluble carbohydrates than are legumes. In silage, glucose and fructose are the principal sugars available to bacteria, because fructosans, starches, and sucrose are easily broken down to glucose and fructose monomers by activity of plant enzymes or simple hydrolysis in the silo (Edwards and McDonald, 1978).

The amount of water-soluble carbohydrate available for fermentation varies widely. Factors that are thought to influence the level or availability of water soluble-carbohydrate in forage are weather conditions (King, 1983), fertilizer application (Jones, 1970), plant species (Church and Pond, 1974), field conditioning (Gibson et al., 1958), wilting (Kung et al., 1982), time of day and wilting conditions (Woolford, 1984), breakdown of hemicellulose (Dewar et al., 1963), and addition of sugars (Thomas, 1978). The structural carbohydrates appear to be of little importance in the ensiling process, though Dewar et al.

(1963) reported some breakdown of hemicellulose through acid hydrolysis or action of plant hemicellulases.

The available water soluble-carbohydrate is utilized by silage lactic acid bacteria by way of two fermentable pathways, which differ in fermentation products and efficiency with which they produce lactate (Whittenbury et al., 1967) (Table 1). The most desirable bacteria are of the homofermentative type, which under anaerobic conditions utilize one mole of glucose or fructose to produce two moles of lactate. The other type is the heterofermentative pathway, yielding one mole of lactate, one mole of ethanol, and one mole of carbon dioxide when glucose is the substrate (Equation D). When fructose is the substrate, the products are lactate, mannitol, acetic acid, and carbon dioxide (Equation E). Homofermentative and heterofermentative microorganisms both utilize pentoses to yield one mole of lactate and one mole of acetate (Equations C and F).

The dry matter recoveries from the homolactic fermentation of glucose and fructose are 100% and 99.3%, respectively, indicating a very efficient utilization. On the other hand, a heterolactic fermentation with glucose as substrate results in a substantial loss of dry matter (24%), but because of the formation of high energy compounds (such as ethanol) energy losses are small (1.7%) (McDonald et al., 1973); with fructose as the substrate there is a 5% dry matter loss and a 1% energy loss. The products of the

heterofermentative pathway are reduced compounds (such as mannitol and ethanol) that are not further metabolizable by silage bacteria, so they do not contribute further to fermentation.

Therefore it is clear that the type of lactic acid bacteria present and the glucose to fructose ratio exert large effects on the efficiency of water-soluble carbohydrate utilization for fermentation. The situation is further complicated when one considers that lactic acid-producing bacteria can use organic acids for substrate.

Table 1. Homofermentative and Heterofermentative Equations.

Homofermentative Equations

A 1 glucose $\longrightarrow$ 2 lactic acid

B 1 fructose $\longrightarrow$ 2 lactic acid

C 1 pentose $\longrightarrow$ 1 lactic acid + 1 acetic acid

Heterofermentative Equations

D 1 glucose $\longrightarrow$ 1 lactic acid + 1 ethanol + 1 carbon dioxide

E 3 fructose $\longrightarrow$ 1 lactic acid + 2 mannitol + 1 acetic acid

F 1 pentose $\longrightarrow$ 1 lactic acid + acetic acid

2.5 Organic Acid as a Substrate for Silage Fermentation

A large number of organic acids are present in herbage, principally citrate, malate, phosphate, and glycerate. Organic acids and their salts are the chief constituents in the buffering systems of plants. Organic acid concentrations in legumes are higher than in grasses. Fauconniau and Jarrige (1954), cited by Edwards and McDonald (1978), reported concentrations of organic acids of 0.2 to 0.6% in grasses and 0.6 to 0.8% in legumes on a dry matter basis. McDonald et al. (1964), found the organic acid content of alfalfa and red clover to be nearly twice as high as that seen in grasses. Plant organic acids buffer best within the 4 to 6 range of pH and this corresponds to the critical range for silage making. The buffering capacity of herbage can be quantified by the amount of lactic acid necessary to titrate to a pH of 4 (McDonald et al., 1964; Playne and McDonald, 1966). In grasses (cocksfoot, timothy, and ryegrass), this is generally equivalent to about 3% of dry matter as lactic acid, and for legumes (alfalfa, red clover), it is almost twice as high, or around 6% lactic acid.

The greater buffering capacity in legumes is primarily due to the higher organic acid content, but other contributing factors could include the high level of cations, such as Ca^{++} and Mg^{++}, and higher levels of

protein. High buffering capacity and low sugar content in legumes are consistent with field observations of difficulty in successfully ensiling alfalfa and clovers.

The early stages of fermentation are characterized by the dissimilation of organic acids by lactic acid bacteria. Homofermentative and heterofermentative organisms utilize organic acids in a variety of ways. The main products of citrate and malate fermentation by lactic acid bacteria were given by Whittenbury (1967) (Table 2). More detailed pathways have been described by Edwards and McDonald (1978). The overall action results in either neutral products (acetone, 2,3 butanediol, and ethanol) or the release of cations, carbon dioxide, and formation of acetate and lactate. These latter compounds increase the buffering power of ensilage and the amount of acid required to lower the pH.

Lactic acid bacteria can also ferment pentoses, xylose, and arabinose which are formed from degradation of hemicellulose (Dewar et al., 1963). One effect of wilting is to decrease the buffering capacity of fresh herbage. Red clover wilted from 14% to 32% dry matter was shown to have an 18% decrease in buffering capacity. This in turn lessens the increase of buffering capacity typically occurring early in fermentation (Playne and McDonald, 1966).

Table 2. Fermentation Equations Utilized by Lactic Acid Producing Bacteria with Citrate or Malate as Substrate.

A. 1 citric acid ⟶ 2 acetic acid + 1 formic acid + 1 carbon dioxide

OR

2 citric acid ⟶ 2 acetic acid + 1 acetone (or 2,3 butanediol) + 4 carbon dioxide

OR

2 citric acid ⟶ 3 acetic acid + 1 lactic acid + 3 carbon dioxide

B. 1 malic acid ⟶ 1 acetone (or 2,3 butanediol) + 4 carbon dioxide

OR

2 malic acid ⟶ 1 acetone (or 2,3 butanediol) + 4 carbon dioxide

OR

1 malic acid ⟶ 1 acetic acid (or ethanol) + 1 formic acid + 1 carbon dioxide

2.6 Fate of Plant Protein During Fermentation

The harvesting of forage is associated with a rapid and extensive period of proteolysis. The degradation is initiated by the activity of plant enzymes which remain active until a sufficiently low pH or high dry matter is attained. The nitrogenous changes occurring during wilting are characterized by a decrease in true plant protein with increases in water-soluble nitrogen (Marsh, 1979), as well as a redistribution of the amino acid pattern. Kemble and MacPherson (1954) reported a 20% breakdown in plant protein to free amino acids during the first three days of wilting. The degradation products of protein appear to be larger polypeptides with only limited amounts of free amino acids or ammonia (Stallings et al., 1981). Proteolysis due to plant enzymes continues in the silo during the early period of the ensiling process. Bergen et al. (1974) reported a 27.2% increase in soluble nitrogen during the first 10 days of ensiling in corn silage. These increases where slowed as the fermentation advanced and silage pH decreased. Increases in ammonia-nitrogen during the ensiling period has been well documented (Church and Pond, 1974).

After anaerobiosis is achieved, proteolysis can continue when clostridial bacteria become active (Watson and Nash, 1960). Clostridial growth is governed principally by silage pH and water content. It is the activity of lactic

acid-producing bacteria that lowers silage pH to a level that clostridial growth is inhibited. The silage pH necessary to inhibit clostridial growth is inversely related to the water content of the silage.

The metabolic products associated with a clostridial-derived proteolysis are volatile fatty acids, ammonia, and a wide variety of amines. Some have stated that a DM content above 31% and/or a pH below 4.5 will suppress clostridial growth (Woolford, 1984).

In addition, lactic acid-producing bacteria are able to ferment amino acids (Rodwell, 1953). Brady (1966a) demonstrated that L. plantarum and L. brevis can deaminate serine, arginine, glutamine, and asparagine.

Apparently, even under the most ideal conditions some proteolysis will occur. The rate of fermentation is of primary importance in minimizing the extent of proteolysis from plant enzymes and clostridia. Stimulation of silage with suitable lactic acid bacteria that will hasten the rate of acid production could minimize proteolysis.

2.7 Aerobic Stability of Silage

After plant material has undergone fermentation and the silo is opened, the silage surface is exposed to oxygen. In practice, this occurs between the time the silage nears the surface of the exposed face and when the animal consumes it, a period lasting a few hours up to several days. Upon exposure to oxygen, conditions become favorable for the proliferation of microorganisms, molds, and yeasts, but bacterial species are also present and are believed to be first to proliferate (Woolford, 1984). Treating silage with antibiotics which were either antimycotic or antibacterial inhibited the deterioration of silages (Woolford and Cook, 1978), suggesting that bacteria rather than yeasts and molds were responsible for deterioration. Bacteria isolated from these silages were lactobacilli, which apparently used lactic acid as a substrate for growth. Other organisms which have been identified include Candida krusei, Pichia fermentans, and Ansenula anomala (Honig and Woolford, 1979; Ohyama et al., 1979). Identical or closely related species were identified by Ohyama and Hara (1975) and Moon and Ely (1979). High yeast counts were associated with unstable silages (Ohyama and Hara, 1975) which earlier had exhibited increases in temperature, pH, and losses of water soluble carbohydrate. Other workers (Britt and Huber, 1975; Britt et al., 1975) identified the molds in deteriorating corn

silage as aspergillus, penicillin, and mucor. Ohyama and Hara (1975) showed that silages with high counts of molds exhibited a delayed but higher maximum temperature, accompanied by greater losses of lactic acid.

Microorganisms can be indigenous to the silage and have survived fermentation in the dormant form, they could be airborne or a result of inoculation from handling and unloading machinery (Watson and Nash, 1960). As the microbes grow, they utilize plant components or fermentation products for substrate, resulting in chemical changes which decrease the nutritive quality, and animal acceptability of silage. During deterioration, easily oxidizable compounds are utilized by growing aerobic and facultatively anaerobic bacteria to produce heat, carbon dioxide, and water; with more extensive deterioration, ammonia is released through deamination. The loss of carbon from the system (as carbon dioxide) represents dry matter loss. The amount of dry matter loss during the feeding out period is variable and ranges from essentially 0 up to 32% of the original silage dry matter (Honig and Woolford, 1979).

Crawshaw and Woolford (1979), discussed the factors influencing the stability of a silage or its susceptibility to deterioration. The type of silo, the size of the exposed silage face, the rate of filling the silo, density of the silage, extent of the disturbance of the silage face, the length of chop, and the rate at which the silo is emptied

all influence the penetration of oxygen into the silage mass. Ohyama et al. (1975) state that slow silo filling facilitates the growth of aerobic microorganisms, some of which can remain dormant during the fermentation period, but resume growth upon reexposure to oxygen. Disturbance of the face of bunker silos by upward movement of buckets on tractors to obtain the silage for feeding can greatly increase oxygen penetration into the face area (Woolford, 1984). The popular practice in the United States of wilting herbage before ensiling also facilitates the establishment of aerobic microorganisms on plant material.

The extent and type of fermentation that the plant material has undergone exerts an influence on the stability of silage after exposure to air. Silages that are well fermented with a high content of fermentation acids are generally more stable than those that have undergone a more restricted fermentation (high dry matter or acid treated) (Ohyama and Hara, 1975; Woolford, 1975; Ohyama and Masaki, 1975).

