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thesis entitled

INFLUENCE OF AMMONIA TREATMENT AND TIME
ENSILING ON PROTEOLYSIS AND FEEDING
VALUE OF CORN SILAGE FOR
DAIRY CATTLE

presented by

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has been accepted towards fulfillment
of the requirements for

Masters degree in Animal Science

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Date 16 Nov 1981

INFLUENCE OF AMMONIA TREATMENT AND TIME OF ENSILING ON PROTEOLYSIS AND FEEDING VALUE OF CORN SILAGE FOR DAIRY CATTLE

By

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A THESIS

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

MASTER OF SCIENCE

Department of Animal Science

1981

ABSTRACT

INFLUENCE OF AMMONIA TREATMENT AND TIME
OF ENSILING ON PROTEOLYSIS AND FEEDING VALUE
OF CORN SILAGE FOR DAIRY CATTLE

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Whole-chopped corn plants (32-34% DM) were treated with 0, .25, .52, or 1.08% added N as ammonia. About 3 kg of material were placed in 5 mil polythene bags which were then evacuated and served as experimental silos. Silage samples were taken on days 0, 1, 2, 6, 12 and 54 and analyzed for dry matter, PH, lactic acid, total and water insoluble nitrogen, and free amino acids in the water phase. Added ammonia increased initial pH of silages, but delayed increases in lactic acid. Increasing levels of ammonia decreased proteolysis. Large increases in alanine were observed in the water phase of high ammonia-treated silages, increases which were apparently not related to proteolytic effects (Experiment 1).

In Experiment 2, 24 lactating cows were utilized to determine the relative feeding value of corn silages treated with Cold-flo (.36% NH_3, 82% N) or Pro-Sil (2.4% ammonium solution, 13.6% N). Dry matter and lactic acid were higher for the Pro-Sil treated silage. Apart from a slightly higher intake of NPN by the Pro-Sil group, milk yields, intakes, and body weight changes were similar for both groups.

DEDICATION

To the memory of my beloved mother,

Edna W. Johnson, 1922-1980.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. J. T. Huber, my major Professor, for accepting me as one of his graduate students and providing me with invaluable assistance and guidance throughout my graduate studies. Also, special thanks are due to the members of my committee, Dr. W. G. Bergen and Dr. D. Hillman, for their patience and understanding of my cause.

My thanks also go to all those who aided the cause, particularly the Department of Dairy Science for financial assistance during the course of my graduate studies.

Special thanks also go to the Dairy Nutrition Secretary, Elaine Kibbey, for her generous help throughout the graduate program.

Finally, I must thank my loving and devoted wife, Paulette, not only for her remarkable ability to turn none-too-legible manuscript into beautiful typescript, but also for her encouragement and patience throughout the graduate study period.

TABLE OF CONTENTS

LIST OF TABLES

TABLE		PAGE
11.2	Effect of ammonia concentration and time of ensiling on free amino acids in corn silage water phase	40
11.3	Main effect means, standard errors, and significant differences for free amino acids in corn silage water phase . . .	41
11.4	Effect of ammonia addition and time of ensiling on free amino acid N in corn silage water phase	43
11.5	Effect of ammonia addition and time of ensiling on total free amino acid N in corn silage water phase	44
12.	Dry matter, pH, lactic acid and crude protein of corn silage composites . . .	46
13.	Influence of non protein nitrogen source on dry matter and protein intakes of dairy cows	46
14.	Influence of non protein nitrogen source on milk yields of dairy cows	48
15.	Influence of non protein nitrogen source on milk composition, weight change and feed efficiency of dairy cows	48

Introduction

It is widely accepted that normal corn silage that is well eared and preserved is a high-energy source of forage for cattle, sheep and other ruminants. In addition, it has the potential of substantially reducing feed costs and increasing beef and milk production per acre of crops fed. However, these advantages are partially offset by an insufficiency of protein, a factor which appears to limit voluntary intake. Moreover, degradation of protein (or proteolysis) in the resultant silage may influence the quantity of protein (as opposed to NPN) which is absorbed and available for metabolism in the animal's body.

In addition to ammonia treatment being the preferred method for making up the protein deficit in corn silage, ammonia treatment of the whole chopped corn plant at ensiling has consistently resulted in increased insoluble nitrogen (Henderson and Bergen, 1972; Huber et al., 1973) and possibly decreased proteolysis of plant protein in the resultant silage (Huber et al., 1979c). The importance of reduced proteolysis during ensiling cannot be overemphasized, since reduced degradation of protein to NPN may increase the supply of undegraded plant protein to the abomasum and intestines, and this may enhance intake (Egan and Moir, 1965).

The forms of ammonia most available for silage treatment are Cold-flo (concentrated anhydrous, 82% nitrogen) and Pro-Sil (an ammonium solution containing 13.6% nitrogen). Earlier studies showed depressed intakes in dairy and beef cattle fed silage treated with concentrated anhydrous ammonia compared to silage treated with more dilute solutions. More studies with Cold-flo and ammonia solutions have not directly compared the forms of treatment in lactating cows. Many dairymen contemplating ammonia treatment question the relative value of the different forms of ammonia, but little information is available.

Therefore, the objective of this study were two-fold:

1. To characterize the nature of the protein breakdown in corn silage ensiled with or without ammonia.
2. To determine the relative feeding value of corn silage treated with two forms of ammonia (Cold-flo vs. Pro-Sil).

Literature Review

Brief History of Ensiling

The process of ensiling is old and has been intensively studied during the last century (Watson and Nash, 1960). The object of storing green crops as silage is to preserve the material with a minimum loss of nutrients. Various ensiling practices have been used to achieve this end. In 1843, Johnston recommended a German method for harvesting and storing green fodder; the direct cut material was packed into trenches which were then covered with boards and earth to facilitate the exclusion of air. Also, as pointed out by Jenkins, 1884, the practice of storing grain in pits or underground trenches dates back to the Greeks and Egyptians.

Goffart's work in France had an important influence on the popularization of the practice in the late 19th century. He recognized the rapidity with which crop maize ferments within the silo. He also stressed the importance of reducing the length of chop so as to facilitate better packing and exclusion of air from the silage mass. Miles (1918) gave Reihlen, a German, credit for being the first to preserve the whole corn plant utilizing a silo.

Corn silage is a basic ingredient in both dairy and beef cattle rations in many regions of North America and Europe. Corn harvested for silage in North America has increased from 47.7 million tons in 1954 (Waldo and Jorgensen, 1981) to 111.1 million tons in 1980 (U.S. D.A., 1981). Although the ensiling of forage maize as a winter feed for livestock has been practiced in Europe for at least 100 years (Wilkinson, 1978) the quantity of maize harvested for silage remained low during the first fifty years of the this century due to inadequate harvesting machinary. The introduction of the forage harvester into Europe during the 1960's revolutionized the technology of the harvesting and ensiling operation (Wilkinson, 1978).

The current energy crisis and world food shortage with the resulting emphasis on animal production from forage has created the proper climate for reevaluating silage production.

Silage Fermentation

Once a producer has grown a good, high-yielding crop, his next objective is to preserve the crop for livestock feeding. As Barnett (1954) pointed out, the aim of silage making is "to achieve within the ensiled mass sufficient concentration of lactic acid, produced as a result of the presence of microorganisms within the cut crop, to inhibit other forms of microbial activity and thus preserve the material until such time as it is required". Research has conclusively shown that the process of ensiling green crops is the most effective and efficient method of storing and preserving the nutritive value of the whole corn plant. However, it must be borne in mind, that the feeding value of silage is largely affected by microbiological, chemical, and physical factors. The ensiling process is not 100%

efficient and energy losses during the process in the form of heat and carbon dioxide evolution can be extensive. Therefore, it becomes extremely important to understand the silage fermentation process in order to consistently produce high quality silage.

Although there is no clear-cut division for the various phases of the fermentation process, Barnett (1954) originally desribed the process as consisting basically of four phases and possibly a fifth phase depending on the course of fermentation. These phases are:

Phase 1. Plant cells continue to respire, utilizing available intermediary metabolites and simple sugars, resulting in the production of CO_2 and heat.

Phase 2. A relatively short phase of acetic acid production by coliform bacteria (and others).

