INFLUENCE OF SOURCE AND LEVEL OF NON-PROTEIN NITROGEN ADDITIONS ON THE NUTRITIVE VALUE OF CORN SILAGE

By

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ABSTRACT

INFLUENCE OF SOURCE AND LEVEL OF NON-PROTEIN NITROGEN ADDITIONS ON THE NUTRITIVE VALUE OF CORN SILAGE

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Four experiments were conducted to investigate the relationship of non-protein nitrogen additions to fermentation and the subsequent influence on the nutritive value of corn silage. A common objective of all experiments was to vary the level of NPN addition to corn silage and to compare ammonia with urea and determine their effects on fermentation or ruminant nutritional parameters.

The first experiment (1-A) was designed to study the effect of urea, aqua-ammonia or an ammonia solution, each at three levels (.23, .46 or .92%N) of addition, on silage nitrogen and organic acid fractions during fermentation. The nitrogen sources and levels were compared to a control corn silage receiving no additive. An added dimension was to compare mineral and disaccharide additives alone or in combination with each low level of nitrogen source. Silages were evacuated of air and stored in 56 kg portions inside experimental silos. Silos were opened, sampled (500g) and re-evacuated of

air on days 1, 3, 5, 10, 15, 20 and 40 of fermentation. Temperature of silage during fermentation decreased over time and was not affected by nitrogen source or level. Silage final pH on day 40 was increased ($P<.05$) by nitrogen addition, with the pH of the highest level of the aqua-NH_4 and NH_4-solution treated silages remaining above 6.0. Lactic acid was increased ($P<.05$) in silage containing the low levels of added nitrogen; However, lactic acid production was essentially eliminated at the highest level of addition to silage by the two sources of ammonia. Acetic acid levels were increased ($P<.01$) by the same treatments which depressed lactate. Total nitrogen and total soluble nitrogen content of corn silage was increased ($P<.05$) by each incremental level of added nitrogen. Water insoluble nitrogen levels in this experiment were unchanged by nitrogen additions to corn silage. Mineral and disaccharide additives had no consistent influence on silage fermentation parameters except that $CaCO_3$ caused elevated lactic acid levels especially incombination with ammonia and lactose tended to cause lower silage pH values.

The second experiment (2-A) was designed to study the effect of three levels of anhydrous ammonia (.24, .48 and .72%), with three levels of added water (0, .8 and 1.6%), on silage fermentation parameters and nitrogen recovery of corn silage harvested at 30, 35, 40, and 45% dry matter. Duplicate silage treatment combinations (56 kg each) were stored as in experiment 1-A. Silos remained sealed until day 40 of fermentation.

Ammonia and water were mixed and added to silage at the silage blower to simulate farm conditions. Recovery of added ammonia, after 40 days of fermentation, decreased with advanced silage maturity and increasing amounts of added ammonia. Over all recovery was 78, 69, and 59% for the incremental levels of NH_3 and 91, 68, 60 and 54% for the advancing stages of plant maturity (DM%). Silage pH was increased with increasing silage dry matter and by the incremental amounts of added NH_3. Lactic and acetic acids were highest ($P < .05$) for the 30% dry matter silage and were not affected by NH_3 or H_2O level. Water insoluble nitrogen (WIN) levels of corn silage were increased ($P < .05$) at the highest dry matter (45%) and WIN levels were higher for all NH_3 levels than for the control silage. It is theorized that the higher dry matter silages and NH_3 both increase WIN or "true protein" content of corn silage by preventing proteolysis.

The third experiment (1-B) was designed to study the effect of urea (.5 and 1%) and anhydrous ammonia (.3, .6, and .9%) on silage fermentation and lactation parameters. The five silages were compared to control corn silage which was fed with either a 20% or a 9% crude protein (CP) concentrate. The NPN treated corn silages were also fed with 9% CP concentrate. All concentrates were fed at .4 kg/1kg daily milk. Thirty-five cows were blocked according to production into five groups. Cows from each block were randomly assigned to one of the seven different treatment groups for a 10-week feeding trial. Water insoluble nitrogen tended to be higher ($P < .10$) for the .6% NH_3 treated silage. Lactic acid was higher for all NPN silage treatments compared to control. Milk yield was maintained at higher ($P < .05$) levels for the cows on the positive than negative control ration (20% vs. 9% CP conc) while cows fed NPN treated silages had intermediate yields. Yields of 4% fat-corrected milk (FCM) were not different but cows receiving the negative control ration tended to eat less silage dry matter and to give less 4% FCM. Ruminal VFA levels were highest for cows receiving the positive control and the .6% NH_3 rations. Blood urea N levels and daily yield of milk protein were highest for cows fed the positive control and the two highest levels of NPN.