Silages that have undergone a non-lactate type of fermentation possess greater stability than lactate fermentations. This stability has been attributed to the presence of larger chain organic acids such as propionate and those of greater carbon length (Moon and Ely, 1979; Moon et al., 1980; Woolford, 1975). Ohyama and Masaki (1977) reported that no deterioration occurred in Italian ryegrass

silages that contained more than 0.5% butyric acid. He compared C_1 to C_{12} acids (formic to lauric) and found that antimicrobial activity increased with chain length. This effect was enhanced at lower pH values.

High ambient temperature also increases the rate at which microorganisms respire and grow, which in turn determines the rate of deterioration. Ohyama and Masaki (1975) observed that Italian ryegrass silages exposed to ambient temperatures of 25 to 30 C were less stable than those at 10 to 15 C; at the lower temperatures no deterioration was observed. Honig and Woolford (1979) showed that maximum temperature and sum of temperature over time correlated well with dry matter loss.

A number of investigators have explored relationships between the chemical composition of silage and its propensity for deterioration (Ohyama and McDonald, 1975). Of the parameters investigated (silage dry matter content, residual water-soluble carbohydrates, volatile basic nitrogen, and lactic acid), none were consistently correlated with silage stability. As was mentioned earlier, there is evidence that longer chain volatile fatty acids impart stability to silage. Silage having propionic acid at ensiling is stable when fed and has no aerobic deterioration (Yu and Thomas, 1975b).

Changes in silage during deterioration are decreased dry matter, water soluble-carbohydrate, and most fermentation acids and increases in pH, CO_2 production, and silage temperature (Ohyama et al., 1975). The dissimilation of fermentation acids results in a corresponding increase in silage pH.

As dry matter is lost, mainly at the expense of the non-structural carbohydrate, there is a corresponding increase in the concentration of crude fiber, crude protein, and ash (Honig and Woolford, 1979). In the more advanced stages of deterioration, nitrogen may be lost from the deamination of amino acids by the action of proteolytic bacteria and yeasts. This loss is usually at the expense of true protein (Honig and Woolford, 1979; Moon et al., 1980; Ohyama et al., 1971; Ohyama et al., 1975).

The effect of lactic acid bacteria additions on aerobic stability of silages has shown conflicting results. Moon et al. (1980) studied aerobic deterioration of wheat, lucerne, and maize silages prepared with Lactobacillus acidophilus and a yeast (Candida). Greater increases in pH and temperature at 48 hours were shown with the treated silages. Theuninck et al. (1981) treated corn silages prepared with an unspecified lactic acid bacteria and reported greater peak temperatures and lower recovery of digestible energy compared to controls after eight days of exposure to air. In another trial an additive consisting of a mixture of

lactic acid bacteria and Aspergillus oryzae improved stability of corn silage in silos evacuated of oxygen while showing no improvement in unevacuated silos. On the other hand, Ohyama et al. (1973) observed that the impairment of fermentation by the introduction of oxygen during the first four days of the fermentation period could be offset with the addition of Lactobacillus plantarum and glucose at the time of ensiling.

3.0 MATERIALS AND METHODS

3.1 Fermentation of Microbial Inocula or Ammonium Hydroxide Treated Alfalfa Ensiled in Small Experimental Silos

First cutting alfalfa-grass (60:40) was cut and wilted to about 43% dry matter. Four lots of this chopped forage weighing approximately 200 kg each, were shoveled into a portable feed mixer and mixed with treatments listed in Table 3.

The inoculant was an air dried, mixed bacterial culture from Furst McNess Co., Freeport, IL containing Lactobacillus plantarum (homofermentative), L. brevis (heterofermentative), and Pediococcus acidilactici (homofermentative), purported to contain at least 8×10^9 colony forming units (CFU) of each organism in each pound (0.45 kg) of inoculum. This gives a total of 24×10^9 organisms added in a pound (0.45 kg) of inocula. The 0.05% inocula treatment corresponds to the manufacturer's recommendation, the higher level (0.15%) represents three times that recommendation.

Microbiological counts were not performed on any of the treatments, therefore actual microbial counts are not

available on the inoculum, plant material, or resultant silages.

Inocula or ammonium hydroxide was applied as the chopped forage was being mixed. All batches were mixed for five minutes, then the chopped forage was packed into double-lined polyethylene bags. Bags were evacuated of air with a large vacuum cleaner and sealed immediately upon removal of the vacuum hose by twisting the bag and folding the twisted section upon itself and tying with a string. Each bag was checked for punctures and stored in a covered barn. Experimental silos in this experiment averaged 27 kilograms (60 lb).

Plant material was fermented in silos for 0, 3, 7, or 50 days. Samples were taken by mixing the entire silo bag contents and subsampling from at least five random locations.

Table 3. Treatments Used in Experimental and Concrete-Stave Silos.

A. Experimental Silos (3.1 and 3.2)

1) Control
2) .05% Bacterial Inoculum*
3) 1.4% Ammonium Hydroxide (fresh forage basis)

B. Concrete Stave Silos (3.3 and 3.4)

1) Control
2) Ammonium Hydroxide .66% NH_3 (at 40% dry matter)
3) Dried microbial inoculant* .05% (on fresh forage basis)

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*Source of inocula in all trials was the Furst and McNess Co., Freeport, IL 61032.

3.2 Fermentation of Microbial Inocula or Ammonium Hydroxide Treated Alfalfa Ensiled at Four Dry Matter Levels

Second cutting alfalfa was harvested as direct cut forage and brought to the MSU Dairy Research and Teaching Center. It was spread thinly upon an asphalt surface and allowed to dry to four dry matters: 28.8%, 40.4%, 51.4%, and 58.4%. This was achieved by wilting 3, 5, 7, and 19 hours, respectively. Inoculant was applied at 0.05% on a fresh forage basis to forages at all four dry matter concentrations to give inocula concentrations of 83.3, 59.4, 46.7, and 41.1 x 10^6 CFU/kg forage dry matter. Likewise, 1.4% ammonium hydroxide on a fresh forage weight basis was applied to forage of each dry matter content. This supplied ammonia concentrations on a dry matter basis of 2.5%, 1.78%, 1.4%, and 1.23% for the four forage dry matters from 28.8%, 40.4%, 51.4%, and 58.4%, respectively. Herbages were mixed with treatments and packed into plastic bag silos in accordance with the description in Experiment 3.1. Each experimental silo weighed approximately 13 kg.

3.3 Large Silo Experiment (1981) — Fermentation and Nutritive Characteristics of Alfalfa Silage Treated with Bacteria Inocula or Ammonia

3.3.1 Silo Filling

Three concrete stave silos (3 x 12 meters) were filled with wilted alfalfa-grass forage (33% dry matter). The total amount of forage ensiled was approximately 46 tons. The silage treatments were as shown in Table 3.

The diluted ammonium hydroxide was applied by delivering a calibrated amount at the blower mouth as the silo was filled. The dry inoculum was applied from a can perforated with holes to allow continuous delivery of inoculum to the plant material as it entered the blower. The proper amount was weighed and put on as each load was unloaded. Alternate wagon loads were placed in the two treated silos in one day, and the untreated silo was filled the following day. Forage dry matter blown into each of the silos was approximately 14 tons. While filling, samples from each wagon load were taken, composited for every three wagon loads, and frozen at -20°C until subsequent analyses.

3.3.2 Silo Temperatures

During filling, thermocouples were placed near the center of the silos' diameters through small holes in silo

doors at elevations corresponding to the lower, middle, and upper portions of each silo. Slack wire was left near the inside of each silo door to allow for settling of the silo contents. Despite this, three thermocouples broke inside the door the day after installation. These thermocouples were replaced with difficulty by boring a hole with sections of pipe through holes in the silo doors. Silo temperatures were monitored daily for the first eight days of ensiling.

3.3.3 Aerobic Stability

Aerobic stability of treated and untreated haylages was studied during weeks four and eight of the feeding out period. Thirteen kg of haylage was placed in 30 liter plastic buckets, compacted by using a 10 pound weight and allowed to deteriorate in a warm room for 0, 1, 3, 5, or 7 days. Two thermocouples were placed in the center of each bucket, duplicate buckets were sampled for all treatments at each time interval. Samples were collected by mixing the entire bucket contents and taking random subsamples of approximately 100 g. Samples were immediately taken to the laboratory for pH determination. Changes in temperature and pH served as indices of haylage stability.

3.3.4 Steer Trial

Forty-eight young Holstein steers, averaging 220 kg body weight, were brought to the MSU Beef Cattle Research Center for a growth trial. Upon arrival, the steers were weighed, ear tagged, and placed on an all-haylage diet. The steers were blocked according to body-weight and randomly assigned to six pens of eight animals each (two pens per treatment).

After a seven day pretreatment period, the steers were fed the experimental diets (Table 4). During the first five weeks (period one), the steers received haylage ad libitum supplemented with minerals and vitamins A and D. For the final five weeks (period two), high moisture shelled corn was added at a rate of 1% of group's mean body weight for each treatment. Amount fed was adjusted weekly. Composition of experimental diets are listed in Table 4.

Steers were weighed at 14 day intervals for the first four weeks and once weekly for the last six weeks. Steers were fed once daily. Feed was offered in amounts that would maintain feed refusal at about 10%. Haylage samples were collected three days per week and composited weekly.

3.3.5 Dairy Cow Trial

Twenty-four lactating Holstein cows were blocked into three groups according to milk production during a 14 day

pretreatment period. The three treatment groups were balanced for number of days in lactation and assigned one of three diets containing untreated, inoculated or ammonia-treated haylage. Concentrate was also fed at a rate of 1 kg for every 4 kg milk produced. Ingredient composition is listed in Table 4. Untreated haylage was fed to all cows during a seven-day adjustment period and the comparison trial followed for the next 70 days. Amount of feed was adjusted bi-weekly and feed refusal was kept at 10%. Milk yields and feed intakes were recorded daily. Milk samples were taken bi-weekly and were composited for the morning and evening milkings. These samples were transported to the Michigan DHIA laboratory for determination of total solids, protein, and butterfat. Solids-non-fat was calculated by difference between the total solids and milk fat. Samples of haylage and total mixed ration were taken three times per week, composited, and frozen for subsequent analyses. Cows were weighed on two consecutive days during the pre-trial period and again on the final two days of the 70 day trial period. The steers and cows were fed concurrently.

Table 4. Ingredient Composition of Experimental Diets Fed During Lactation and Growth Experiments — Large Silo Experiments (3.3 and 3.4).

Lactating Cows

Ingredients	Experiment 3.3.5 (1981)	Experiment 3.4.3 (1982)
Animal/treatment	12	12
Alfalfa silage (% as fed)	73.3	70.8
High moisture corn (% as fed)	25.0	25.0
Soybean meal[a] (% as fed)	1.1	3.8
Trace mineralized salt (% as fed)	0.22	0.22
Magnesium oxide (% as fed)	0.13	0.13
Monosodium phosphate (% as fed)	0.22	0.22
Vitamin A (% as fed)	0.0022	0.0022
Vitamin D (% as fed)	0.0044	0.0044

Growing Steers

Ingredients	Experiment 3.3.4 (1981)	Experiment 3.4.2 (1982)
Animal/treatment	16	16
Alfalfa silage	ad libitum	ad libitum
High moisture corn[b]	1% of body weight	
Trace mineralized salt	30 g/steer/day	30 g/steer day
Monosodium phosphate	28 g/steer/day	28 g/steer/day
Vitamin A[c]	20,000 IU/steer/day	20,000 IU/steer/day
Vitamin D[c]	25,000 IU/steer/day	5,000 IU/steer/day

[a]44% crude protein.