Phase 3. The conversion of soluble carbohydrates to lactic acid by lactobacilli and streptococci, with most silages reaching this stage two to three days after filling.

Phase 4. Stage of quiescence of silage mass. In the absence of buffer additions, lactic acid production continues and reaches a peak at 1 to 3 percent of the fresh weight for corn silage. The pH of the ensiled material declines to below 4.0 when the lactic acid producing bacteria cease fermentative activity. The ensiled material, if undistured, is now quite stable for prolonged storage.

Phase 5. Proliferation of butyric acid producers. This normally occurs in silages where the acidity is not high enough to prevent the conversion of residual carbohydrates and lactic acid to butyric acid by clostridial bacteria. Ammonia from deamination of protein, carbon dioxide and heat often accompany this phase, particularly when air is introduced into the silo mass (Huber, 1979a).

As mentioned before, losses due to the ensiling process can be very extensive, and a large share of these losses can be attributed to the action of plant and microbial cell respiration (Barnett, 1954; Kempton, 1958; Watson and Nash, 1960). When the plant material is ensiled, the living cells continue to respire until all the oxygen is used up. The production of lactic acid is an anaerobic process, and therefore it is essential that anearobic conditions become established as soon as possible within the ensiled mass. Previous research has shown that delays in establishing anaerobic conditions can be detrimental to the silage fermentation (Miller et al., 1961; Langston et al., 1962; Ohyama et al., 1970). Obligate anaerobes, such as clostridia and yeasts have been known to compete with facultative lactic acid microbes for available water soluble carbohydrates under aerobic conditions (Takahashi, 1970). Utilizing sterilized corn inoculated with bacteria to make silage, Peterson et al.,(1925), demonstrated that plant cell respiration was nonessential and that bacteria are mainly responsible for the production of acids from sugar and starches.

Two major compositional changes occur in the corn plant during ensiling; namely, the conversion of water-soluble carbohydrates to short-chain organic acids and the degradation of plant protein to non-protein nitrogenous compounds (Johnson et al., 1967, Demarquilly and Andrieu, 1973; Bergen et al., 1974).

Carbohydrate Fermentation

In silage making, the green chop is preserved by lactic acid formed in the fermentation process. The production of lactic acid requires comparatively large amounts of fermentable carbohydrates in the ensiled material. Thus, the amount of fermentable carbohydrates in the green chop becomes of prime importance in producing the desired type of fermentation (Melvin, 1965, Ohyama et al., 1973; Ohyama and Masaki, 1974). The carbohydrates available for fermentation are primarily those within the water soluble carbohydrate fraction (Zimmer, 1971).

It is generally accepted that as the corn plant matures, soluble carbohydrates contained in the leaves and stems are converted to insoluble starch in the kernels; thus, the total level of soluble carbohydrates decreases, and in turn reduces fermentation as well as the level of lactic acid produced. However, McCullough (1973) summarized the soluble carbohydrate content of nine crops (including maize), and reported that under most ensiling conditions, the crops contained a sufficient amount of available carbohydrate (about 6 to 8% of the dry matter content). Ensiling the whole maize plant at 30 to 35% of dry matter has also been shown to supply adequate soluble carbohydrates for fermentation (Hillman, 1977).

Prolonged cell respiration under aerobic conditions can significantly decrease the quantity of available carbohydrates remaining for the anaerobic fermentation phase (Zimmer, 1971; McCllough, 1973). However, the soluble carbohydrates surviving aerobic metabolism are fermentable by a variety of micro-organisms, of which lactic acid bacteria are the most important (Whittenbury _et al._, 1967). Since the desired fermentation is a rapid production of lactic acid, the desired changes would be a low pH with a minimum loss of carbohydrates. Johnson _et al._, (1966) reported decreases in corn plant soluble carbohydrate ranging from 39 to 83%, and concurrent conversion to lactic acid. Both lactic and acetic acids contribute towards avoiding undesirable fermentations. However, lactic acid is the more important, it being present in larger amounts than acetic acid and exerting a greater effect on pH (Greenhill, 1964).

In summary, the most striking feature of carbohydrate fermentation is the production of organic acids of which lactic acid is the most important. Although there are many factors that can influence the extent of lactic acid production, the chief determining factor will be the amount of water soluble carbohydrates available for fermentation.

Protein Degradation

The degradation of plant protein to non-protein nitrogenous compounds during ensiling has long been a subject of discussion. It is noteworthy that even in well-preserved silages about 50% net breakdown of the protein may take place (McDonald and Whittenbury, 1973). Various factors have been known to affect the course and extent of the breakdown. Some of those implicated are, dry matter or moisture content of the crop at the time of ensiling, pH of the silage mass, lactic acid content, level of oxygen, temperature, and most of all enzymatic activity.

The importance of dry matter content of the whole plant material at the time of ensiling cannot be overemphasized, it being the most decisive factor influencing the extent of protein degradation (Bergen et al., 1974). Geasler (1970), working with corn silage material, found that material ensiled at 48 and 60% dry matter contained 30 and 26% of total nitrogen in water soluble form, respectively. A similar phenomenon was observed by Bergen et al., (1974) using corn silage material ranging from 32 to 85% dry matter, and by Hawkins (1969) using alfalfa silage material. Immature silage (low dry matter) tend to be higher in protein content, but a larger portion is degraded to non-protein nitrogen during fermentation (Hillman and Fox, 1977). According to Andrieu and Demarquilly (1974), protein degradation is inversely related to the dry matter content at ensiling.

The pH value is the most universal measure used in assessing silage quality. In general, pH values associated with well-preserved silages are between the ranges of 3.8 to

4.2 (Greenhill, 1964; Bergen, 1980), while that of poor quality silages are above 4.2 (Greenhill, 1964). McCullough (1961), is of the opinion that the high pH usually associated with undesirable silage fermentation is a result of the apparent protein breakdown with the resulting effect of increased buffering capacity. Later, McDonald and Henderson (1962) suggested that the contribution from the protein fraction resulted from protein degradation to decarboxylated amino acid bases. De Vuyst et al., (1971) have made a comprehensive study of the changes in amino acid composition of grass and alfalfa during ensilage. They found that in silages ranging in pH from 4.9 to 5.7, considerable degradation of amino acids occurred, especially the basic amino acids (lysine, histidine and arginine). In the same experiment, these workers also observed that the amount of alanine almost doubled in some silages. Hughes (1971) in a study of high pH silages observed a decline in amino acid nitrogen with a concomitant increase in volatile nitrogen which was mostly ammonia.

The preservation of plant material as silage is dependent on the accumulation of lactic acid and a corresponding decrease in pH. However, even if a rapid drop in pH is achieved and the growth of proteolytic bacteria is inhibited, some ammonia is still formed in the silo (Mo and Fyrileiv, 1979). Some lactic acid producing bacteria are also capable of both deaminating and decarboxylating amino acids (Whittenbury et al., 1967). Brady (1966) isolated lactic acid bacteria from farm silages and showed that Lactobacillus *plantarum* and Pediococcus *sp*. deaminated serine to pyruvate and arginine to ornithine. Ammonia nitrogen levels in high lactate silages are frequently of the order of less than 10% of total nitrogen (McDonald and Edwards, 1976).

In addition to deterioration of silage during the utilization period, high amounts of oxygen during ensiling have been shown to result in the formation of large quantities of volatile nitrogen from the deamination of amino acids (Ruxton and McDonald, 1974).

Temperature is one of the factors affecting the success of silage making, since the lactic acid bacteria have an optimum temperature for growth. However, according to Nilsson *et al*., (1956), the optimum temperature of 80 to 100°F for lactic acid producing bacteria is also within the temperature range of proteolytic bacteria. Thus, it was not surprising when Wieringa, (1960) reported that the ammonia fraction in silages stored at 68°F was almost as high as at 95°F. Nilsson *et al*., (1956) further stressed that temperature becomes an important factor in silage fermentation when the crop is of high-protein and low-sugar content.

Both plant and microbial enzymes have also been implicated as affecting the course and extent of protein degradation during the ensiling process. The general consensus is that protein degradation is a two phase process; the first involving the rapid breakdown of plant protein into peptides and amino acids, primarily by endogenous plant proteases

(Barnett, 1954; Kemble, 1956; Watson and Nash, 1960; McDonald and Whittenbury, 1973; Bergen et al., 1974), and the second involving the subsequent degradation of amino acids to volatile nitrogen and other nitrogenous compounds by clostridial activity (McDonald and Whittenbury, 1973; Oshima and McDonald, 1978).