The fourth experiment (2-B) was designed to compare the effects of ammonia and urea additions to: 1) freshly chopped corn silage prior to ensiling; or 2) fermented control corn silage prior to feeding. Eight rumen fistulated Holstein steers were used in two 4 X 4 Latin squares. Digestibility of dry matter and nitrogen balance was lower for those silages to which NPN was added prior to ensiling. Rumen liquor VFA concentrations and pH were unchanged. Ruminal NH_3 levels were lower ($P < .05$) for NPN additions at ensiling. Water insoluble N was higher ($P < .05$) for NH_3 added at ensiling but there were no discernable advantages manifest in this trial.

DEDICATION

To Rosella and Our Children:

Robert

Douglas

Deborah

Diana

Paul

Thomas

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INTRODUCTION

Corn will produce more digestible nutrients per hectare than any other food crop. Harvesting the entire corn plant as silage, instead of only for grain, nearly doubles the nutrient yield for ruminant feeding. Green-chopped corn ensiles remarkably well due to a high carbohydrate content which yields substantial amounts of organic acids upon fermentation. Corn silage is a highly palatable, concentrated forage of consistently high digestibility.

Corn silage is an excellent energy source for ruminants but it is notariously low in crude protein content (8% of dry matter). Corn silage rations must be supplemented with either expensive plant protein or a source of non-protein nitrogen (NPN). Urea has traditionally been the NPN supplement for increasing crude protein content of corn silage. Anhydrous ammonia is cheaper than urea and not only augments crude protein but also has been shown to increase the water-insoluble or "true protein" content of corn silage.

Therefore, the objectives of this study were:

(1) To evaluate urea and anhydrous ammonia alone or with mineral buffers and disaccharides as corn silage fermentation ammendments:

(2) To compare recovery of anhydrous ammonia at varying stages of corn silage maturity and follow fermentation

parameters;

(3) To evaluate urea and ammonia treated corn silage with minimal plant protein supplementation as a feed for lactating dairy cows; and

(4) To determine nitrogen balance and digestibility of corn silage treated with urea or ammonia at ensiling and just prior to feeding.

LITERATURE REVIEW

Importance of Corn Harvested as Silage

Corn (Zea mays L.) is grown within a wide climatic range. It is grown from 58 north latitude in Canada and the USSR to 40 south in South America, and from below sea level in the Caspian Plain to 3,600 m and beyond in parts of the Andes. Despite this adaptability to vastly different environmental conditions, a minimum amount of moisture must be available and temperatures must be above freezing during the growing season. Corn is a warm-weather crop requiring considerable warmth after tasseling. Once it is above ground it will tolerate only very brief and light frosts (Jenkins, 1941).

Temperate areas, specifically the USA Corn Belt, the La Plata corn belt of Argentina, the Garonne Basin of France, Italy's Po Valley, and the Hungarian and Walachian Basin, produce three-fourths of the world's corn and are considered to have almost ideal climatic and soil conditions for the crop. Summer temperature should be about 24° C, with warm nights (average 14° C), and rainfall should be abundant, from 460 to 600 mm during the growing period (Van Royen, 1954).

Corn will produce more digestible nutrients per unit of land surface where it is well adapted, than any other food crop that can be grown. Sorphum is a close second. Both have high levels of net photosynthesis with a minimum of energy loss through photorespiration (Wittwer, 1974).

Corn uses 56% less water per unit of dry matter produced than alfalfa (Coppock, 1969). Green-chopped corn ensiles remarkably well due to a high carbohydrate content which yields substantial amounts of organic acids upon fermentation. Corn silage is a palatable, concentrated forage of consistently high digestibility (65-75% of the dry matter). Corn as silage is harvested but once during the growing season, and lends itself to mechanical harvesting, storing, and feeding.