[b]Added at a rate of 1% of the average body weight of each pen, adjusted at each weighing. This was fed for the last 35 days (Experiment 3.3.4).

[c]IU/day.

3.4 Large Silo Experiment (1982) — Fermentation Characteristics and Animal Performance when Fed Alfalfa Silage Treated with Bacterial Inocula

3.4.1 Silo Filling

Two concrete stave silos (4 x 15 meters) were filled with wilted alfalfa-grass forage (40% dry matter). One silo was untreated while the other received commercial microbial inoculum at a rate of 454 g per ton of chopped forage.

The dry inoculum was applied from a can perforated with holes to allow continuous delivery of inoculum to the plant material as it entered the blower.

The two silos were filled by alternating wagon loads between them. One day was required to fill both silos. While filling, samples from each wagon load were taken, composited for every three wagon loads, and frozen at -20°C until subsequent analyses.

3.4.2 Steer Trials

Thirty two Charolais steers, averaging 227 kg body weight, were brought to the MSU Beef Cattle Research Center for a growth trial. Upon arrival, all steers were ear-tagged, dewormed, weighed, and put on an all-haylage diet. The steers were randomly assigned to groups according to initial body weight.

Four pens of eight steers each were used. Animals in the first and third pens received treated haylage, while those in the second and fourth pens received untreated haylage.

After a seven day adjustment period, the steers were fed the experimental diets (Table 4).

Steers were weighed at 14 day intervals for the 6 week trial period. Steers were fed once daily and the amount fed was adjusted to allow 10% feed refusal. Haylage samples were collected three days per week and composited weekly.

3.4.3 Dairy Cow Trial

Twenty lactating Holstein cows were blocked into two groups during a seven day pre-treatment period. Groups were balanced for number of days in lactation and assigned one of two diets containing untreated or inocula treated haylage. Concentrate was also fed at a rate of 1 kg for every 4 kg of milk produced. Experimental diet compositions are listed in Table 4. The comparison trial lasted 56 days. Feed intakes were adjusted bi-weekly and set to allow for 10% feed refusal. Milk samples were collected bi-weekly and composited for the morning and evening milking. Milk samples were handled as described in Section 3.3.5.

3.5 Preparation of Samples

3.5.1 Water Extract Preparation

For each sample, 20 g of silage was added to 100 ml of distilled water, placed in a plastic cup and allowed to stand 15 minutes before the pH determination on a Beckman pH meter equipped with a glass electrode. Contents of each cup were then homogenized in a Sorvall Omnimixer for one minute, strained through four layers of cheesecloth, and centrifuged at 27,000 x gravity for 20 minutes. The supernatant was stored at -20°C until thawed for determinations of water-soluble components. To inhibit mold growth during storage, two or more grains of thymol were added.

3.5.2 Dry Matter Determinations

Dry matter was determined on forage and silage samples by drying at 60°C in a forced air oven for 48 hours. Samples for fiber and certain other analyses were air-dried by spreading approximately 500 g on a tray for 48 to 72 hours. To facilitate drying, a fan was placed nearby to circulate air and the silage was occasionally stirred. Samples dried in this manner were then ground in a Wiley mill through a 1 mm screen. Dry matter contents of air-dried samples were determined in a 100°C forced air oven for 24 hours. The air-dried samples were used in the

determination of *in vitro* dry matter digestibility, acid detergent fiber (ADF), and acid detergent insoluble nitrogen (ADIN).

3.5.3 Nitrogen Fractions

Total and soluble nitrogen were determined by the Kjeldahl procedure (A.O.A.C., 1975) using fresh silage samples and 10 ml of water extracts, respectively. ADIN was determined by Kjeldahl on the ADF residue. Ammonia nitrogen was measured with a specific ion ammonia electrode using an appropriate aliquot of the water extract.

3.5.4 Measurements of Other Fermentation Changes

Water soluble carbohydrate was assayed colorimetrically using the method of DuBois et al. (1951) and DuBois et al. (1956) by using appropriate aliquots of the water soluble extract. Water soluble carbohydrate was quantified by regression on a standard curve made of different concentrations of a 50:50 mixture of glucose and xylose.

Lactic acid was determined using appropriate aliquots of water soluble extract according to the procedure of Barker and Summerson (1941). Acid detergent fiber was determined using a modification of the procedure of Goering and Van Soest (1970) used in this laboratory. *In vitro* dry

matter digestibility was by the Tilly-Terry Method, and modified by Tinnet and Thomas (1976).

3.5.5 Statistical Analysis

Statistical analysis of fermentation parameters was made by using repeat measurement design, with mean comparisons by Bonnferroni's T-test, as described by Gill (1978). Animal performance data were analyzed as a repeat measurement design with blocking of subject. Steers were blocked according to body weight while dairy cows were blocked by pre-trial milk production. Means of the variables measured in the diary trial were co-varied with measurements of the pretrial period.

4.0 RESULTS AND DISCUSSION

4.1 Results and Discussion of Experiment 3.1

The influence of inoculation of lactic acid bacteria and ammonia additions on the various fermentation parameters measured in this experiment are described in Tables 5 through 9.

Dry matter content of fresh chopped herbage averaged 44.1% for all treatments. Dry matter for inocula treatments was increased ($P<.05$) on day 0, 7, 50, and overall (Table 5). This may have been a result in part of the addition of dry inocula, though it cannot account for the entire increase. Differences in initial dry matter content of the forages may have been due to the sequence in which treatments were prepared. Control silos were packed first, followed by ammonium hydroxide, 0.1% inocula, and finally 0.05% inocula. During the 3 to 3½ hours necessary to complete the filling of experimental silos, there may have been a progressive drying of the chopped herbage, resulting in higher initial dry matter content for the inoculated forage ensiled later in the afternoon.

At 50 days of ensiling, the percent dry matter was lower ($P<.05$) for inocula treated silages when compared to the control silage. Weight loss (kg) of the ensiled mass during the 50 day ensiling period (Table 5) was not greatly different among treatments, but dry matter loss expressed as a percent of initial was greatest ($P<.05$) for treated silages (Table 5). This suggests either a greater loss of dry matter through gaseous losses, primarily CO_2, in treated silages, or more likely an error in dry matter determination due to the volatilization of fermentation products in the more fermented silages. This would result in an artifactual dry matter loss. Increased dry matter loss with ammonia treatment measured in this experiment would contradict the studies of Goering and Waldo (1980), who reported a 5% higher recovery of dry matter and Honig and Zimmer (1975), who observed lower CO_2 production during ensiling of ammonia treated corn silages. Increased dry matter loss has been reported by other workers, who inoculated silages with lactic acid bacteria (Podkowka and Pauli, 1973; Buchanan-Smith and Yao, 1980, 1981). This is not a consistent finding, however, as others have found decreased dry matter recoveries (Rakshit and Voelker, 1981; Ely et al., 1979; Drake et al., 1981).

Due to its alkalinity, the ammonium hydroxide treatment resulted in higher mean pH values ($P<.05$) each day but especially for the 0 time samples (Table 6). Inoculated

samples also tended to be higher in initial pH values than were controls; this increase was significant ($P<.05$) for the high level of inoculation. Days of ensiling was significant for pH, since the pH decreased in all silages over time as expected. At days 3 and 7, the pH of inoculated silages had decreased more rapidly than the control ($P<.05$), suggesting a more rapid rate of fermentation in microbially stimulated silages. Silages inoculated at the high rate reached a stable pH by day 3, while other treatments did not reach a stable pH until day 7. At the end of the measured ensiling period, silages had a mean pH below 4.8 and all were of excellent quality, as determined by visual inspection and odor. The inoculated silages were lower in pH on days 3, 7, and 50, but only one was significant. Ammonium hydroxide did not alter the silage pH for day 50 though undoubtedly much of the acid produced in these silages was neutralized by the ammonia. The decrease in pH from day 0 was greatest for the ammoniated silage.

Initial water soluble carbohydrate (WSC) concentrations were not different for the four haylages (Table 6). By day 3 of ensiling the residual WSC of treated silages tended to be lower than controls ($P<.05$) but by days 7 and 50 all three treated silages had less WSC than control ($P<.05$). At day 3 treated silages had greater lactic acid concentration (Table 7). Ammonia treatment increased lactic acid concentration on days 3, 7, and 50 ($P<.05$) compared to

control. Inoculated silages had more lactic acid than control after 3 days but the differences were not always significant ($P<.05$). Of the original WSC, 41.3% was utilized in the fermentation process by day 3 (Table 6), with no differences found due to treatment. On day 7 all treatments resulted in greater utilization ($P<.05$) of the original WSC (73 to 75%) when compared to control, which averaged 43.1%. The increased WSC utilization in treated silages was apparently related to greater lactic acid contents. The trend of less residual WSC, more utilization of original WSC, and greater lactic acid content in all treated silages persisted at day 50, although not always significant ($P<.05$).

Crude protein and soluble nitrogen means are reported in Table 7. The chopped forage used in this experiment had a high proportion of grass and this observation is probably responsible for the relatively low crude protein values of the silages. Average crude protein for initial control plant material was 11.3% and all treatments had values greater than this ($P<.05$). The increase in crude protein due to non-protein nitrogen addition as ammonium hydroxide was expected and has been well documented in the literature (Church and Pond, 1974). On day 3 there must have been a sampling or laboratory error, since control silage had an unexpected high value of 13.3% protein, making it greater than values for inoculated silage. On days 7, 50, and

overall ammonia treatment increased protein content. Fluctuations observed in crude protein means also occurred with soluble nitrogen (Table 7) and soluble nitrogen expressed as a percent of total nitrogen (Table 8) are difficult to explain; they were most likely caused by a sampling error.

Water soluble nitrogen (WSN) increased ($P<.01$) with addition of ammonia and duration of ensiling. Inoculated and control silages showed similar values for WSN content as a percentage of dry matter. Progressive increases in WSN during the ensiling period is a common finding in silage; Bergen et al. (1974) attributed this to progressive proteolysis caused by the action of plant enzymes followed by acid hydrolysis and perhaps by some lactic acid bacteria. In this experiment, WSN increased by 68% in control silages during the 50 day fermentation period, whereas the increase in ammonium-hydroxide treated silages was only 49%. This is consistent with investigations of Johnson et al. (1982), who observed a decrease in WSN with ammonia additions to corn silage when compared to control.

Few differences between treated and control silages were observed when WSN was expressed as a percent of total nitrogen (Table 8). The exceptions to this were the ammonia treatment on day 7, which was lower, and the inocula treatment at day 50, which was higher.

Treatments differed in their effect on the ammonia-nitrogen concentration of alfalfa silages in this experiment (Table 9). Ammonium hydroxide treatment increased ammonia-nitrogen concentrations; when expressed on a dry matter basis, percent of total nitrogen (Table 9) and ammonia gained from day 0 ($P<.05$). Microbially inoculated silages, in contrast, exhibited lower values at days 3, 50, and overall.