Non-Protein-Nitrogen Treatment of Corn Silages

In periods of high cost of oilseed meals, simple nitrogen-containing substances such as urea, ammonia or ammonium salts are added to cattle rations to replace plant protein supplements. These compounds are referred to as a group as non-protein nitrogen (NPN).

The notable advantages of corn silage are partially offset by its well known nutritional insufficiency with respect to protein. Nevertheless, being high in energy and organic acids, fermented silage make an ideal carrier of NPN in ruminant rations. Urea has traditionally been the most widely utilized from of NPN for treating silages. However, since anhydrous ammonia is a cheaper source of NPN, researchers became interested in its application to corn silage at ensiling. This program was initiated at the Michigan State University in the years 1967 and 1968. Evolving from the original use of anhydrous ammonia mixed with water (aqueous ammonia) were aqua ammonia (21-23%N), ammonia-molasses mineral-solution (now bearing the trade name Pro-Sil) and Cold-flo ammonia. These three sources of ammonia have been hound to give results superior to urea, and allow for higher intakes of NPN without decreasing milk production (Huber *et al.*, 1980).

This section of the paper will consider data available on the effects of using ammonia as an additive to the chopped corn plant material at ensiling, and subsequent effects in feeding trials.

Effects of Ammonia Addition on Silage Fermentation

Although ammonia is added principally as a nutritive supplement to furnish crude protein to cattle, it also exerts a profound effect on silage fermentation. It was noted earlier, that under natural ensiling conditions energy losses in the form of heat and carbon dioxide evolution can be extensive. Treating the chopped corn plant at ensiling with ammonia has shown to reduce such losses when compared to the untreated material (Juengst *et al.*, 1975; Huber *et al.*, 1979b). Juengst *et al.*, (1975) reported that the untreated silage produced more carbon dioxide during the first 24 hours than the ammonia (Pro-Sil) treated silage produced during the entire 11 days of fermentation.

Initial increases in pH and higher lactic acid concentrations have also been reported for ammonia-treated corn silages (Cash 1972; Henderson and Bergen 1972; Huber *et al.*, 1973; Juengst *et al.*, 1975). This phenomenon has been attributed to the increased buffering capacity resulting from ammonia treatment, thus enabling the lactic acid-producing bacteria to survive longer (Britt and Huber, 1975). However, this phenomenon has not been found to be operative at all levels of ammonia additions. Levels of ammonia addition above 1% of silage dry matter have been found to decrease lactic acid production (Huber et al., 1979), and total inhibition is achieved at 2.5% of the dry matter (Britt and Huber, 1975).

Also worthy of note, is the increased water insoluble nitrogen concentrations resulting from ammonia treatment (Henderson and Bergen, 1972; Huber et al., 1973). Bergen et al., (1974), suggested that this increased water insoluble nitrogen fraction might be related to decreased proteolysis of the original plant protein. More recent studies (Huber et al., 1979c, 1980a) revealed that 40% of the added ammonia was recovered as ammonia in the water insoluble nitrogen fraction in unfermented silage. In the fermented silage, 28% of the added ammonia was recovered in the insoluble nitrogen fraction, as revealed by ^{15}N analyses (Huber et al., 1979c); this accounted for only 59% of the total increase in the insoluble nitrogen fraction. The remainder was presumably due to decresed breakdown of original plant protein as suggested by Bergen et al., (1974).

Apart from its influence on the silage fermentation process, ammonia treated silages have also been shown to be more stable during the utilization period (Britt and Huber, 1975; Soper and Owen, 1977; Buchanan-Smith, 1980). Britt (1973), has attributed this increased stability to the antifungal action of ammonia and ammonium salts.

<u>Feeding Trials</u>

The ability of the ruminant to utilize NPN as a partial replacement for the preformed protein in the ration is well documented. However, it must be borne in mind that with increasing levels of dietary nitrogen, rumen ammonia increases more rapidly with NPN than with natural protein (National Research Council, 1976). The ammonia nitrogen released from NPN compounds as the result of rumen microbial enzyme action may be utilized in the synthesis of microbial protein (Reid, 1953), but the extent to which this can be done will depend on the energy concentration in the ration (Satter and Roffler 1975; Chalupa, 1978). A deficiency in intake of metabolize energy may result in decreased animal performance (Wilkinson, 1978). However, as pointed out by Huber and Kung (1981), inclusion of NPN in dairy cattle rations is profitable as long as production is not diminished and utilization for microbial protein synthesis is assured.

For the past 13 years, several investigations have been made by workers at the Michigan State University to determine the relative feeding value of corn silage treated with the various forms of nitrogen-containing compounds at ensiling. Many of the studies have revealed that milk production (Huber and Santana, 1972; Huber et al., 1973; Boman, 1980; Huber <u>et al</u>., 1980b) and body weight gains (Henderson and Bergen, 1972; Cook and Fox, 1976) in cattle fed silages treated with ammonia solutions prior to ensiling, were comparable or higher than those fed untreated or urea-treated silages. Other workers (Honig and Zimmer, 1975; Buchanan-Smith, 1980) have reported similar results in beef cattle studies.

However, a closer look at the relative feeding value, particularly in dairy cows, of corn silage treated at ensiling with the different forms of anhydrous ammonia (gaseous, aqueous, and ammonia-molasses-mineral solution) reveals that the apparent differences are within the realm of experimental error. A look at the summary in Table 1, of some data on dairy cow performance on ammonia treated corn silage will tend to support this view. Nevertheless, the data in Table 1 reveals that the forms of ammonia can be safely added at ensiling to corn silages varying in dry matter content from 30 to 42% without depressing milk yields; ammonia (Pro-Sil) addition to 53% dry matter depressed milk yields as shown in experiment 2 on Table 1. Urea addition to high dry matter silages (above 42%) have consistently resulted in lower milk yields of lactating dairy cows than addition to lower dry matter silages (Huber <u>et al</u>., 1968; Van Horn <u>et al</u>., 1968).

Also worthy of note in Table 1, experiment 3, is the significant ($P<.05$) depression in intake of dry matter with the more concentrated ammonia (gaseous NH_3 - 82%N). According to the reported literature, silage protein was lower for the gaseous ammonia than for the Pro-Sil treatment (12.1 vs. 14.2% of dry matter), but the total dietary protein for the gaseous ammonia treatment was 13.9% which

TABLE 1. Summary of dairy cow performance on corn silage treated with ammonia at ensiling

Treatments	Dry matter	% added	Milk Production		Persistency	Dry Matter Intake		Milk Dry Matter
			Pre-exptl.	Exptl.		Silage	Total	
	%		kg	kg	%	% body weight		kg
Expt. 1(1969,8 cows/trt)								
Aqua NH_4[a]	31	1.0	30.5	26.8	88.3	1.27	2.91	1.52
ProSil[b]	31	2.0	30.0	27.3	91.4	1.25	2.85	1.50
	42	2.5	29.8	27.0	90.2	1.24	2.88	1.53
Expt. 2(1970,7 cows/trt)								
ProSil	30	2.0	25.2	23.0	90.4	1.34	2.69	1.29
	53	2.6	24.7	18.4	75.2	1.65	3.02	0.96
	35	4.0	25.1	21.0	84.4	1.41	2.80	1.20
Expt. 3(1972,8 cows/trt)								
Gaseous NH_3[c]	34.4	0.375	27.1	24.8	93.0	1.24	2.73	1.43
ProSil	31.8	2.0	26.5	24.3	91.0	1.52	2.97	1.29
Expt. 4(1973,8 cows/trt)								
Gaseous NH_3	32.6	0.35	24.6	20.5	82.7	1.25	2.60	1.26
	33.7	0.77	23.9	20.7	85.8	1.40	2.67	1.17
ProSil	32.2	2.0	24.1	20.4	84.2	1.20	2.46	1.22
	32.9	4.0	24.6	20.4	83.5	1.07	2.40	1.29

[a]Contained 22% N.
[b]A mixture containing 13.6% N (as NH_3), 55.1% molasses, 5.8% NaCl, plus Ca, P, S, Mg, Zn, Cu, Co, and I.
[c]Contained 82% N.
Reference: Huber et al., 1973 and 1979b.

was not low enough to depress intake (Huber _et al_., 1979b). However, the occurrence was not evident in experiment 4, Table 1; highest intakes were recorded for the group receiving the .77% gaseous ammonia treated silage (Huber _et al_., 1979b).