Corn silage is the most important silage crop in Michigan with over four million metric tons produced in 1973 (Mich. Ag. Stats., 1974) which was 57% greater than 1959 due to 25% increases in both yield per hectare and total hectares harvested. US corn silage production for 1973 (USDA Ag. Stats., 1974) was 100 million metric tons with increased production trends similar to Michigan for the last 14 years (USDA Ag. Stats., 1972). The state of Wisconsin alone produces 10 million metric tons and with the seven other North-central states accounts for nearly one-half the total US corn silage production (USDA Ag. Stats., 1974). Corn harvested for all purposes (approx. 25 million hectares) in 1969 represented 22.1% of all harvested US cropland and only 12% of this was harvested as silage (USDC Census of Ag., 1969). When harvested as silage instead of grain the total dry matter for ruminant feeding is nearly doubled. It would appear that this idea ought to be actively promulgated among dairymen and cattlemen. The cultural practices of sod-seeding and minimum tillage should permit corn to be grown on land which, because of inclined terrain, has heretofore been used for less productive crops.

Historical Aspects of Corn Silage

Coppock and Stone (1968) reviewed the literature and credited the Egyptians and Greeks for having stored grain in pits or underground trenches. Johnston (1843) presented as worthy of acceptance a German method for harvesting and storing green fodder by packing the direct-cut material into trenches which are then covered with boards and earth to facilitate sealing. Reihlen of Germany was credited (Miles 1918) with being the first to use a silo to preserve the whole corn plant. Goffart (1877), of Burtin, France having gained experience with silage preservation for 20 years was the first to describe in practical terms in 1873 the important aspects of making corn silage successfully. He recommended that the length of cut be one cm and that bricks or stone be placed on top of the silage to aid in packing and exclusion of air.

Among the first to preserve corn as silage in the United States were Miles of Illinois in 1875 and Morris of Maryland in 1976 (Miles, 1918). By 1882 a USDA special report (Nesbit, 1882) summarized the experience of 91 respondents who were then using corn silage. Corn silage has been fed to dairy cattle at Michigan State University since 1881, but research in this area did not start until the introduction of hybrid corn in 1940 (Huffman, 1960). The late C.F. Huffman deserves much acclaim for his pioneering investigations of corn silage during the 1950's from which he attested to the abundant yield of total digestible nutrients per hectare, to the milk-stimulating capacity for lactating cows, and he strongly suggested that "corn silage should not be considered a true roughage, but a mixture of roughage and grain" (Huffman 1954a and b, 1956, 1959 and 1960).

Fermentation of Corn Silage

One of the first observations of production of organic acids in corn silage was by Brandeau who analyzed the corn silage of Goffart. The volatile acid was designated acetic acid and the non-volatile, lactic acid (as reported by Annett and Russell, 1908). Analysis of the whole corn plant before and after ensiling led Annette and Russell (1908) to conclude that the major changes which take place during fermentation are: disappearance of most of the sugars; production of carbon dioxide and a number of organic acids; reduction in protein nitrogen by one-half and a concomitant increase in non-protein nitrogen. However, there was little change in fiber content. Subsequently, Russell (1908) identified in corn silage the volatile fatty acids (formic, acetic, and butyric) and the non-volatile acids (lactic, malic, and succinic) plus a number of amino acids and purine bases.

The five aliphatic acids, formic through valeric, were quantitatively identified by Dox and Neidig (1912), with acetic comprising about 90 percent of the total. Propionic acid was second in importance and butyric acid was found only in samples characterized by some spoilage. One year later the same workers (Dox and Neidig, 1913) reported that lactic acid was normally present in corn silage at levels 25% higher than the volatile acids. They further characterized the lactic acid of corn silage as an optically inactive racemic mixture. Neidig (1914) took samples over a two week period and reported the concentration of both volatile and non-volatile acid reached a plateau in about eight to ten days, which indicated that fermentation was essentially complete after two weeks. The amount of acid produced was indirectly related to maturity, owing to the higher sugar and moisture content of the immature corn plant.

Barnett (1954) outlines the changes during silage fermentation as a four or possibly five-phase process:

Phase 1. The continued respiration of the plant cells results in the production of CO_2, the utilization of simple carbohydrates, and the evolution of heat;

Phase 2. Acetic acid is produced in small quantities by organisms of the coliform group (This phase is short and merges into the third phase);

Phase 3. Lactic acid organisms, lacto-bacilli and streptococci, supported by adequate carbohydrate, initiate a lactic acid fermentation;

Phase 4. Quiescence in the silage mass during which lactic acid production passes its peak and remains constant at 1 to 1.5% of the fresh weight, with a constant pH less than 4.2. These four phases occur over a three-week period with the first three being complete after three days. Insufficient lactic acid production or incorporation of air into the mass may cause development of the fifth phase;

Phase 5. Butyric acid-producing organisms attack both residual soluble carbohydrates and the lactic acid which has been formed. In extreme cases, there may be deamination of amino acids with the formation of higher volatile fatty acids and ammonia as well as decarboxylation leading to the formation of amines and carbon dioxide.