Mean values for *in vitro* dry matter digestibility of 50 day silages averaged 54.2% and treatments had no significant effect. The data presented for Experiment 3.1 suggest that all silage samples were well fermented. The addition of lactic acid bacteria tended to increase the rate of fermentation, as noted by the earlier production of lactic acid, earlier decline in pH, and WSC concentration. There was no evidence to indicate a dose response with greater numbers of lactic acid bacteria added as inoculum in this experiment. The deamination of silage amino acids was lowered in silages treated with inocula. This may have been a function of the more rapid fermentation and earlier achievement of stability in the silages than in control. General proteolytic activity in these silages was apparently unaffected, however, as WSN levels were essentially similar to controls.

Ammonium hydroxide treatment also influenced the fermentation pattern of silage. A greater rate and extent

of lactic acid formation was observed, concurrent with greater utilization of the water-soluble carbohydrate fraction of the plant material.

Treatment of alfalfa with both ammonium hydroxide and lactic acid bacteria resulted in greater wet silage dry matter losses expressed as kg silage (as is) and as a percent, over the 50 day fermentation period. The treated silages in this experiment can be presumed to have had more of their dry matter represented as volatile fermentation products as shown by their greater concentrations of lactate and lower pH. These volatiles may have been driven off during the dry matter determination at 60°C in a forced air oven, resulting in artifactually lower dry matter percents. An alternative explanation would be that there was no error in the laboratory dry matter determination and that there was truly more dry matter loss in the treated silages. The lack of significant difference in weight loss of experimental silos indicates that differences were not actually a function of treatment.

Table 5. Influence of Inoculation or Ammoniation and Time of Ensiling on Dry Matter Percentage, Silage Weight Loss, and Dry Matter Loss for the Four Alfalfa Silages[d].

Treatment[f]	Days of Ensiling[e] 0	3	7	50	Treatment Mean
Dry Matter Content (%)					
CTRL	43.4[a]	42.5[b]	42.6[b]	43.4[ac]	43.0
AMM	43.8[a]	42.6[bc]	43.2[ab]	42.1*[c]	43.0
INOC-low	44.7*[a]	44.8*[a]	44.7*[a]	41.2*[b]	43.8*
INOC-high	44.5*[a]	42.0[b]	41.8*[b]	41.5*[b]	42.4*
Day mean[g]	44.1	43.0	43.0	42.0	
Weight Loss of Silage Mass $Wt(kg)_x - Wt(kg)_o$					
CTRL	--	0.6[a]	0.7[ab]	1.3[b]	0.8
AMM	--	--	0.9[a]	0.8[a]	0.8
INOC-low	--	1.7*[a]	1.5*[a]	1.2[a]	1.5*
INOC-high	--	0.9[a]	0.8[a]	1.0[a]	0.9
Day mean[g]	--	1.1	1.0	1.1	
Loss of Dry Matter $((wt.(kg)_x \times DM(\%)_x) - (wt(kg)_o \times DM(\%)_o))/(wt(kg)_o \times DM(\%)_o)$					
CTRL	--	0.3[a]	0.8[a]	1.2[a]	0.8
AMM	--	--	1.2[a]	3.5[b]	1.6*
INOC-low	--	2.2*[a]	1.9*[a]	12.0*[b]	5.3*
INOC-high	--	4.4*[a]	2.8*[b]	4.7*[ac]	3.9*
Day mean[g]	--	2.3	1.7	5.3	

[abc] Time means within treatments (rows) with unlike superscripts differ ($P<.05$).

* Treatment means within time (columns) are different than control ($P<.05$).

[d] Experiment 3.1 (1981).

[e] Tabular entries represent averages from two experimental silos on a dry matter basis.

[f] Standard error of treatment means for dry matter (%), weight loss of silage mass, and loss of dry matter is .09, .11, and .17, respectively.

[g] Standard error of time means for dry matter (%), weight loss of silage mass, and loss of dry matter is .09, .10, and .14, respectively.

Table 6. Influence of Inoculation or Ammoniation and Time of Ensiling on pH, Water Souble Carbohydrate (Percent of Dry Matter), and Percent Loss of Water Soluble Carbohydrate in Four Alfalfa Silages[d].

Treatment[f]	Days of Ensiling[e]				Treatment Mean
	0	3	7	50	
pH of Silages					
CTRL	5.81[a]	4.98[b]	4.76[c]	4.62[c]	5.05
AMM	7.98[a]*	5.09[b]	4.83[c]*	4.76[bc]*	5.67*
INOC-low	5.96[a]*	4.53[b]*	4.28[c]*	4.39[bc]*	4.79*
INOC-high	6.02[a]*	4.46[b]*	4.37[b]*	4.44[b]*	4.82
Day mean[g]	6.45	4.72	4.52	4.56	
Water Soluble Carbohydrate, DM%					
CTRL	9.7[a]	6.0[b]	5.5[c]*	2.3[c]	5.9
AMM	9.9[a]	5.9[b]*	2.7[c]*	1.2[d]	4.9*
INOC-low	10.0[a]	5.6[b]*	2.6[c]*	1.4[c]	4.9*
INOC-high	9.3[a]	5.5[b]*	2.3[c]*	1.9[c]	4.7*
Day mean[g]	9.7	5.7	3.3	1.7	
Loss of Water Soluble Carbohydrates, % $WSC_o - WSC_x / WSC_o$					
CTRL	38.1[a]	43.1[a]*	75.9[b]*	52.3*	
AMM	40.5[a]	73.0[b]*	88.2[b]*	67.2*	
INOC-low	43.6[a]	74.3[b]*	85.5[b]*	67.9*	
INOC-high	40.9[a]	75.0[b]*	80.0[b]*	65.3*	
Day mean[g]	41.3	65.4	82.4		

[abc]Time means within treatments (rows) with unlike superscripts differ ($P<.05$).

*Treatment means within time (columns) are different than control ($P<.05$).

[d]Experiment 3.1 (1981).

[e]Tabular entries represent averages from two experimental silos on a dry matter basis.

[f]Standard error of treatment means for pH, water soluble carbohydrate (% of dry matter), and percent loss of water soluble carbohydrate is .02, .03, and .19, respectively.

[g]Standard error of time means for pH, water soluble carbohydrate (% of dry matter, and percent loss of water soluble carbohydrate is .02, .03, and .17, respectively.

Table 7. Influence of Inoculation or Ammoniation and Time of Ensiling on Crude Protein, Water Soluble Nitrogen, and Lactic Acid in Four Alfalfa Silages[e].

Treatment[g]	Days of Ensiling[f] 0	3	7	50	Treatment Mean
Crude Protein, % DM Tot. N x 6.25					
CTRL	11.3^{ac}_{*ad}	13.3^{b}_{*b}	11.8^{c}_{*c}	12.2^{c}_{*cd}	12.1_{*}
AMM	15.0^{*a}	16.3^{*ab}	13.7^{bc}	14.3^{ab}	14.8^{*}
INOC-low	12.9^{*}	12.1^{*}	12.2	12.3	12.2
INOC-high	13.5	12.2	12.2	12.3	12.5
Day mean	13.2	13.1	12.0	12.8	
Water Soluble Nitrogen, % DM					
CTRL	0.8^{a}_{*a}	1.3^{a}_{*a}	1.2^{a}_{*b}	1.3^{a}_{*c}	
AMM	1.5^{a}	1.5^{*ab}	0.9^{ab}	2.2^{b}	
INOC-low	0.8^{a}_{*a}	1.1^{*a}	1.2^{*a}	1.5^{b}	
INOC-high	0.9^{*a}	1.2^{*a}	1.1^{*a}	1.5^{b}	
Day mean	1.0	1.2	1.1	1.7	
Lactic Acid, % DM					
CTRL	0.1^{a}	1.6^{b}_{*b}	1.2^{b}_{*b}	3.4^{c}_{*c}	1.7^{*}
AMM	0.3^{a}	3.1^{b}	3.4^{b}	4.9^{*c}	2.9^{*}
INOC-low	0.3^{a}	2.5^{b}	2.8^{b}_{*b}	4.6^{*c}	2.6^{*}
INOC-high	0.3^{a}	2.6^{b}	3.4^{*b}	4.5^{bc}	2.7^{*}
Day mean	0.2	2.3	2.8	4.4	

[abcd] Time means within treatments (rows) with unlike superscripts differ ($P<.05$).

[*] Treatment means within time (columns) are different than control ($P<.05$).

[e] Experiment 3.1 (1981).

[f] Tabular entries represent averages from two experimental silos on a dry matter basis.

[g] Standard error of treatment means for crude protein, water soluble nitrogen, and lactic acid is .12, .15, and .17, respectively.

[h] Standard error of time means for crude protein, water soluble nitrogen, and lactic acid is .12, .15, and .17, respectively.

Table 8. Influence of Inoculation or Ammoniation and Time of Ensiling on Soluble Nitrogen as a Percent of Total Nitrogen and Insoluble Nitrogen in Four Alfalfa Silages[e].

Treatment[g]	Days of Ensiling[f] 0	3	7	50	Treatment Mean
Water Soluble Nitrogen, % Total Nitrogen					
CTRL	27.8[a]	38.5[ab]	40.0[bc]	44.1[bc]	37.6
AMM	38.2[*ad]	33.5[ab]	24.1[*bc]	47.6[d]	35.9
INOC-low	26.1[a]	36.3[ab]	41.7[b]	49.9[bc]	38.5
INOC-high	25.9[a]	37.1[a]	33.4[a]	56.2[*b]	38.2
Day mean[h]	29.5	36.4	34.8	49.5	
Insoluble Nitrogen	Insoluble N equals total nitrogen-soluble nitrogen (% DM)				
CTRL	1.3[a]	1.3[a]	1.1[a]	1.1[a]	1.2
AMM	1.5[a]	1.6[*a]	1.7[*a]	1.0[b]	1.4[*]
INOC-low	1.5[a]	1.2[*ab]	1.1[b]	1.1[b]	1.2
INOC-high	1.6[a]	1.2[ab]	1.3[a]	0.9[b]	1.2
Day mean[h]	1.5	1.3	1.3	1.0	

[abcd]Time means within treatments (rows) with unlike superscripts differ ($P<.05$).

[*]Treatment means within time (columns) are different than control ($P<.05$).

[e]Experiment 3.1 (1981).

[f]Tabular entries represent averages from two experimental silos on a dry matter basis.

[g]Standard error of treatment means for water soluble nitrogen and insoluble nitrogen is 1.45 and .05, respectively.

[h]Standard error of time means for water soluble nitrogen and insoluble nitrogen is 1.45 and .05, respectively.

Table 9. Influence of Inoculation or Ammoniation and Time of Ensiling on Ammonia Nitrogen, Ammonia Nitrogen as a Percent of Total Nitrogen, and Ammonia Gained in Four Alfalfa Silages[d].