Materials and Methods

Experiment 1. Silage Fermentation Study

Whole corn plants (32-34% dry matter) were field chopped and brought to the Beef Cattle Research Center where 30 kg portions were treated with 0, .25, .52, or 1.08% added N as ammonia (40% N). This required the addition of 0, .63, 1.3 or 2.7% ammonia (of silage dry matter).

To ensure complete mixing and uniform application of treatments, each 30 kg portion of the chopped material was weighed, then placed into a small feed mixer where the specified treatments were applied as the material rotated. Immediately after treatment, duplicate samples of the freshly chopped material, of about 3 kg each, were hand packed into double layered polythene bags. The bags were evacuated to remove air, tied securely with string, then placed in a container packed with dry ice. These constituted day 0 samples after harvest. The same procedure was repeated for the other samples, except that they were placed in 200 liter covered plastic barrels to ensile for 1, 2, 6, 12 and 54 days in a protected area at about 25^{o}C. Total time elapsed between harvesting and sealing of the bags was about 2 to 3 hours. Bagged samples were removed at the various times and frozen at -20^{o}C until analyzed. Parameters under investigation are shown in Table 2.

Silage Analyses

Silage samples were removed from the freezer and allowed to thaw at room temperature. Thereafter, dry matter was determined on 50 gram portions by drying in a forced-air oven at 60°C for 48 hours. The remaining portion of the silage sample was comminuted by a commerical food cutter.

Table 2. Silage Parameters under Investigation

Dry matter
pH
Lactic acid
Total nitrogen
H_2O insoluble nitrogen
H_2O soluble nitrogen
Individual free amino acids (AA)
Essential and non-essential AA
Branched chain AA
Total free AA

Twenty-five gram portions of the comminuted sample were then homogenised in 100 ml double-distilled water for 2 minutes in an Ommi-mixer. The homogenizer cup was immersed in ice during the process. The pH was determined on the homogenate, and 12 grams were removed and centrifuged at 20,000 x g for 20 minutes. The residue was washed with double-distilled water and recentrifuged. This was repeated three times. The residue was then analyzed for insoluble nitrogen by macro-Kjeldahl. Total nitrogen was also determined by macro-Kjeldahl on 12 grams of the homogenate. Soluble nitrogen was determined by difference between the total and insoluble nitrogen. Fifteen ml of the supernatant was deproteinized with 50% sulfosalicyclic acid (1 part SSA to 10 parts supernatant) by centrifugation at 15,000 x g for 20 minutes, and analyzed for free amino acids as described by Bergen et al., (1974) and for lactic acid according to Barker and Summerson (1941).

Statistical Analysis

To facilitate studying the effects of treatment and time on the parameters studied (Table 2), a split-plot repeat measure design was utilised (Table 3). Error "a" tested treatment effects, and error "b" tested both the time (day) effect and interaction of time with treatment. Response to treatment was measured using orthogonal polynomial contrasts.

Table 3. Analysis of Variance for Split-Plot Analysis

Source of Variance	Degrees of Freedom
Treatments[a]	3
Batches/treatment (Error a)	4
Days	5(2)[b]
Treatment x Days	15(6)
Batches x Days (Error b)	20(8)

[a] Treatment = Control (no ammonia), .25, .52, and 1.08% ammonia.
[b] Numbers in parenthesis represent degrees of freedom for analysis of amino acids (only 3 days were analyzed).

Dunnett's test as described by Gill (1978) was used to compare ammonia treatments with the control, and other days with day 0.

Experiment 2. Feeding Trial

Whole-chopped corn plant (32 to 37% dry matter) was harvested for silage and placed in two 20 x 60 ft. silos located at the Michigan State University Dairy Cattle Center. Before ensiling, the chopped material (½ inch) was treated with either 2.4% Pro-Sil (13.6% N) or .36% anhydrous ammonia (82% N) by the Cold-flo process.

The Pro-Sil was metered on to the chopped material as it moved from self-unloading wagons into the blower to be conveyed into the silo. The gaseous ammonia was also metered on at the blower; however, it was first recondensed to a liquid at -28°F in a simple cooling chamber before it was metered on to the chopped material. The composition of Pro-Sil is as shown in the footnotes of Table 1.

For 14 days prior to the feeding trial, 24 lactating Holstein cows were fed a pretreatment ration (14% crude protein) of corn silage, .33 kg concentrate per kg milk and 4.5 kg of alfalfa haylage. At the end of the pretreatment period, cows were paired for milk yield, stage of lactation, breeding groups and age. Within each pair, the two experimental diets (Table 4) were randomly assigned, resulting in a total of 12 cows per diet. Diets were fed at 10% in excess of appetite during the treatment period which lasted for 12 weeks.

Silage samples were collected three times per week during the feeding period and composited into three-week

Table 4. Ration composition of experimental diets

Feedstuff	Cold-flo		Pro-Sil	
	Dry Matter[a]	Crude Protein[b]	Dry Matter[a]	Crude Protein[b]
Corn silage	49.2	6.41	50.1	6.61
Alfalfa hay	9.9	1.37	10.0	1.38
Ground ear corn	30.4	3.01	31.0	3.07
42% Soy supplement[c]	7.4	3.49	7.4	3.49
Dicalcium phosphate	1.0	....	.2	....
Limestone	.1	....	.3	....
Calcium sulfate	1.3	....	.7	....
Trace mineral salt[d]	.7	....	.3	....
Total composition	100.0	14.28	100.0	14.55

[a] % of total
[b] % contributed
[c] Guaranteed chemical analysis: 42% protein (11.9% NPN), 2% Ether extract, 8.5% CF, 3% Ca, 1.05% P, 2.06% NaCl, 1.15% K, .9% Mg, and .6% S. Each Kg soy supplement supplemented with 4400 IU vit. A and 2000 IU vit. D.
[d] Guaranteed to contain: .35% Zn, .2% Mn, .2% Fe, .03% Cu, .005% Co, .007% I, and 96% salt.

composites. Feed intakes and milk weights were recorded daily, and an AM and PM composite milk sample was taken biweekly. Cows were weighed on two consecutive days, seven days after beginning and at the end of treatment.

Silages were analyzed for dry matter, pH, lactic acid and crude protein as in Experiment 1. Milk fat was determined according to Babcock, milk protein by micro-Kjeldahl and milk solids by AOAC (1965).

The paired T test as described by Gill (1978) was used to statisically analyze the measured parameters.

Results and Discussion

Experiment 1. Silage Fermentation Study

Results from this study are presented in Tables 5 through 11.5. Analysis of variance of data for Tables 5 through 10, 11.2, and 11.3 did not reveal significant interactions between treatment and time, so main effect means are presented in addition to treatment combination means. In Table 11.1, only treatment combination means are presented since interactions were significant for the data.

Dry Matter: Initial dry matter content of silages was not significantly altered by treatments or time of ensiling (Table 5). This observation is consistent with other studies (Britt and Huber, 1975; Huber *et al.*, 1979c; Boman, 1980). However, a tendency towards a decrease over time is evident, and this appears to be greater in the controls than the treated silages. More water production coupled with a greater

TABLE 5. Effect of ammonia concentration and time of ensiling on % dry matter in corn silage

Treatment	Added N as NH_3	Days of ensiling[a] 0	1	2	6	12	54	Treatment mean
	% DM							
Control	0	33.80	32.52	32.21	31.68	30.81	29.95	31.84[b]
Ammonia	0.25	32.90	31.96	32.45	32.65	30.73	30.48	31.86
	0.52	32.58	32.32	32.20	32.08	31.74	30.61	31.90
	1.08	32.43	32.32	32.00	32.27	31.45	30.39	31.80
Day mean[c]		32.95	32.26	32.21	32.17	31.18	30.36	

[a] Tabular values represent averages from two experimental silos with two determinations per silo.
[b] Standard error of any treatment mean is ± 1.02.
[c] Standard error of any day mean is ± .28.

carbon dioxide evolution in controls than ammonia treated silages might explain the higher decreases observed for the controls. This parameter was not under investigation in the present study, but Juengst _et al_., (1975) reported that untreated silage produced more carbon dioxide during the first 24 hours than the ammonia treated silage had produced during 11 days of fermentation.