Peterson *et al.* (1925) noted that the first chemical changes incident to ensiling corn were that after four to five hours oxygen disappeared and for about 48 hours carbon dioxide rapidly increased. They observed the presence of large numbers of lactic acid-producing bacteria, as fermentation products appeared in 25 to 48 hours after ensiling. Utilizing sterilized corn inoculated with bacteria they demonstrated that corn silage could be made without the benefit of plant cell respiration. Their conclusion was that bacteria are the chief agents involved in the production of silage acids from the sugar and starches of the ensiled plant. A similar conclusion regarding the production of acids was made by Hunter (1921), but he felt that the formation of ammonia was due to both plant exzymes and bacteria.

A complete review of the microbiology of silage is presented by Kempton (1958) who found that less than .1% of the bacteria on the crop at time of ensiling were capable of growing on lactobacillus selection medium.

Degradation of Carbohydrates

Organic acids are formed due to the action of microorganisms on the carbohydrates of the ensiled plant material (Hunter, 1921; Watson and Nash, 1960). This is considered the most salient feature of the ensiling process. Johnson et al. (1966) reported decreases in corn plant soluble carbohydrate ranging from 39 to 83% and concurrent conversion to lactic and acetic acids. Peterson et al. (1925) observed that starch of the corn plant material decreased from 10 to 30% with ensiling. Iowa researchers (Dox and Neidig, 1912) also noted considerable degradation of starch, while others (Dexter et al. (1959) showed no appreciable breakdown.

Geasler (1970), using laboratory silos to evaluate fermentation parameters over a 10-week harvesting period with silage dry matter ranging from 22 to 48%, showed that as dry matter and/or maturity increased the lactic acid content of corn silage was significantly ($P < .01$) reduced from a high of 5.8% to a low of 2.2% of the dry matter. Acetic acid was also significantly ($P < .01$) reduced with advancing corn plant maturity. Soluble carbohydrate level followed that of the organic acids and was probably the reason for the decrease in organic acids. Other researchers have noted similar declines with advancing maturity of the corn plant (Gordon et al., 1968; Johnson and McClure, 1968).

Degradation of Protein

Plant protein breakdown and a consequent increase in water soluble NPN during ensiling is regarded as an inevitable forfeiture. Various factors may alter the extent of proteolysis. Ordinarily proteolytic enzymes of plant cell origin are directly responsible for this hydrolysis (Barnett 1954; Watson and Nash 1960). Russell (1908) treated corn silage with chloroform or toluene to arrest plant cell and micro-organism activity and attributed the resultant proteolysis to plant "tryptic" enzymes. Hunter (1921) analyzed field pea and oat, corn, and corn plus soybean silages and concluded that the bacteriological and chemical changes were similar for all silages. The field pea and oat silage ensiled in glass jars was subjected to either control treatment, 2% chloroform, or sterilization by heating and inoculation with silage juice. Chloroform treatment essentially halted organic acid production via microbial inhibition but did not change the level of proteolysis ascribed to the plant enzymes. Sterilization of the silage mass and subsequent inoculation with silage juice teeming with characteristic silage bacteria resulted in normal organic acid production but proteolysis was arrested due to plant enzyme inhibition. Mabbit (1951) approached this question by ensiling timothy grown in a microbe free chamber. Chemical analysis after ensiling revealed the absence of organic acids, a fourfold increase in amino acid nitrogen, and an elevenfold increase in volatile bases. This effect on fermentation was attributed to the plant enzymes since bacteria were absent Watson and Nash (1960) conclude that protein degradation to amino acids is primarily the action of plant enzymes, but add that certain butyric acid bacterial enzymes may decompose plant protein of crops low in soluble carbohydrates during initial fermentation. Refermentation of silage, associated with putrefying changes when air is introduced into silage, elicits further bacterial degradation of amino acids to keto acids, amides, amines, carbon dioxide and ammonia (Barnett, 1954)

Some loss of plant protein during ensiling is unavoidable since the bacteria which produce the acid necessary for silage preservation require nitrogen for growth. In fact, nitrogen may have to be added to crops which have a low content of natural protein. Cullison (1944) showed that a more rapid fermentation of sweet sorghum was acheived by adding 0.5% urea to the fresh material. The keeping quality, as measured by lower silage temperature and increased titratable acidity, was also enhanced. Barnett (1954) suggests that chopping and crushing plants prior to ensiling induces earlier lactic acid fermentation which in some cases has increased the crude protein content of the resultant silage.