Treatment[f]	Days of Ensiling[e] 0	3	7	50	Treatment Mean
Ammonia Nitrogen					
CTRL	0.033^{a}	0.199^{b}	0.182^{b}	0.340^{c}	0.169*
AMM	0.464^{*a}	0.457^{*a}	0.607^{*b}	0.881^{*c}	0.602*
INOC-low	0.051^{a}	0.085^{ab}	0.128^{b}	0.251^{*c}	0.128*
INOC-high	0.047^{a}	0.101^{a}	0.242^{b}	0.208^{*b}	0.149
Day mean[g]	0.149	0.153	0.244	0.420	
Ammonia Nitrogen Gained	NH_3-N (DMB)$_x$ - NH_3-N (DMB)$_o$				
CTRL	--	0.087^{a}	0.149^{a}	0.307^{b}	0.181
AMM	--	0.067^{a}	0.143^{b}	0.417^{*c}	0.189*
INOC-low	--	0.035^{a}	0.077^{a}	0.185^{*b}	0.100*
INOC-high	--	0.054^{a}	0.195^{b}	0.160^{*b}	0.136*
Day mean[g]	--	0.046	0.141	0.267	
Ammonia Nitrogen, % Total Nitrogen					
CTRL	1.82^{a}	5.65^{b}	9.63^{c}	17.48^{d}	8.64*
AMM	19.29^{*a}	17.50^{*a}	27.75^{*b}	38.38^{*c}	25.73*
INOC-low	2.46^{a}	4.40^{ab}	7.06^{b}	12.82^{*c}	6.68*
INOC-high	2.20^{a}	5.15^{a}	12.41^{b}	10.38^{*b}	7.58
Day mean[g]	6.44	6.84	12.28	19.82	

[abc]Time means within treatments (rows) with unlike superscripts differ ($P<.05$).

*Treatment means within time (columns) are different than control ($P<.05$).

[d]Tabular entries represent averages from two experimental silos on a dry matter basis.

[e]Experiment 3.1 (1981).

[f]Standard error of treatment means for ammonia nitrogen, ammonia gained, and ammonia nitrogen as a percent of total nitrogen is .01, .04, and .43, respectively.

[g]Standard error of time means for ammonia nitrogen, ammonia gained, and ammonia nitrogen as a percent of total nitrogen is .01, .04, and .43, respectively.

4.2 Discussion of Experiment 3.2

The experimental treatments were described in Table 3. The results are given in Tables 10 through 14.

The dry matter content (Table 10) of the silages averaged 28.9, 40.4, 51.4, and 58.4% for the four dry matter contents, similar to the range of silage dry matter used by Michigan farmers (30 to 60%). Treatments within dry matter levels did not influence dry matter content, with the exception of the 40 and 50% dry matter silages, which were increased with the addition of the dry inocula ($P<.05$). Dry matter content of silages did not change over time for any treatment.

Means of pH are presented in Table 10. The initial pH's were very similar for the non-ammonia treatments over the four dry matter contents ($P>.05$). In control and inocula-treated silages, there was a tendency for initial pH to increase with increasing dry matter.

Ammonia treatment was applied on a fresh weight basis, so the drier plant material received less ammonia per unit of dry matter and this is reflected in the initial pH (8.49 > 8.19 > 7.63 and 7.72). The same was true for the inocula treatments.

Silage pH for all silages was significantly affected by time ($P<.05$), as expected. The time necessary to reach a stable pH increased as the dry matter content of the silages

increased. Wetter silages (30 and 40% DM) took three days to reach a stable pH, while drier silages took seven days. This suggests that the rate of fermentation decreased as the dry matter of forage increased.

Inocula treatment did not hasten the rate of pH decrease, except in the 60% dry matter silages and had almost no effect on the other three. Ammonia treatment resulted in pH values greater than controls for all dry matters at 3, 7, and 21 days.

Final silage pH after 21 days of ensiling for treated silages was not lower than controls at any dry matter level, with the exception of inocula treatment at 60% dry matter ($P<.05$). These results contrast with those in Experiment 3.2.

Initial water soluble carbohydrate (Table 11) concentration levels were significantly affected by dry matter levels ($P<.05$). The average WSC contents were 11.4, 9.8, 8.7, and 7.8 for 30, 40, 50, and 60% dry matter levels, respectively. However, this loss may not occur as extensively under field conditions, since the alfalfa plant is only crimped, whereas in our study, the chopped plant was wilted. This may have led to increased respiration losses.

Water soluble carbohydrate decreased over time for all treatments, with a tendency of the higher dry matter silages to decrease more slowly. Fermentation appears to have been most extensive in the 40% dry matter silage. All 21 day

silages ensiled at 40% dry matter had residual WSC below 2.0%, while all other dry matter levels were greater than 2.0% ($P<.05$).

Ammonia-treated 21 day silages tended to have lower WSC contents than controls, but this was only significant in the 60% dry matter material ($P<.05$). Inocula treatment significantly lowered WSC content in 60% DM silages ($P<.05$).

Lactic acid contents of silages are shown in Table 11. Lactic acid increased with time in all silages ($P<.05$). By day 3 of ensiling, treated silages had formed more lactic acid than had control silages and similarly for day 7 except for the wettest silage. These differences at day 3 were significant for ammonia treated silage at 30, 40, and 50% dry matter, and for inocula at the 40% dry matter ($P<.05$). Lactic acid contents of 21 day ammonia treated silages were greater than control for all four dry matters but significantly greater ($P<.05$) than controls for only the 40 and 60% dry matter silages.

The 40% dry matter level was most conducive to lactic acid formation for all treatments. Dry matter content significantly affected the formation of lactic acid, with lower production in the drier silages ($P<.05$).

Crude protein (Table 12) was increased with the addition of ammonia ($P<.05$). Control and inocula treated silages were not different.

Ammonia treated silages had higher water soluble nitrogen contents overall due to the added ammonia (Table 12), but did not increase over the 21 day fermentation period as did the others. This result suggests that plant proteolytic enzymes were inhibited by ammonia. Inoculation of silages had no effect on plant proteolysis, when compared to controls.

As dry matter content of silage increased, there was a trend for lower water-soluble nitrogen concentrations when expressed as a percent dry matter (Table 12) or as a percent of total nitrogen (Table 13).

As determined by water soluble nitrogen, the proteolysis of plant protein is most active between days 0 and 3. After three days of ensiling, there was little change in WSN content in the silage. This is in agreement with the results of Bergen et al. (1974) and Stallings et al. (1981).

As expected, the content of ammonia-nitrogen expressed as a percent of dry matter (Table 13) and percent total nitrogen (Table 14) in ammonia treated haylages increased ($P<.05$). The wetter silages (30 and 40% DM) tended to form ammonia more rapidly and to a greater extent than did the drier silages (50 and 60%). Ammonia nitrogen increased in all silages with time ($P<.05$). Inoculation of silage did not affect ammonia-nitrogen content of silage as a percent of dry matter or total nitrogen.

There was no consistent effect of treatment on *in vitro* dry matter digestibility of alfalfa silages (Table 14). Inoculation of silage resulted in lower IVDMD in the 30 and 60% DM silages. Ammonia treatment increased IVDMD in 50% dry matter silages. There was a tendency for decreased IVDMD with increased dry matter content.

The greater water soluble carbohydrate utilization and lactic acid production noted in this experiment suggest that the fermentation is maximized at 40% DM. Ammoniation of forage stimulated lactic acid production and inhibited proteolysis, which agrees with the work of Johnson et al. (1982). The increased lactic acid with ammoniation was not accompanied by an increased utilization of WSC, possibly due to a decrease in plant respiration and/or a development of a more homofermentative population of bacteria or a increased breakdown of hemicellulose providing more lactate precursors. Inocula did not stimulate fermentation of forage unless ensiled at 60% DM and had no effect on proteolysis.

Table 10. Influence of Inoculation or Ammoniation and Time of Ensiling on Dry Matter Content and pH in Four Alfalfa Silages Having Different Dry Matter Contents[e].

Treatment[f]	Days of Ensiling[g]				Treatment Mean
	0	3	7	21	
Dry Matter Content					
CTRL	28.3^{aA}	28.7^{aA}	27.4^{aA}	27.6^{aA}	28.0^{A}
AMM	28.9^{aA}	28.4^{aA}	28.0^{aA}	27.5^{aA}	28.3^{A}
INOC	29.4^{aA}	28.7^{aA}	28.5^{aA}	27.3^{aA}	28.5^{A}
CTRL	39.6^{aB}	38.9^{aB}	38.2^{aB}	37.7^{aB}	38.6^{B}
AMM	39.6^{aB}	39.7^{aB}	39.1^{aB}	38.5^{aB}	39.2^{B}
INOC	41.9^{aB}	41.7^{a*B}	41.3^{a*B}	40.9^{a*B}	41.4^{*B}
CTRL	49.9^{aC}	51.0^{aC}	50.0^{aC}	50.1^{aC}	50.2^{C}
AMM	51.0^{aC}	51.7^{aC}	51.2^{aC}	50.5^{aC}	51.1^{C}
INOC	53.4^{a*C}	53.9^{a*C}	53.6^{a*C}	53.6^{a*C}	53.6^{*C}
CTRL	58.2^{aD}	59.2^{aD}	58.8^{aD}	58.6^{aD}	58.7^{D}
AMM	58.3^{aD}	58.2^{aD}	57.9^{aD}	58.0^{aD}	58.1^{D}
INOC	58.6^{aD}	58.8^{aD}	58.8^{aD}	58.4^{aD}	58.7^{D}
pH					
CTRL	5.74^{aA}	4.87^{bA}	4.60^{cAC}	4.57^{cA}	4.95^{A}
AMM - 29%	8.49^{a*A}	5.16^{b*A}	5.03^{bc*A}	4.91^{c*A}	5.90^{*AD}
INOC	5.62^{a*A}	4.68^{b*A}	4.78^{bc*AC}	4.90^{c*AC}	4.99^{AC}
CTRL	5.74^{aAB}	4.72^{bB}	4.46^{cB}	4.54^{cA}	4.86^{B}
AMM - 40%	8.19^{a*B}	5.16^{b*A}	5.02^{c*A}	4.94^{c*A}	5.83^{*B}
INOC	5.77^{aB}	4.64^{bA}	4.46^{cB}	4.62^{bdB}	4.87^{B}
CTRL	5.79^{aBC}	5.12^{bC}	4.71^{Cc}	4.58^{dA}	5.06^{C}
AMM - 50%	7.63^{a*C}	5.60^{b*B}	4.93^{c*A}	4.83^{c*A}	5.25^{*C}
INOC	5.78^{aB}	5.14^{bB}	4.70^{cC}	4.61^{cB}	5.06^{C}
CTRL	5.86^{aC}	5.47^{bD}	4.97^{cD}	5.11^{dB}	5.35^{D}
AMM - 60%	7.72^{a*D}	5.83^{b*C}	4.99^{cA}	4.91^{c*A}	5.86^{*BD}
INOC	5.84^{aB}	5.20^{bB}	4.79^{cC}	4.81^{c*C}	5.16^{*D}

^{ABCD}Dry matter means within treatment and time (rows) with different superscripts differ ($P<.05$).

*Treatment means with time and dry matter (columns) are different from control ($P<.05$).

^{abcd}Time means within treatments and dry matter (rows) with different superscripts differ ($P<.05$).

[e]Experiment 3.2 (1981).

[f]Standard error of treatment means for dry matter content and pH is .17 and .01, respectively.

[g]Standard error of time means for dry matter content and pH is .29 and .02, respectively.

Table 11. Influence of Inoculation or Ammoniation and Time of Ensiling on Lactic Acid and Water Soluble Carbohydrate in Four Alfalfa Silages Having Different Dry Matter Contents[e].