In our study, percent dry matter decreases from 0 to 54 days were 11.6, 7.4, 6.0 and 6.3% for the control, low, intermediate and high ammonia additions, repectively. Smaller percentage decreases for the ammonia-treated silages suggest a lower energy loss during ensiling, and higher conservation of the energy originally present in the chopped corn plant.

_pH_: Initially, ammonia treatment of silages resulted in higher ($P<.01$) pH values than controls (Table 6). This observation is consistent with those of other studies (Henderson and Bergen, 1972; Britt and Huber, 1975; Huber _et al_., 1979b), and is thought to be associated with a longer fermentation period and higher lactic acid. However, by day 12 of ensiling pH values of all silages were similar and appeared to plateau about 4.0. In general, pH increased with added levels of ammonia but decreased with time of ensiling.

Lactic Acid: Ammonia additions tended to delay increases in lactic acid during the early stages of ensiling, but as ensiling progressed increases were greater for ammonia-treated

TABLE 6. Effect of ammonia concentration and time of ensiling on pH in corn silage

Treatment	Added N as NH_3	0	1	2	6	12	54	Treatment mean[b]
	% DM							
Control	0	5.72[i]	5.16	6.25	5.72	3.86[d]	3.98[d]	5.12[g]
Ammonia	0.25	7.73[e]	5.86[d]	4.26[d]	3.95[d]	4.04[d]	4.00[d]	4.97
	0.52	8.69[e]	8.20[f]	6.86[d]	4.12[d]	4.18[d]	4.16[d]	6.03
	1.08	8.55[e]	9.16[f]	8.82[e]	5.63[d]	4.58[d]	4.60[d]	6.89
Day mean[h]		7.67	7.10	6.55	4.85[c]	4.16[d]	4.18[d]	

a Tabular values represent averages from two experimental silos with two determinations per silo.
b Significant linear response ($P<.01$).
c Significantly lower than day 0 ($P<.05$).
d Significantly lower than day 0 ($P<.01$).
e Significantly higher than control ($P<.05$).
f Significantly higher than control ($P<.01$).
g Standard error of any treatment mean is $\pm$ .45.
h Standard error of any day mean is $\pm$ .30.
i Standard error of any treatment combination mean is $\pm$ .58.

TABLE 7. Effect of ammonia concentration and time of ensiling on lactic acid in corn silage

Treatment	Added N as NH_3	Days of ensiling[a]						Treatment mean[b]
		0	1	2	6	12	54	
	% DM	% of dry matter						
Control	0	.03[g]	.75	2.85[c]	4.54[c]	6.32[c]	4.25[c]	3.12[e]
Ammonia	0.25	.03	1.10	2.15[c]	5.21[c]	7.46[c]	6.52[cd]	3.74
	0.52	.04	.03	.56[d]	4.40[c]	6.36[c]	7.28[cd]	3.11
	1.08	.03	.02	.05[d]	1.97[cd]	4.46[c]	5.14[c]	1.94
Day mean[f]		.03	.48	1.40	4.03[c]	6.15[c]	5.80[c]	

[a] Tabular values represent averages from two experimental silos with four determinations per silo.
[b] Significant linear response ($P < .05$).
[c] Significantly higher than day 0 ($P < .01$).
[d] Significantly defferent from control ($P < .05$).
[e] Standard error of any treatment mean is $\pm$ .50.
[f] Standard error of any day mean is $\pm$ .50.
[g] Standard error of any treatment combination mean is $\pm$ .91.

than control silages (Table 7). This differential response to treatment was probably due to the increased buffering capacity resulting from ammonia treatment, thus requiring more lactic acid to reduce the pH to a low of 4.0. At day 54, lactic acid content of silages was higher ($P<.01$) for the low and intermediate levels of added ammonia, but similar to controls for the high ammonia treatment (Table 7).

A lower lactic acid content throughout the ensiling period for silages treated with the high ammonia (Table 7) suggests that lactic acid production is inhibited at this level of added ammonia. Other researchers have reported similar results for ammonia-treated silages (Britt and Huber, 1975; Huber et al., 1979c; Boman, 1980).

Total Nitrogen: Total nitrogen (% of dry matter) increased over time in controls and treated silages (Table 8). This was probably due to the carbon dioxide loss during fermentation and lower nitrogen retention by unfermented silages, as suggested by Huber et al., (1979c). However, total nitrogen for subsequent days was not different ($P<.10$) from that at day 0.

Comparing mean values of ammonia treated silages with those of the controls, the highest level of added ammonia resulted in higher ($P<.05$) nitrogen (1.32 vs 2.45% of dry matter) . Similar results have been reported previously (Huber et al., 1973, 1979b,c, 1980b).

Insoluble Nitrogen: Water insoluble nitrogen (% of dry matter)

TABLE 8. Effect of ammonia concentration and time of ensiling on total nitrogen in corn silage

Treatment	Added N as NH_3	Days of ensiling[a] 0	1	2	6	12	54	Treatment mean[b]
	% DM	-------------% of dry matter					----------	
Control	0	1.19	1.31	1.28	1.27	1.37	1.51	1.32[d]
Ammonia	0.25	1.47	1.58	1.55	1.54	1.57	1.71	1.57
	0.52	1.78	1.89	1.74	1.85	1.97	2.02	1.88
	1.08	2.33	2.31	2.44	2.47	2.54	2.64	2.45[c]
Day mean[e]		1.69	1.78	1.75	1.78	1.86	1.97	

a Tabular values represent averages from two experimental silos with two determinations per silo.
b Significant linear response ($P<.01$).
c Significantly higher than the control ($P<.05$).
d Standard error of any treatment mean is $\pm$ .14.
e Standard error of any day mean is $\pm$ .07.

increased linearly ($P<.01$) with added ammonia (Table 9), but tended to decrease over time. Water insoluble nitrogen was maximized at the highest level of added ammonia, and the corresponding mean values were higher ($P<.05$) than the controls (1.15 *vs*. .87% of dry matter).

When treatment combination means were expressed as a percentage of total nitrogen as shown in Table 9, decreases over time greater for the controls than treated silages. Decreases in percent of total nitrogen as water insoluble nitrogen over time were 27, 16, 8, and 3 for the control, low, intermediate and high ammonia, respectively (Table 9).

Similar results have been reported by others (Henderson and Bergen, 1972; Huber *et al*., 1979c, 1980b), and it was suggested that the increased insoluble nitrogen concentrations resulting from ammonia treatment was due to decreased proteolysis (Bergen *et al*., 1974), increased synthesis of microbial protein (Juengst *et al* ., 1975), or possibly a binding of ammonia by insoluble compounds in silage (Huber *et al*., 1980b). A higher water insoluble nitrogen fraction has been found to be associated with increased milk yields of cows on ammonia compared to urea-treated silages, particularly when they are fed large amounts of non-protein nitrogen (Huber *et al*., 1980a).

Water Soluble Nitrogen: The water soluble nitrogen content of silages was not determined directly, but by difference between the total (Table 8) and water insoluble nitrogen fraction (Table 9).

TABLE 9. **Effect of ammonia concentration and time of ensiling on water insoluble nitrogen in corn silage**

Treatment	Added N as NH_3	Days of ensiling[a] 0	1	2	6	12	54	Treatment mean[b]
	% DM	% of dry matter						
Control	0	.92(77)[f]	.88(67)	.95(74)	.85(67)	.85(62)	.76(50)	.87[d]
Ammonia	0.25	.97(66)	1.04(66)	1.01(65)	.98(64)	.99(63)	.86(50)	.97
	0.52	1.04(58)	1.13(60)	1.01(58)	.98(53)	1.02(52)	1.00(50)	1.03
	1.08	1.07(46)	1.23(53)	1.20(49)	1.13(46)	1.14(45)	1.14(43)	1.15[c]
Day mean[e]		1.00	1.07	1.04	.99	1.00	.94	

[a] Tabular values represent averages from two experimental silos with two determinations per silo.
[b] Significant linear response ($P < .01$).
[c] Significantly higher than the control ($P < .05$).
[d] Standard error of any treatment mean is $\pm$ .04.
[e] Standard error of any day mean is $\pm$ .04.
[f] Numbers in parentheses represent % of total nitrogen for that fraction.