The magnitude of proteolysis of ryegrass (*Lolium perenne*) silage, harvested just prior to head emergence and after seven days of ensiling, ranged from 18 to 29% depending on the silage dry matter (Brady, 1965). Water soluble

NPN constituted from 37 to 47% of the total N after 133 days of ensiling. Hughes (1970) estimated that 20% of total N of fresh perennial ryegrass was NPN compared to a determined average value of 65% NPN for eleven samples taken 2-18 months after ensiling. Hawkins (1969) observed 54% of total N as soluble NPN in 22% DM alfalfa silage. The amount of soluble N decreased as silage dry matter was increased due to wilting.

Crude protein of freshly chopped corn silage consists of only 13-15% NPN (Cash, 1972; Salas, 1971). Protein degradation to ammonia and other NPN compounds consequent to ensiling elevates NPN to 36-48% of total N (Bergen, Cash and Henderson 1974; Cash, 1972; Salas, 1971). Johnson et al. (1967) reported tungstic-acid-supernatant nitrogen nearly doubled during ensiling over a range of 20-46% dry matter while there was essentially no change when harvested at 55% dry matter.

Proteolysis is extremely difficult to eliminate except with mineral acids. Watson and Nash (1960) credit A.I. Virtanen with having shown experimentally that neither plants nor the anaerobic microorganisms contain proteolytic enzymes capable of action below pH 4.0. Addition of hydrochloric acid to clover silage to give a pH of 3.6 after four weeks resulted in soluble and ammonia nitrogen levels equivalent to freshly chopped material, while the non-acid control treatment magnified the respective nitrogen levels 3 and 15 times. Addition of hydrochloric acid to achieve a final pH of 4.3 led to proteolysis intermediate between fresh material and control, while a final pH of 4.6 was similar to control (Virtanen, 1933). Other mineral acids including sulfuric and phosphoric have been used alone and in combination with hydrochloric (Watson and Nash, 1960), but the corrosive nature of the mineral acids for men and equipment is a serious problem which tends to limit their use (Owen, 1971).

Organic acids have been used on a limited scale to inhibit proteolysis and enhance corn silage preservation. Huber _et al._ (1972) employed pilot silos (plastic bags inside 200 liter metal barrels) to investigate additions of formic, acetic, propionic, and lactic acids to 24-28% DM corn silage. Formic acid markedly decreased silage lactic acid and NPN, while causing a concurrent increase in water insoluble nitrogen. All other acid treatments had a similar but less pronounced influence on silage nitrogen fractions. Feeding trials with dairy (Huber _et al._, 1973b) and beef (Cash _et al._, 1971) animals demonstrated no advantage in animal performance from .5 to .6% formic acid at ensiling to 31 to 39% DM corn silage, even though proteolysis and bacteria fermentation were reduced. Again (Huber _et al._, 1973b) formic acid more effectively inhibited proteolysis and lactic acid production than either acetic or propionic. Huber (1971) demonstrated increased silage intake and greater milk production of 43% DM urea-treated corn silage when formic acid was added prior to ensiling at 1.5% of the dry matter. These animal responses were negatively related to the extent of bacterial fermentation and silage proteolysis.

A more attractive method of controlling proteolysis of corn silage would be the addition of NPN. Johnson et al. (1967) demonstrated that additions of urea and limestone, each at .5% of corn silage green weight, further decreased the percent of total N as tungstic-acid-precipitable nitrogen (true protein nitrogen) when harvested at 27% dry matter and below, but increased the amount of true protein at dry matter levels of 34% and above. Coppock and Stone (1968) cite German and Russian workers who have shown that urea additions to corn silage at ensiling increased the true protein and ammonia content when compared to control silage. Addition of ^{15}N labeled urea at .6% of silage fresh weight increased true protein 22% more than in control corn silage. Approximately 30% of this increase in true protein was attributed to microbial synthesis of protein from ^{15}N labeled urea. The remainder of the true protein increase was ascribed to a sparing action of the urea since it appeared to reduce proteolysis during silage fermentation. Another Russian experiment cited by these same reviewers (Coppock and Stone, 1968) demonstrated that .65% urea added to corn silage provided true protein values approximately 35% greater than control corn silage.