Treatment[f]	Days of Ensiling[g]				Treatment Mean
	0	3	7	21	
Lactic Acid	(% of Dry Matter)				
CTRL	0.3^{a}	3.8^{Bb}	5.4^{Cc}	5.3^{C}	3.7^{C}
AMM	0.3^{a}	5.4^{C*b}	6.2^{Cb}	6.1^{Bb}	4.5^{C*}
INOC	0.3^{a}	4.6^{Bb}	5.2^{Cb}	4.4^{Bb}	3.6^{C}
CTRL	0.3^{a}	5.0^{Cb}	6.2^{Cc}	8.2^{Dd}	4.9^{D}
AMM	0.2^{a}	7.9^{D*b}	8.6^{D*b}	10.1^{C*c}	6.7^{D*}
INOC	0.2^{a}	6.4^{C*b}	6.6^{Db}	7.5^{Cb}	5.2^{D}
CTRL	0.4^{a}	0.8^{Aa}	2.6^{Bb}	4.3^{Bc}	2.0^{B}
AMM	0.4^{a}	2.8^{B*b}	4.1^{Bc}	4.6^{Ac}	3.0^{AB*}
INOC	0.4^{a}	0.9^{Aa}	3.2^{Bb}	4.7^{Bc}	2.3^{B}
CTRL	0.3^{a}	0.4^{Aa}	1.3^{Ab}	1.2^{Ab}	0.8^{A}
AMM	0.3^{a}	1.3^{Ab}	2.4^{Ac}	3.7^{A*d}	1.9^{A*}
INOC	0.3^{a}	0.7^{Aab}	1.5^{Abc}	2.5^{Ac}	1.2^{A}
Water Soluble Carbohydrates					
CTRL	10.7^{Ca}	5.5^{Ab}	2.4^{Bc}	2.2^{Bc}	5.2^{A}
AMM	12.1^{C*a}	5.4^{Bb}	2.5^{Bc}	2.1^{Bc}	5.7^{C}
INOC	11.3^{Ca}	3.8^{A*b}	1.9^{Ac}	1.6^{Ac}	4.7^{AB*}
CTRL	10.9^{Ca}	5.6^{Ab}	1.5^{Ac}	1.7^{Ac}	4.9^{A}
AMM	9.2^{B*a}	5.7^{Bb}	1.2^{Ac}	1.0^{Ac}	4.3^{A*}
INOC	9.3^{B*a}	5.2^{Bb}	1.4^{Ac}	1.9^{ABc}	4.4^{A}
CTRL	8.9^{Ba}	5.5^{Ab}	3.7^{Bc}	2.9^{Bc}	5.3^{A}
AMM	8.7^{ABa}	3.9^{A*b}	2.9^{Bb}	2.3^{Bc}	4.5^{AB*}
INOC	8.4^{ABa}	5.3^{BCb}	3.7^{Bc}	2.7^{Bc}	5.0^{B}
CTRL	7.7^{Aa}	5.9^{Ab}	6.6^{Cb}	6.1^{Cb}	6.6^{B}
AMM	7.8^{Aa}	5.3^{Bb}	4.5^{C*b}	2.6^{B*c}	5.1^{B*}
INOC	7.9^{Aa}	6.2^{Cb}	5.4^{C*b}	4.2^{C*a}	6.0^{C*}

^{ABCD}Dry matter means within treatment and time (rows) with different superscripts differ ($P<.05$).

*Treatment means with time and dry matter (columns) are different from control ($P<.05$).

^{abcd}Time means within treatments and dry matter (rows) with different superscripts differ ($P<.05$).

[e]Experiment 3.2 (1981).

[f]Standard error of treatment means for lactic acid and water soluble carbohydrate is .09 and .09, respectively.

[g]Standard error of time means for lactic acid and water soluble carbohydrate is .15 and .16, respectively.

Table 12. Influence of Inoculation or Ammoniation and Time of Ensiling on Crude Protein and Water Soluble Nitrogen in Four Alfalfa Silages Having Different Dry Matter Contents[e].

Treatment[f]	Days of Ensiling[g]				
	0	3	7	21	Treatment Mean
Crude Protein					
CTRL	18.5^{Aa}	17.9^{Aa}	17.6^{Aa}	18.1^{Aa}	18.0^{A}
AMM	20.6^{Aa}	21.5^{A*a}	22.5^{A*a}	22.8^{A*a}	21.8^{AB*}
INOC	18.6^{Aa}	17.8^{Aa}	17.8^{Aa}	18.5^{a}	18.2^{A}
CTRL	17.5^{Aa}	16.9^{Aa}	16.9^{Aa}	17.0^{Aa}	17.1^{A}
AMM	22.0^{A*a}	20.4^{A*ab}	24.9^{A*b}	20.9^{AB*ac}	22.0^{B*}
INOC					
CTRL	19.9^{Aa}	17.0^{Aa}	17.9^{Ab}	17.7^{Ab}	18.4^{A}
AMM	21.1^{Ba}	19.3^{AB*a}	19.4^{Ba}	19.9^{Ba}	19.6^{BC*}
INOC	15.9^{B*a}	17.3^{Aa}	17.3^{Aa}	17.0^{Aa}	16.9^{B*}
CTRL	16.0^{Aa}	16.2^{Aa}	16.0^{Aa}	16.3^{Aa}	16.1^{B}
AMM	18.1^{BAa}	17.7^{Ba}	18.2^{Ba}	19.1^{Ba}	18.7^{C*}
INOC	16.6^{Aa}	16.9^{Aa}	15.9^{Aa}	16.7^{Aa}	16.6^{B}
Water Soluble Nitrogen					
CTRL	0.8^{Aa}	1.9^{Ab}	1.7^{Ab}	1.8^{Ac}	1.6^{A}
AMM	1.8^{A*a}	1.9^{Aab}	1.8^{Aa}	2.1^{Ab}	1.9^{A*}
INOC	1.1^{A*a}	1.7^{A*b}	1.6^{A*b}	1.8^{Ab}	1.5^{A}
CTRL	0.8^{Aa}	1.4^{Bb}	1.4^{Bb}	1.3^{Bc}	1.2^{B}
AMM	1.5^{B*a}	1.7^{AB*b}	1.7^{Ab}	1.7^{Bb}	1.6^{AB*}
INOC	0.7^{Ba}	1.3^{Bb}	1.5^{Ab}	1.4^{Bb}	1.2^{B}
CTRL	0.8^{Aa}	1.3^{Bb}	0.8^{Ca}	1.4^{Bb}	1.1^{C}
AMM	1.4^{BC*a}	1.5^{BC*a}	0.9^{Bb}	1.5^{Cc}	1.3^{BC*}
INOC	0.8^{Bac}	1.2^{Bb}	1.0^{Bc}	1.2^{BCbd}	1.1^{C}
CTRL	0.8^{Aa}	1.0^{Cb}	1.0^{Cb}	1.1^{Cb}	0.9^{D}
AMM	1.2^{C*a}	1.4^{B*b}	1.0^{Bb}	1.3^{Cac}	1.2^{C*}
INOC	0.7^{Ba}	1.2^{B*b}	1.1^{Bab}	1.1^{Cb}	1.0^{C}

^{ABCD}Dry matter means within treatment and time (rows) with different superscripts differ ($P<.05$).

*Treatment means with time and dry matter (columns) are different from control ($P<.05$).

^{abcd}Time means within treatments and dry matter (rows) with different superscripts differ ($P<.05$).

[e]Experiment 3.2 (1981).

[f]Standard error of treatment means for crude protein and water soluble nitrogen is .02 and .02, respectively.

[g]Standard error of time means for crude protein and water soluble nitrogen is .04 and .03, respectively.

Table 13. Influence of Inoculation or Ammoniation and Time of Ensiling on Soluble Nitrogen as a Percent of Total Nitrogen and Ammonia Nitrogens in Four Alfalfa Silages at Different Dry Matter Contents[e].

Treatment[f]	Days of Ensiling[g]				Treatment Mean
	0	3	7	21	
Soluble Nitrogen, % TN					
CTRL	28.0^{aA}	66.1^{bA}	61.3^{bA}	62.7^{bA}	54.5^{A}
AMM	52.2^{a*A}	55.2^{ab*A}	51.2^{ab*A}	55.8^{bA}	53.6^{A}
INOC	36.2^{aA}	57.9^{bA}	54.8^{bA}	59.5^{bA}	52.1^{A}
CTRL	28.1^{aA}	50.8^{bB}	53.4^{bA}	47.8^{bB}	45.0^{B}
AMM	41.6^{a*B}	52.2^{bA}	43.9^{abA}	53.4^{bA}	47.8^{B}
INOC	27.9^{aA}	48.4^{bAB}	55.2^{bA}	47.0^{bB}	44.6^{B}
CTRL	23.5^{acA}	49.2^{bB}	27.3^{cB}	47.9^{bdBC}	37.0^{C}
AMM	44.6^{a*AB}	48.9^{acA}	27.5^{bB}	46.1^{cAB}	41.8^{C}
INOC	30.9^{a*A}	44.2^{b*B}	36.3^{ab*B}	43.9^{b*B}	38.8^{C}
CTRL	30.2^{aA}	39.8^{abB}	37.5^{abC}	41.3^{bC}	37.2^{C}
AMM	42.5^{ab*AB}	48.6^{acA}	32.7^{bB}	42.3^{bcB}	41.5^{C}
INOC	26.7^{aA}	44.8^{bB}	41.5^{bB}	41.0^{bB}	38.5^{C}
Ammonia-Nitrogen (Percent of Dry Matter)					
CTRL	$.04^{Aa}_{B*a}$	$.31^{Bb}_{B*a}$	$.33^{Ab}_{B*a}$	$.46^{Bb}_{C*b}$	$.29^{B}_{BC*}$
AMM	$.74^{Aa}$	$.74^{Ab}$	$.80^{bB}$	1.53^{Bb}	1.28^{B}
INOC	$.07^{Aa}$	$.31^{Ab}$	$.37^{bB}$	$.42^{Bb}$	$.29^{B}$
CTRL	$.04^{Aa}_{B*a}$	$.13^{ABa}_{B*a}$	$.19^{Aa}_{B*a}$	$.32^{ABb}_{B*b}$	$.17^{AB}_{AB*}$
AMM	$.81^{B*a}$	$.88^{B*a}$	1.01^{B*a}	1.11^{B*b}	$.95^{AB*}$
INOC	$.05^{Aa}$	$.12^{Aa}$	$.19^{ABa}$	$.24^{ABa}$	$.15^{A}$
CTRL	$.04^{Aa}_{A*a}$	$.12^{ABa}_{B*b}$	$.13^{Aa}_{A*a}$	$.17^{Aa}_{A*a}$	$.11^{A}_{AB*}$
AMM	$.43^{A*a}$	$.88^{B*b}$	$.54^{A*a}$	$.67^{A*a}$	$.63^{AB*}$
INOC	$.04^{Aa}$	$.08^{Aa}$	$.12^{ABa}$	$.17^{ABa}$	$.10^{A}$
CTRL	$.04^{Aa}_{A*a}$	$.05^{Aa}_{A*a}$	$.28^{Aa}_{Aa}$	$.09^{Aa}_{A*a}$	$.11^{A}_{A*}$
AMM	$.29^{A*a}$	$.47^{A*a}$	$.34^{Aa}$	$.53^{A*a}$	$.41^{A*}$
INOC	$.04^{Aa}$	$.05^{Aa}$	$.08^{A*a}$	$.09^{Aa}$	$.07^{A}$

[ABCD] Dry matter means within treatment and time (rows) with different superscripts differ ($P<.05$).