TABLE 10. Effect of ammonia concentration and time of ensiling on water soluble nitrogen in corn silage

Treatment	Added N as NH_3	Days of ensiling[a] 0	1	2	6	12	54	Treatment mean[b]
	% DM	--------------------	--------	% of dry matter	--------	--------	--------	
Control	0	.27(23)[i]	.43(33)	.33(26)	.42(33)	.52(38)	.75(50)	.45[g]
Ammonia	0.25	.51(34)	.54(34)	.54(35)	.56(36)	.58(37)	.85(50)	.60
	0.52	.74(42)	.76(40)	.73(42)	.87(47)	.95(48)	1.02(50)	.85[c]
	1.08	1.26(54)	1.08(47)	1.24(51)	1.34(54)	1.40(55)	1.50(57)	1.30[d]
Day mean[h]		.70	.70	.71	.80[e]	.86[f]	1.03[f]	

[a] Tabular values represent the difference between total (Table 8) and water insoluble (Table 9) nitrogen.
[b] Significant linear response ($P<.01$).
[c] Significantly higher than the control ($P<.05$).
[d] Significantly higher than the control ($P<.01$).
[e] Significantly higher than day 0 ($P<.05$).
[f] Significantly higher than day 0 ($P<.01$).
[g] Standard error of any treatment mean is $\pm$ .10.
[h] Standard error of any day mean is $\pm$ .03.
[i] Numbers in parentheses represent % total nitrogen for that fraction.

Water soluble nitrogen (% of dry matter) increased with added ammonia ($P<.01$) and time of ensiling. Both intermediate and highest level of added ammonia resulted in higher ($P<.05$ and .01, respectively) mean values than controls (Table 10).

Means for days 6, 12, and 54 were higher ($P<.05$ and .01) than day 0. A strong negative correlation (-.85) between day means for pH (Table 6) and water soluble nitrogen (Table 10) suggests that the apparent increase in water soluble nitrogen over time was inversely related to the rate of pH fall. Therefore, it would appear that the more rapid the decline in pH, the less protein breakdown. Evidence of this has been obtained where crop material was acidified with formic acid during harvesting (McDonald and Edwards, 1976).

From the percentage values for water soluble nitrogen (% of total nitrogen) given in Table 10 for day 0 samples, it would appear that extensive proteolysis occurs from the time the crop is harvested, and is accentuated during the ensiling process. Although higher mean values were recorded for ammonia treated silages, it is apparent that increases are higher for controls than treated silages.

Free amino acids in water phase: Aggregate concentrations of non essential amino acids (Table 11.1) contributed to a greater proportion of the total free amino acids than essential amino acids (Tables 11.2 and 11.3). Of particular importance, was the increase noted for alanine (Table 11.1) on high ammonia treatment between days 0 and 54; alanine concentration increased approximately eight-fold above initial values.

In terms of % distribution this value would account for 70% of total free non essential amino acids (Table 11.1) and 49% of total free amino acids in the water phase (Table 11.2). The decrease in aspartic acid (Table 11.1) suggests some conversion to alanine, since aspartic acid can be decarboxylated by aspartate-1-decarboxylase to alanine (Kemble, 1956). However, the relatively small loss in aspartic acid compared to the large increase in alanine discounts aspartic acid as the major contributor (Table 11.1). A more plausable explanation for the increased alanine seems through pyruvate, since pyruvate can be reduced to lactic acid, or transaminated to alanine. From the low lactic acid levels (Table 7) recorded for the high ammonia treatment, it seems possible that some of the pyruvate produced was diverted to alanine. Similar observations have been made in continuous culture studies (Erfle _et al_., 1977) where it was shown that alanine concentration rises rapidly when the ammonia concentration is high in the rumen. Those workers suggested that the alanine in the rumen was probably acting as a reservoir for ammonia.

TABLE 11.1 Effect of ammonia concentration and time of ensiling on free amino acids in corn silage water phase

Amino acids	Days of ensiling	Added N as NH_3 (% DM) 0	.25	.52	1.08	SEM[a]
		-------------ug/ml H20 phase	-------------			
Aspartic-acid	0	74.54	69.88	76.53	75.21	12.93
	1	97.83	66.55	77.87	65.21	
	54	171.04[c]	141.09[c]	127.11[b]	58.57[d]	
Threonine	0	30.37	20.85	21.44	22.63	3.68
	1	44.07[b]	30.37	23.82	17.27	
	54	75.04[c]	45.86[cd]	42.28[cd]	30.97[d]	
Serine	0	39.94	27.85	28.38	31.53	4.99
	1	38.37	12.62[c]	26.28	24.70	
	54	88.81[c]	39.41[e]	44.14[be]	24.70[e]	
Proline	0	32.81	31.08	29.93	33.26	8.62
	1	53.52	41.44	38.56	32.81	
	54	150.21[c]	104.74[cd]	77.69[ce]	65.03[be]	
Glycine	0	9.76	9.39	8.26	8.64	2.52
	1	17.65[b]	14.65	9.39	5.64	
	54	41.69[c]	31.55[c]	22.53[cd]	22.53[cd]	
Alanine	0	85.09	90.00	76.19	72.62	15.93
	1	148.53[b]	146.13[b]	98.91	65.49[d]	
	54	204.49[c]	170.19[c]	234.78[c]	584.05[ce]	

TABLE 11.1 (continued)

Amino acids	Days of ensiling	Added N as NH_3 (%DM) 0	.25	.52	1.08	SEM[a]
		---------------ug/ml H_2O phase			---------------	
Valine	0	28.11	24.59	26.35	26.94	6.66
	1	46.84	33.96	31.03	27.52	
	54	128.23^{c}	81.97^{c}	66.75^{cd}	55.04^{bd}	
Methionine	0	0.00	0.00	0.00	0.00	1.27
	1	0.00	0.00	0.00	0.00	
	54	33.57^{c}	27.61^{cd}	23.87^{ce}	21.64^{ce}	
Iso-leucine	0	19.03	14.43	13.78	5.25	4.25
	1	32.80^{b}	16.40	15.75	15.09	
	54	79.38^{c}	42.64^{cd}	33.46^{be}	26.24^{ce}	
Leucine	0	39.36	24.93	28.87	9.84	12.74
	1	73.47	34.11	30.18	26.24	
	54	228.29^{c}	127.27^{ce}	119.39^{ce}	74.79^{ce}	
Tyrosine	0	34.43	21.75	19.93	26.28	5.26
	1	40.77	12.69	12.69	13.59	
	54	32.62	41.68^{b}	35.34	17.22	
Phenyl-alanine	0	25.61	15.69	19.83	17.35	4.59
	1	38.82	21.48^{d}	14.05^{d}	14.04^{d}	
	54	123.90^{c}	70.21^{cd}	50.39^{cd}	33.04^{bd}	

TABLE 11.1 (continued)

Amino acids	Days of ensiling	Added N as NH_3 (%DM) 0	.25	.52	1.08	SEM[a]
		------------ug/ml H_2O phase			------------	
Histidine	0	12.42	5.43	7.76	8.54	3.55
	1	6.21	9.31	7.76	5.44	
	54	40.35[c]	25.61[cd]	10.87[e]	15.52[e]	
Total non-essential	0	307.37	291.24	281.81	283.55	39.66
	1	402.08	337.15	310.48	237.91	
	54	715.60[c]	547.88[c]	560.68[c]	833.31[c]	
Branched chain	0	86.49	63.95	68.99	42.03	17.38
	1	153.11[b]	86.97	76.96	68.85	
	54	435.90[c]	251.88[ce]	219.60[ce]	156.06[ce]	

[a] SEM = Standard error of mean
[b] Significantly different from day 0 ($P<.05$)
[c] Significantly different from day 0 ($P<.01$)
[d] Significantly different from control (0-NH_3) ($P<.05$)
[e] Significantly different from control (0-NH_3) ($P<.01$)

Concentrations of methionine and phenylalanine (Table 11.1) were relatively low in the water phase, being less than 8% of the total free amino acids (Tables 11.2 and 11.3).