Ammoniated corn silage also evokes higher true protein levels than the same material ensiled without additives. Huber and Santana (1972) showed that compared to control corn silage, urea and an anhydrous ammonia-water mixture elevated water-insoluble nitrogen (true protein) 34 and 49%, respectively. A subsequent investigation (Huber _et al._, 1973c) revealed that urea, aqua ammonia, and an ammonia-molasses-mineral suspension all increased water-insoluble nitrogen 38, 45, and 54%, respectively, when compared to control corn silage. Other researchers have reported similar increases in water-insoluble nitrogen when either urea or ammonia is added to corn silage at ensiling (Beattie, 1970; Cash, 1972).

Bergen _et al._ (1974) filled 4-liter glass jars with either control or corn silage treated at 2.25% of green weight with an ammonia-molasses-mineral suspension (13.6% nitrogen). Buffered extracts from these laboratory silages were used as proteolytic enzyme sources. Proteolytic activity (m equiv. nitrogen solubilized per 5 ml of incubation system per 420 minutes) of NPN treated silage was 50, 20, 42, and 56% lower than control silages on days 0, 1, 2, and 5 respectively. This observation would imply that NPN spares corn plant protein from degradation.

Corn Silage Maturity

The most important single factor affecting digestible nutrient yield per hectare and voluntary silage intake by dairy cattle is the stage of growth at which corn is harvested for silage. Field and storage losses are affected by stage of maturity, but digestibility of dry matter bears little relation to stage of growth (Coppock & Stone, 1968).

Dry matter harvested per hectare

Dry matter accumulation (average of adapted hybrids grown in central Iowa) as related to stage of growth, dry matter percent, and the relative proportion of plant parts is depicted in Figure 1 (Hanway, 1966). While this graphic representation is not universally applicable, it does point out that harvesting too early diminishes the total dry matter yields. This reduction would be almost entirely due to less grain which in itself enhances silage energy value. The corn kernel at physiological maturity is 65 to 70% dry matter (Daynard and Duncan, 1969; Hanway, 1966) which, in effect, dries out the corn plant. Silage made from corn harvested at this stage of growth (35% dry matter) will not cause seepage problems in the silo and will be readily consumed by cattle.

Physiological maturity of normally developed corn coincides with the formation of an abscission-type layer near the tip of the kernel. This closing layer (ranging in color from brown to black) reportedly forms within a 3-day period and is a useful indicator of cessation of nutrient deposition in the kernel coincident to maturation of the corn plant. Normal kernels should be obtained from the central portion of the ear on healthy green plants to most accurately characterize physiological maturity (Daynard and Duncan, 1969; Rench and Shaw, 1971; Daynard, 1972). This simple method has proven effective in Kentucky (Daynard and Duncan, 1969), Iowa (Rench and Shaw, 1971; Baker, 1971) and with early planted corn in Ontario, Canada (Daynard, 1972).