[*] Treatment means with time and dry matter (columns) are different from control ($P<.05$).

[abcd] Time means within treatments and dry matter (rows) with different superscripts differ ($P<.05$).

[e] Experiment 3.2 (1981).

[f] Standard error of treatment means for soluble nitrogen as a percent of total nitrogen and ammonia nitrogen is .84 and .02, respectively.

[g] Standard error of time means for soluble nitrogen as a percent of total nitrogen and ammonia nitrogen is 1.46 and .04, respectively.

Table 14. Influence of Inoculation or Ammoniation and Time of Ensiling on Ammonia Nitrogen as a Percent of Total Nitrogen and In Vitro Dry Matter Digestibility[e].

Treatment[f]	Days of Ensiling[g]				
	0	3	7	21	Treatment Mean
Ammonia/Total N					
CTRL	1.5^{aA}	10.9^{bA}	11.6^{bB}	16.1^{bA}	10.0^{A}
AMM	22.4^{ac*A}	59.9^{b*A}	22.2^{c*}	42.0^{d*}	36.6^{A}
INOC	2.3^{aA}	10.9^{bA}	13.0^{bA}	14.4^{bA}	10.1^{A}
CTRL	1.5^{aA}	4.8^{abAB}	7.1^{abAB}	11.7^{bAB}	6.3^{AB}
AMM	23.2^{a*}	27.1^{ab*B}	29.9^{ab*}	33.2^{b*}	28.4^{B}
INOC	2.0^{aA}	4.4^{aAB}	7.1^{aAB}	8.8^{aAB}	5.6^{B}
CTRL	1.3^{aA}	4.3^{aAB}	4.5^{aAB}	5.9^{aB}	4.0^{B}
AMM	13.5^{ac*B}	28.5^{b*B}	17.5^{c*}	21.2^{bc*}	20.2^{c}
INOC	1.7^{aA}	2.9^{aB}	4.4^{aB}	6.1^{aB}	3.7^{B}
CTRL	1.5^{aA}	1.8^{aB}	3.1^{aB}	3.5^{aB}	2.4^{B}
AMM	10.3^{a*B}	16.7^{a*C}	11.5^{a*}	17.3^{a*}	14.0^{D}
INOC	1.7^{aA}	1.9^{aB}	3.3^{aB}	3.5^{aB}	2.6^{B}
In Vitro Dry Matter Digestibility					
CTRL				65.3	
AMM				66.4	
INOC				66.7	
CTRL				67.0	
AMM				65.9_{*}	
INOC				64.9^{*}	
CTRL				61.9_{*}	
AMM				64.7^{*}	
INOC				60.8	
CTRL				60.0	
AMM				56.7_{*}	
INOC				55.8^{*}	

^{ABCD}Dry matter means within treatment and time (rows) with different superscripts differ ($P<.05$).

*Treatment means with time and dry matter (columns) are different from control ($P<.05$).

^{abcd}Time means within treatments and dry matter (rows) with different superscripts differ ($P<.05$).

[e]Experiment 3.2 (1981).

[f]Standard error of treatment means for ammonia as a percent of total nitrogen and in vitro dry matter digestibility is .67 and .19, respectively.

[g]Standard error of time means for ammonia as a percent of total nitrogen is 1.16.

4.3 Discussion of Experiment 3.3 (1981)

4.3.1 Fermentation Characteristics, Fermentation Temperatures, and Aerobic Stability

Data presented in Table 15 suggest higher temperatures during fermentation of inocula and ammonia treated silages than in the control silages. Fermentation temperatures (expressed as degrees above ambient) after four days of ensiling averaged 13.5, 17.6, and 21.0°C for control, ammonia, and microbial inocula treated silos. Higher temperatures in treated silos may be indicative of a more active microbial population, though caution in the interpretation of these temperature data is warranted because of lack of replication of silos and thermocouples as well as the small number of data points. Temperatures in all silos tended to be greater at day 4 than when measured at day 8. This observation suggests that the peak rate of fermentation occurs before the eighth day of ensiling and is consistent with experimental silo studies which found that most of the lactic acid formation had occurred by day 7 (Kung et al., 1981). Poorer compaction in the upper section of the silo may allow for less oxygen exclusion and increased oxidative processes, which would explain the greater production of heat in this silo area (Yu and Thomas, 1975a).

Chemical composition of composite haylage samples collected for 12 weeks during the emptying of silos is shown in Table 16. Even though an attempt was made to achieve equal dry matter (DM) for all haylages, the control silage was lower than treated silages (34.1 < 40.0 and 39.7). The treated silages were ensiled as alternate loads on one day and the control ensiled the following day. This appears to be the reason for these differences in DM. Treated haylages had lower pH, residual water-soluble carbohydrate (WSC) content, water-soluble nitrogen (WSN), acid detergent insoluble nitrogen (ADIN), and in vitro dry matter digestibility (IVDMD) than did the control. Treatments increased the lactic acid content of silages and these silages were drier than controls. Generally, silages of lower DM will contain more lactic acid, but this was not the case, which suggests that inocula and ammonia treatments increased lactate concentration, though these differences may also be due to forage source and environmental effects due to differences in date of ensiling. Ammonia treatment increased the crude protein content 1.52% units above the non-ammonia treated haylages.

Deterioration of haylage, defined by increasing silage pH and temperature during aerobic storage was affected by treatments (Table 17). Inocula-treated silages increased in pH at a more rapid rate and to a greater extent than did control or ammonia silages ($P<.05$). Concurrently, there was

increased silage temperature ($P<.05$) in inoculated silage. Ammonia-treated silages, conversely, were observed not to change appreciably in pH during the 7 day deterioration period. In addition, silage temperatures were cooler for ammonia-treated silage than temperatures measured in control or inocula treated silages ($P<.05$). The lapsed time required for silage pH to reach 5.5 was 5, 7, and 3 days for control, ammonia, and inocula treated silages, respectively. Instability in control and inocula treated silages has been a feature of other investigations (Moon et al., 1980; Theuninck, 1981). The mechanism for this instability is unclear and has not been a constant finding in experiments that added lactic acid bacteria to forage (Ohyama et al., 1975). Possibly, the higher levels of lactic acid in inoculated silages serves as a substrate for the yeasts and bacteria responsible for aerobic deterioration. Alternatively the inocula could contain organisms responsible for increasing aerobic instability. Woolford (1984) found that lactobacilli can and do utilize lactic acid when WSC is low and limiting. Ammonia has been implicated to impart stability to silages during conditions of aerobic storage in the work of Huber and Britt (1975; Britt et al., 1975) and others. Antimycotic properties of ammonia have been described by Bothast (1973).

4.3.2 Production of Lactating Cows

Ingredient composition of experimental diets fed to cows is given in Table 4. Diets were formulated to meet the cows' recommended requirements (NRC, Dairy, 1978).

Responses by dairy cows fed during a 70 day trial is shown in Table 18. There was no significant difference between treatment groups in daily dry matter consumption, though there was a tendency for greatest intakes by cows fed inocula-treated and control silage and least in those fed ammonia treated silage. Milk yields were greater ($P<.05$) for cows receiving treated silages than for the control group. Composition of the milk produced by cows was similar for the three treatment groups. Milk fat percentage was slightly depressed in cows fed treated haylages, though fat corrected milk yields were not different. Efficiency of feed utilization tended to be greatest for cows fed ammonia-treated silage and least for the control group, with inoculated silage intermediate. Body weight loss during this trial was greatest for cows fed ammonia treated haylage which, in part, may explain the tendency for higher conversion of feed to milk for this group. This group also had the lowest dry matter intake.

Overall, inocula or ammonia treatment of alfalfa forage did not influence the performance of lactating cows when fed as the primary dietary ingredient.

4.3.3 Growth Trial with Holstein Steers

Ingredient composition of diets fed to steers was presented in Table 4 while results are shown in Table 19. Little gain was observed for either group of steers for the first period. All steers consumed an average 5.9 kg of dry matter daily. The energy provided in the dry matter daily consumed as wilted ensiled alfalfa was approximately 12.4 Mcal, sufficient to sustain an ADG of 0.5 kg/d (NRC, Beef, 1976). Some steers scoured while adapting to the all haylage diet and this may have restricted their growth or reduced their body weight.

Greatest gains and DM intakes ($P<.05$) were observed for steers fed inocula treated haylage for the last 35 days (high moisture corn supplemented). There was also a tendency for more efficient feed conversion by steers fed inoculated haylage when compared to control and ammoniated haylage. Ammonia-treated haylage fed steers tended to have lower dry matter intakes and converted feed to gain more efficiently than did control steers, but the differences were not statistically significant.

The results of this trial show a significant benefit of inocula treated silages when fed to steers, provided grain is also fed. Krause and Clanton (1977) reported improvement in ADG and feed efficiency of steers fed alfalfa silage treated with a microbial inocula. Olson and Voelker (1961)

observed greater growth in dairy heifers and calves when fed inocula treated alfalfa, however, others have reported no improvement in the performance of growing animals (Burghardi et al., 1977; Woods et al., 1967). A possible reason for the improved performance is an increased digestibility of nutrients with inocula treated silages. Waldo and Goering (1976), using a commercial microbial inoculum, found improved digestibility of alfalfa silage, as did McCullough (1975). It is important to note that inocula treated silages did not differ from control silages in IVDMD in the present study. The differences in this trial only occurred during the last 35 day period. One could also use the first 35 day period data and conclude control and inoculated silages were equal. Inconsistent data are frequently obtained when only 30 to 35 day trial periods are used (Thomas, J.W., personal communication). Definite conclusions are apparently not warranted from this experiment because of the short feeding periods employed.

Table 15. Temperatures During the Ensiling of Alfalfa Silages Treated with Microbial Inocula or Ammonium Hydroxide in Concrete Stave Silos[a].

Treatment	Temperatures[b]			
	Day[c]	Lower	Middle	Upper
Control	4	10.6	7.8	22.2
	8	7.2	5.8	22.7
Ammonia	4	12.8	20.0	20.0
	8	9.4	16.9	20.6
Inocula	4	12.8	18.0	33.3
	8	8.9	19.7	28.3

[a]Experiment 4.3.1 (1981).

[b]Temperatures are expressed as degrees Centigrade above that day's ambient temperature.

[c]Days of ensiling.

Table 16. Chemical Composition of Haylages Fed to Growing Steers and Lactating Dairy Cows in Large Silo Experiment 3.3.4 and 3.3.5 (1981).

Analyses	Treatments[a]					
	Control	SD	Inocula	SD	Ammonia	SD
Dry Matter Percent	34.18	2.94	40.01	1.07	39.70	1.94
pH	4.57	0.07	4.50	0.16	5.28	0.50
Lactic Acid, % DM	2.37	0.34	3.07	0.40	2.78	0.47
Water Soluble Carbohydrate, % DM	1.14	0.41	0.80	0.25	0.53	0.49
Crude Protein, % DM	15.70	0.25	15.57	1.27	17.16	0.69
Soluble Nitrogen, % DM	1.65	0.09	1.20	0.10	1.49	0.05
Ammonia Nitrogen, % DM	0.21	0.02	0.19	0.04	0.51	0.06
Acid Detergent Insoluble Nitrogen, % DM	0.31	0.06	0.30	0.04	0.28	0.06
Acid Detergent Fiber, % DM	44.93	4.92	44.66	3.96	45.75	6.56
In Vitro Dry Matter Digestibility, % DM	50.81	1.59	50.04	1.66	50.40	1.37

[a]Mean of 12 weekly composite samples.