Branched chain amino acid (isoleucine, leucine and valine) concentrations in the water phase also increased over time. Mean values at day 54 were higher ($P<.01$) than at day 0 (Table 11.1). Also, mean values for the ammonia treated silages were lower ($P<.01$) than controls.

In general, concentrations of free amino acids in corn silage water phase increased over time (Tables 11.1 to 11.3). Mean values for total free amino acids at day 54 were higher ($P<.01$) than at day 0, but tended to be lower ($P<.01$) with ammonia-treated than control silages (Table 11.3). This was probably due to a decrease in proteolytic activity in the ammonia-treated silages as suggested by Huber _et al._, 1973 and confirmed by Bergen _et al._, 1974 and Huber _et al._, 1979c.

Free Amino acid nitrogen (as percent of original silage N): Table 11.4 shows the effect of ammonia addition and time of ensiling on free amino acid N in corn silage water phase. Nitrogen values are expressed as a percentage of total basal (total N minus added N) nitrogen (Table 8) and provide another way for comparing ammonia treatments with controls.

Total free amino acid N in corn silage water phase increased over time in both controls and treated silages. However, increases were greater for the controls than treated silages. In day 0 samples, total free amino acid N (Table 11.4) in the water phase accounted for 10.32, 8.16, 8.14 and 8.11%

TABLE 11.2 Effect of ammonia concentration and time of ensiling on free amino acids in corn silage water phase.

Amino acids	Days of ensiling	Added N as NH_3 (%DM)			
		0	.25	.52	1.08
		ug/ml H_2O phase			
Glu & Glu-NH_2	0	65.24	63.04	63.04	62.31
	1	46.18	55.78	59.49	44.06
	54	59.37	60.91	54.43	78.43
Lysine	0	77.49	40.21	46.06	46.06
	1	26.32	40.94	36.55	29.24
	54	125.00	100.88	89.18	65.79
Arginine	0	33.10	22.65	25.26	29.62
	1	26.13	20.04	17.42	15.68
	54	32.23	33.97	24.39	17.42
Total essential	0	229.80	190.52	209.26	192.48
	1	335.43	219.29	189.24	164.10
	54	898.59	597.68	495.90	357.65
Total	0	607.47	481.75	491.57	475.58
	1	737.50	556.44	499.72	402.51
	54	1614.19	1145.56	1056.58	1190.96

TABLE 11.3 Main effect means, standard errors, and significant differences for free amino acids in corn silage water phase[a]

Amino acids	Days				Added N as NH_3 (%DM)				
	0	1	54	SEM[b]	0	.25	.52	1.08	SEM[b]
Glu & $GluNH_2$	63.41	51.38	63.28	4.21	56.93	59.91	58.98	61.60	6.28
Lys	52.46	33.26	95.21[c]	1.78	76.27	60.68	57.26	47.03	5.37
Arg	27.66	19.82	27.00	3.71	30.48	25.55	22.36	20.90	2.90
TEAA	223.01	227.01	587.46[d]	64.09	511.27	335.83[e]	298.13[f]	238.08[f]	30.36
Total	514.09	549.04	1251.82[a]	38.57	986.39	727.92[e]	682.62[e]	689.62[e]	59.77

[a] Corresponds to mean values shown in Table 11.2.
[b] SEM = standard error of mean.
[c] Significantly higher than day 0 ($P<.05$).
[d] Significantly higher than day 0 ($P<.01$).
[e] Significantly lower than control (0-NH_3) ($P<.05$).
[f] Significantly lower than control (0-NH_3) ($P<.01$).

of total basal nitrogen for controls, low, intermediate and high ammonia treatments, respectively. By day 54, deamination (as measured by the increase in free amino acids) accounted for 9.3, 7.1, 5.7 and 8.2% of the basal nitrogen for controls, low, intermediate and high ammonia treatments. However, if alanine increases on high ammonia were assumed equal to those of the control silages, deamination between days 0 and 54 would only account for 2.39% of total basal nitrogen (Table 11.5).

Though increases were recorded for threonine, methionine, isoleucine, tyrosine, phenylalanine and arginine, they appeared to be least affected by ensiling; their individual contributions to the total free amino acid N in the water phase (Table 11.4) accounted for less than 1% of total basal nitrogen.

In general, a lower amino acid N content in the water phase of treated silages suggests that there was less deamination in treated than control silages. Moreover, increasing ammonia decreased deamination. These data are the first to show large increases in alanine in the water phase of corn silages treated with high ammonia, increases which were apparently not related to proteolytic effects.

TABLE 11.4 Effect of ammonia addition and time of ensiling on free amino acid N in corn silage water phase

Days	0				1				54			
Added N as NH_3	0	.25	.52	1.08	0	.25	.52	1.08	0	.25	.52	1.08
	% of total N[a]											
Asp	.91	.86	.92	.91	1.08	.75	.86	.80	1.64	1.44	1.28	.57
Thr	.41	.29	.29	.31	.54	.38	.29	.24	.81	.52	.48	.34
Ser	.62	.43	.43	.48	.54	.18	.37	.39	1.08	.51	.56	.30
Pro	.46	.44	.41	.47	.69	.54	.49	.47	1.67	1.24	.90	.73
Gly	.21	.20	.18	.19	.35	.29	.18	.21	.71	.57	.40	.39
Ala	1.55	1.65	1.36	1.32	2.46	2.45	1.63	1.21	2.94	2.60	3.52	8.48
Val	.39	.34	.36	.37	.59	.43	.39	.39	1.40	.95	.76	.61
Met	0	0	0	0	0	0	0	0	.29	.25	.21	.19
Ile	.24	.18	.17	.06	.37	.19	.18	.19	.77	.44	.34	.26
Leu	.49	.31	.35	.12	.87	.39	.34	.33	2.23	1.32	1.22	.74
Tyr	.31	.20	.18	.23	.33	.10	.10	.12	.23	.31	.26	.12
Phe	.25	.15	.19	.17	.35	.19	.12	.14	.96	.58	.41	.26
His	.39	.17	.24	.27	.18	.27	.22	.17	1.00	.67	.28	.39
Glu and Glu NH_2	1.44	1.40	1.37	1.37	.93	1.14	1.19	.99	1.04	1.13	.99	1.38
Lys	1.72	.90	1.00	1.02	.53	.84	.73	.66	2.19	1.88	1.63	1.16
Arg	.93	.64	.69	.82	.66	.52	.44	.44	.71	.80	.56	.39
TEAA	5.13	3.18	3.47	3.37	4.42	3.31	2.81	2.68	10.59	7.72	6.15	4.46
TNEAA	5.19	4.98	4.74	4.74	6.05	5.35	4.72	3.98	9.08	7.49	7.65	11.85
BCA	1.12	.83	.55	.55	1.83	1.01	.91	.91	4.44	2.71	2.32	1.61
Total	10.32	8.16	8.14	8.11	10.47	8.66	7.53	6.66	19.67	15.21	13.80	16.31 (10.5)[b]

[a] total basal nitrogen for that treatment and day (i.e. total N minus added N).
[b] Alanine increase assumed equal to control.

TABLE 11.5 Effect of ammonia addition and time of ensiling on total free amino acid N in corn silage water phase

Item	Added N as NH_3 (% DM)			
	0	.25	.52	1.08
	------------% of total N[a]------------			
Day 0	10.32	8.16	8.14	8.11
Day 54	19.67	15.21	13.80	10.50
Change	9.35	7.05	5.66	2.39

[a] % total basal nitrogen for that treatment and day (i.e. total N minus added N).

Experiment 11: Feeding Trial

Silage Analyses: Table 12 shows the dry matter, pH, lactic acid and crude protein of silage composites collected during the 12 weeks of treatment.

Dry matter content of silages was higher ($P<.05$) for the Pro-Sil than Cold-flo treated (36.44 *vs*. 34.61%). This was probably due to a higher dry matter of corn plant material entering the Pro-Sil silo, or the added minerals and molasses in Pro-Sil, since there was little ($\pm$ .5 units) or no variation from mean values (Table 12) in either silage throughout the entire feeding period.

The lactic acid content of the Pro-Sil treated silage was higher ($P<.02$) than the Cold-flo (7.67 *vs*. 6.30% of dry matter), suggesting that minerals in Pro-Sil were affecting the silage fermentation separate from the ammonia. Evidence of this was obtained by Lomas *et al*., (1978) who found 16% higher lactic acid in corn silage treated at ensiling with anhydrous ammonia (Cold-flo) plus minerals compared to the anhydrous ammonia alone. A lower lactic acid content of Cold-flo than Pro-Sil treated silages was also reported by Fox and Cook, (1977).

Differences in pH and crude protein of silages were small ($P>.10$).

Animal Response: Response of lactating dairy cows to the two NPN treated silages is summarized in Tables 13 through 15.

Dry matter intakes from silage (based on a fixed % of total ration as shown in Table 4) as Kg/day or % of live

TABLE 12. Dry matter, pH, lactic acid and crude protein of corn silage composites[a]

Item	Cold-flo	Pro-Sil	SEM[b]	Effect
Dry matter (%)	34.61	36.44	.33	$P<.05$
pH	4.44	4.37	.12	NS[c]
Lactic acid (%DM)	6.30	7.67	.15	$P<.02$
Crude protein (%DM)	13.03	13.20	.16	NS

[a] Mean values represent averages for 3-four week composites of the silages fed during the 12 weeks treatment period.
[b] SEM = standard error of mean difference.
[c] NS = non significant ($P>.10$).

TABLE 13. Influence of non protein nitrogen source on dry matter and protein intakes of dairy cows[a]

Item	NPN source		SEM[b]	Effect
	Cold-flo	Pro-Sil		
Silage dry matter (Kg/day)	8.56	8.75	.34	NS[c]
(% BW)	1.48	1.49	.05	NS
Total dry matter (Kg/day)	17.39	17.47	.68	NS
(% BW)	3.01	2.95	.10	NS
Total crude protein (Kg/day)	2.51	2.57	.11	NS
Crude protein from NPN (% of total CP)	16.05	16.75	.08	$P<.001$

[a] Mean values represent 12 cows per treatment for 12 weeks.
[b] SEM = standard error of mean difference.
[c] NS = non significant ($P>.10$).

weight were similar for both groups (Table 13). Previous work (Huber <u>et</u> <u>al</u>., 1979b) showed depressed intakes by dairy cattle of silage treated at ensiling with .375% gaseous ammonia (82% N). However, this effect was not found to be consistent (Huber <u>et</u> <u>al</u>., 1979b). Intakes of total dry matter as Kg/day or % of live weight were also similar for both silages (Table 13). Intakes of total crude protein tended to be higher for the Pro-Sil group, but differences were not significant. However, intakes of crude protein from NPN (% of total crude protein) were higher ($P < .001$) for the Pro-Sil than Cold-flo group (Table 13). A higher intake of silage dry matter by the Pro-Sil group, though not significant might explain the higher NPN intake (Table 13).

Milk yields of cows averaged about 28 Kg/day during pre-treatment (Table 14). The decrease in milk yields from pre-treatment to the end of the trial was probably due to cows being put on trial in mid lactation, averaging about 125 days postpartum when treatment commenced. Milk yields and persistencies during treatment were slightly higher for cows on Cold-flo, whereas fat-corrected and solids-corrected milk were slightly higher for those on Pro-Sil, but differences were not significant.

Milk components were all within the normal range, and did not differ significantly between groups (Table 15). Both groups gained .29 kg body weight during treatment, typical of cows after peak lactation. Efficiency of conversion of feed to milk was also similar for both groups (Table 15).

TABLE 14. Influence of non protein nitrogen source on milk yields of dairy cows[a]

Item	NPN source Cold-flo	Pro-Sil	SEM[b]	Effect
Pretreatment (Kg/day)	28.07	28.32		
Treatment (Kg/day)	24.88	24.25	1.32	NS[c]
Persistency (%)[d]	88.96	85.40	2.44	NS
Fat corrected milk (Kg/day)	21.89	22.15	1.22	NS
Solid corrected milk (Kg/day)	22.21	22.27	1.20	NS

[a] Mean values other than pretreatment represent 12 cows per treatment.
[b] SEM = standard error of mean difference.
[c] NS = non significant (P>.10).
[d] Persistency (%) = (treatment milk/pretreatment milk) x 100.

TABLE 15. Influence of non protein nitrogen source on milk composition, weight change and feed effeciency of dairy cows[a]

Item	Cold-flo	Pro-Sil	SEM[b]	Effect
Milk composition				
Protein (%)	3.20	3.06	.11	NS[c]
Fat (%)	3.20	3.44	.13	NS
Solids not fat (%)	8.76	8.75	.13	NS
Weight change (Kg/day)	.29	.29	.08	NS
Feed efficiency[d]	1.43	1.41	.08	NS

[a] Mean values represent 12 cows per treatment for 12 weeks.
[b] SEM = standard error of mean difference.
[c] NS = non significant (P>.10).
[d] Feed efficiency = treatment milk yield/total dry matter intake.

Although there have been no previous comparisons between the feeding value of corn silages treated with Cold-flo and Pro-Sil for dairy cattle, these results compare favorably with those of Donaldson and Thomas, (1978) for Cold-flo treated corn silage, and of Huber et al., (1979b) for Pro-Sil treated corn silage.

SUMMARY AND CONCLUSIONS

This study consisted of two experiments. Experiment 1 determined the nature of the protein break-down occuring in corn silages treated with or without ammonia. Whole chopped corn plants (32-34% DM) were treated with 0, .25, .52, or 1.08% added N as ammonia (of silage dry matter). Immediately after treatment, about 3 kg material were placed in 5 mil polythene bags which were then evacuated and served as experimental silos. Silage samples were taken on days 0, 1, 2, 6, 12 and 54 and analyzed for dry matter, pH, lactic acid, total and water-insoluble nitrogen, and free amino acids in the water phase. The fermentation patterns for dry matter, pH, lactic acid, total and water insoluble nitrogen were similar to those reported by other researchers (Britt and Huber, 1975; Huber et al., 1980b) for ammonia treated corn silage. Alanine concentrations increased substantially over time in the water phase of high ammonia treated silages, a phenomenon which is apparently not related to proteolytic effects. It was suggested that the increased alanine was probably through pyruvate. Increased total amino acid N between days 0 and 54 on high ammonia treatment would account for 2.4% of total basal (original) nitrogen if alanine increase assumed equal to controls. Apart from the large increases in alanine, these data support earlier studies that suggest that ammonia inhibits plant protein breakdown. Further experimentation is needed to more clearly define the nature of alanine increases in ammonia-treated silages, and possible nutritional significance in ruminant nutrition.

In Experiment 2, 24 lactating Holstein cows were employed to determine the relative feeding value of corn silages treated with Pro-Sil or anhydrous ammonia (by Cold-flo process). Prior to going on trial, cows received a standardization ration (14% crude protein) for 14 days. Thereafter, cows were paired based for milk yields, stage of lactation, breeding group and age. Within each pair, experimental diets (49 to 50% of either treated silage) were randomly assigned. The feeding period lasted for 12 weeks, during which time silage samples were collected and analyzed for dry matter, pH, lactic acid and crude protein. Daily dry matter intakes and milk weights were recorded, and biweekly milk samples analyzed for milk fat, protein and total solids. Cows were weighed on two consecutive days, seven days after beginning and at the end of treatment. Dry matter content of silages was higher for the Pro-Sil than Cold-flo treated, which was apparently due to a higher dry matter of corn plant material entering the Pro-Sil silo, or the added minerals and molasses in Pro-Sil. Lactic acid was also higher for the Pro-Sil silage, but differences in pH and crude protein were negligible. Dry matter and total crude protein intakes were similar for both silages. However, the slightly higher intake of silage (based on a fixed % of total ration) by the Pro-Sil group was reflected in a higher intake of crude protein from NPN. No major differences in milk yields, persistencies, milk components or feed efficiencies were observed between groups during treatment. Both groups showed similar weight gains. These data suggest that Cold-flo treated corn silages can be used as efficienty as Pro-Sil treated silages in rations for lactating dairy cows. However, rations containing Cold-flo treated corn silage would need greater mineral supplementation than those with Pro-Sil silage.

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