Table 17. pH and Temperatures of Ammonia and Bacterial Inocula Treated Silages Stored in Aerobic Conditions[d].

Treatment[f]	Days of Aerobic Storage[e]				
	0	1	3	5	7
pH					
Control	4.82[a]	4.94[a]	5.14[a]	6.07[a]	7.94[b]
Ammonia	5.20[a]	5.29[a]	5.28[a]	5.22[a]	5.61[a]
Inocula	4.67[a]	5.25[a]	7.56[*b]	8.48[*b]	8.80[b]

Temperature (Temperatures expressed in degrees above that day's ambient temperature)

Treatment[h]	Days of Ensiling[g]			
	1	3	5	7
Control	-0.5[a]	-9.0[a]	+4.2[b]	+28.7[c]
Ammonia	-3.5[a]	-8.8[a]	+0.7[ab]	+5.7[*bc]
Inocula	+21.0[*ab]	+14.0[*a]	+33.5[*c]	+27.2[bc]

[abc]Time means within treatments (rows) are different than controls ($P<.05$).

[*]Treatment means within time (columns) are different than controls ($P<.05$).

[d]Experiment 3.3.

[e]Standard error of time means for the variable pH is .04.

[f]Standard error of treatment means for the variable pH is .93.

[g]Standard error of time means for the variable temperature is .73.

[h]Standard error of treatment means for the variable temperature is .56.

Table 18. Responses of Holstein Cows Fed Haylages Treated with Inocula or Ammonia[d].

Measurements	Treatments[e]			
	Control	NH_3	Inocula	SEM
Dry Matter Intake (kg/day)	18.28[a]	16.40[a]	17.18[a]	1.42
Milk Production (kg/day)	19.50[a]	21.05[b]	21.59[b]	1.58
Fat-Corrected Milk (kg/day)[f]	18.36[a]	19.31[a]	19.72[a]	1.86
Fat (%)	3.62[a]	3.43[a]	3.47[a]	0.114
Protein (%)	3.19[a]	3.19[a]	3.15[a]	0.086
Feed Efficiency[g]	1.01[a]	1.18[b]	1.11[b]	0.06
Body Weight (kg)	638[a]	592[b]	607[b]	0.162
Change (kg/day)	-0.03[a]	-0.22[a]	+0.01[a]	

[abc]Treatment means (columns) sharing an uncommon superscript are different ($P<.05$).

[d]Experiment 3.3.5 (1981).

[e]8 cows per treatment for 70 days. Means adjusted by covariance, using 7 day pretreatment period.

[f]4% FCM.

[g]Fat corrected milk/dry matter intake.

Table 19. Growth and Intakes[c] of Steers Fed Haylages Treated with Inocula or Ammonia[c].

Measurements	Treatments[d]			
	Control	Ammonia	Inocula	SEM
Initial Weight (day 0)	215	217	219	
Final Weight (day 35)	224	218	218	
(day 70)	252	246	257	
Dry Matter Intake				
day 0 - 35 (kg/steer/day)	5.91	5.75	6.01	
36 - 70	6.08	5.60[a]	7.30[b]	.66
0 - 70	5.99	5.68	6.66	
Average Daily Gain				
day 0 - 35	0.26	0.03	-0.03	
36 - 70	0.83[a]	0.82[a]	1.12[b]	.19
0 - 70	0.59[a]	0.46[a]	0.54[a]	.12
Feed/Gain				
day 0 - 35	22.7	191.0	--	
35 - 70	7.46[a]	6.82[a]	6.58[a]	.97
0 - 70	10.16[a]	12.15[a]	12.33[a]	.72

[ab]Treatment means (columns) sharing uncommon superscripts are different ($P<.05$).

[c]Experiment 3.3.4.

[d]16 steers per treatment.

4.4 Experiment 3.4 (1982)

4.4.1 Fermentation Characteristics of Silages

The chemical composition of inoculated and untreated haylages fed in 1982 growth and lactation experiments are presented in Table 20. Inocula treatment appeared to result in a slight stimulation of lactate production; however, this is not consistent with the slightly higher pH and greater residual WSC values in the inoculated silage. These values are so similar that one should conclude that inoculation did not influence pH, lactic acid, or WSC. The calculated average water soluble nitrogen was least in the inocula-treated haylage samples, but average ammonia-nitrogen was greatest. The percent dry matter, crude protein, ADIN, and acid detergent fiber (ADF) were closely matched between controls and inocula treated composite haylage samples.

4.4.2 Milk Cow Trial — 1982

Milk production parameters measured in the 1982 trial are shown in Table 21. There were no differences in dry matter intake, milk yield, milk composition, or efficiency of feed conversion to milk during the 56 day lactation trial. Body weight changes of cows during the trial were also not different between the two treatment groups.

4.4.3 Steer Trial — 1982

The growth and intakes of steers are in Table 22. There was no difference between control and treated steers in daily dry matter intake, ADG, or feed efficiency for growth. Steers were fed a diet of haylage with a vitamin and mineral supplement (Table 4). All steers gained an average of 0.59 kg/day for the trial period with no treatment difference. The reason why the steers gained weight on a straight haylage diet and steers in 1981 trial (3.3.4) did not may be a difference due to breed, the Charolais crosses used in 1982 would be expected to have a greater propensity for growth than Holstein steers (Garrett, 1980).

The results of the 1982 animal performance trials (milk cows and steers) suggest that feeding livestock inocula treated haylage has no benefits over untreated haylage. These results contradict the 1981 growth trial.

The trials of 1981 and 1982 indicate lactic acid production was slightly stimulated in silages inoculated with silage bacteria, there was no consistent benefit in animal growth performance. Lactating dairy cattle showed no improvement of animal performance using bacterial inoculated silage. Inoculated silage was least stable and ammonia most stable when exposed to air. Ammonia treated silage resulted in lower dry matter intakes by both steers and dairy cows

and production was not affected. Lactic acid was stimulated in inocula and ammoniated silages.

Table 20. Chemical Composition of Haylages Fed to Growing Steers and Lactating Cows in Large Silo Experiments 3.4.2 and 3.4.3 (1982).

Analyses	Treatments[a]			
	Control	SD	Inocula	SD
Dry Matter Percent	48.03	6.38	47.40	5.85
pH	4.79	0.13	4.81	0.33
Lactic Acid, % DM	3.16	0.35	3.45	0.39
Water Soluble Carbohydrate, % DM	0.83	0.29	0.93	0.29
Crude Protein, % DM	16.28	0.52	16.12	0.89
Soluble Nitrogen, % DM	1.29	0.17	1.19	0.10
Soluble Nitrogen, % Total Nitrogen	49.5		46.1	
Ammonia Nitrogen, % DM	0.13	0.02	0.15	0.02
Ammonia Nitrogen, % Total Nitrogen	5.0		5.8	
Acid Detergent Insoluble Nitrogen, % DM	0.23	0.06	0.23	0.07
Acid Detergent Insoluble Nitrogen, % Total Nitrogen	8.8		8.9	
Acid Detergent Fiber	30.24	6.48	29.45	6.15

[a]Mean of weekly composites.

Table 21. Intake and Milk Production of Holstein Cows Fed Control or Inoculated Haylages in Large Silo Experiment 3.4.3 (1982).

Treatments[b]	Control	Inocula	SEM
Dry Matter Intake (kg/d)	18.15[a]	17.79[a]	0.81
Milk Production (kg/d)	22.40[a]	22.52[a]	0.34
Fat Corrected Milk (kg/d)[c]	20.67[a]	20.80[a]	0.41
Fat (%)	3.53[a]	3.50[a]	0.12
Protein (%)	2.88[a]	2.91[a]	0.06
Solids Non-Fat (%)	8.92[a]	8.81[a]	0.17
Milk/kg DM[d]	1.16[a]	1.18[a]	0.06
Weight Change (kg/day)	+0.22[a]	+0.26[a]	3.81

[a]Treatment means (columns) on a line with different superscripts are different ($P<.05$).

[b]10 cows per treatment for 56 days. Means adjusted by covariance, using 7 day pre-treatment period.

[c]4% FCM.

[d]Fat corrected milk/dry matter intake.

Table 22. Growth and Intakes of Steers Fed Control or Inoculated Haylages in Large Silo Experiment 3.4.2 (1982).

Treatments[b]	Control	Inocula	S.E.
Initial Weight, kg	224	231.5	
Final Weight, kg	249.5	256	
Weight Gain, kg	25.5	24.5	
Days Fed	42	42	
Dry Matter Intake (kg/d)	5.98[a]	6.05[a]	0.21
Average Daily Gain (kg/d)	0.61[a]	0.57[a]	0.02
Feed/Gain	14.57[a]	15.63[a]	0.06

[a]Treatment means (columns) with different superscripts are different ($P<.05$).

[b]32 Charolais cross steers, 16 per treatment, divided into two pens per treatment.

5.0 CONCLUSIONS

The results of the experimental silo studies indicate that treatment of alfalfa with ammonia had a consistent but non-significant stimulatory effect on lactic acid production. These observations were over a wide range of silage dry matters. This is consistent with the findings of other researchers who found corn silage treated with ammonia contained an increased concentration of lactic acid (Huber and Santana, 1972; Huber et al., 1973; Huber and Kung, 1981).

The mechanism by which ammonia increases lactic acid production is unknown. It may be through increased buffering from added ammonia which allows lactic acid production to continue before the low pH inhibits silage bacterial growth.

Animal performance trials suggest that ammonia treatment decreases dry matter intake in steers and dairy cows without a decrease in milk production or growth. Decreased dry matter intake has been a finding in the work of others using urea as a non-protein nitrogen source (Huber and Kung, 1981).

Alfalfa silage that had been treated with ammonia was observed not to increase soluble nitrogen during the ensiling period. This suggests that ammonia treatment was sparing plant proteins from the proteolytic aspect of respiration and fermentation. This has also been observed in corn silage (Huber et al., 1979).

Ammonia treatment of alfalfa resulted in a silage that was more stable when exposed to air. This is similar to the findings of other investigators working with corn silage (Britt and Huber, 1975).

The results of these experiments suggest fermentation of alfalfa can be positively influenced by the addition of ammonia. Further study into how ammonia treatment can best be utilized in alfalfa is warranted.

Inoculation of alfalfa with commercial silage bacteria preparation in experimental silos resulted in inconsistent results with regard to lactic acid production. Likewise, there was no clear indication that inoculation of alfalfa decreased the breakdown of plant protein during fermentation.

Inocula treatment of alfalfa resulted in a less stable silage when exposed to air, as evidenced by rapid rise in temperature and silage pH. This instability was manifest within 24 hours after silo opening and so may be an important aspect in the field use of this product as a fermentation aid.

An effect upon animal performance from feeding inocula treated haylage was not clearly shown. Significantly greater weight gains in steers fed inocula treated haylage in 1981 were followed in 1982 by no difference. Production of dairy cows was generally unaffected by inocula treatment of the silage. The lack of consistent results in experimental silo studies and animal production trials and the observation of decreased aerobic stability may not warrant the expense of using this commercial inoculum.

Since these investigations were performed, other inoculation products have been developed and investigated. Some show promise of consistently hastening development of lactic acid production and pH decrease.

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