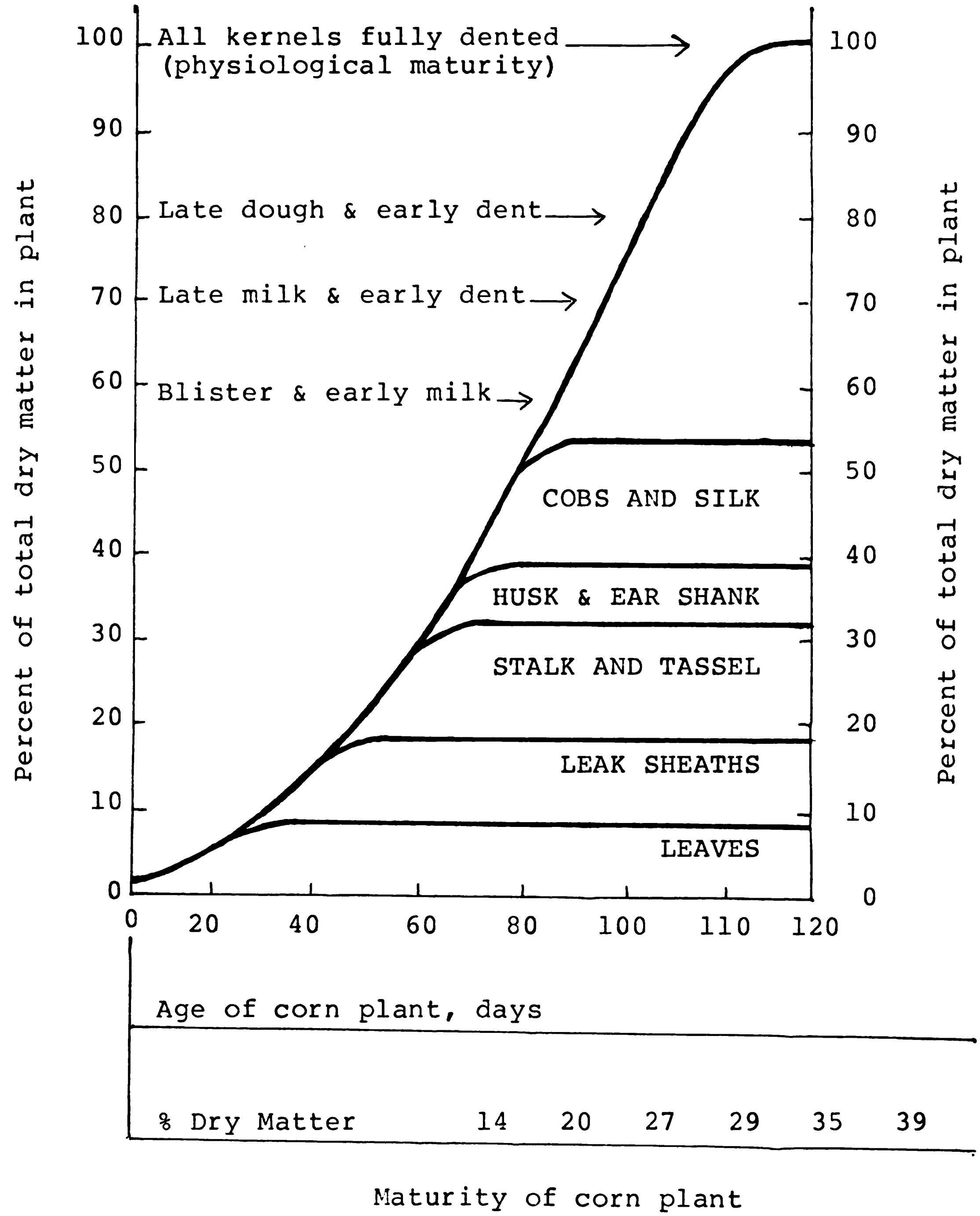
Dry matter content of normally developed corn kernels is approximately 70% at completion of black layer formation. However, this phenomenon also occurs subsequent to a deficit of available assimilate due to frost-damage of leaves, drought, desease, or a nutrient deficiency (Daynard and Duncan, 1969; Daynard, 1972) and therefore cannot always be relied upon to predict when to harvest corn for silage.

Total dry matter content of the corn plant also reflects the degree of physiological maturity. Descriptive terms for kernel development such as milk, dough, dent, or glaze while less objective than dry matter percent do, however, assist in characterizing stage of maturity. The dry matter content of the whole corn plant at physiological maturity is 32 to 38% and the kernel is in a hard dent to glazed state and it is at this point that maximum yield of grain and silage dry matter is realized (Jorgensen and Crowley, 1972).

The proximate composition of the corn plant changes with increasing maturity. Dry matter content of the total plant increases, but expressed as a percent of the total dry matter, there are decreases in crude protein and crude fiber while both ether extract and nitrogen free extract increase (Hooper, 1925; Stallcup et al., 1964).

Harvesting corn for silage before physiological maturity reduces potential nutrient yield. On the other hand, delaying harvest beyond physiological maturity augments field loss of leaves, ears, and in extreme cases the stalks and whole plant may be lost because of lodging. Byers and

Figure 1. Effect of maturity of corn plant on total dry matter accumulation.



Source: Hanway, 1966

Ormiston (1964) harvested late planted corn at 32% dry matter and 45 days later at 55% dry matter. The last harvest yielded 11% less dry matter per hectare mainly due to plant lodging. USDA researchers (Gordon et al., 1968) harvested corn silage in two successive years at the hard dough (26 and 30% DM) and seven to eight weeks later after full maturity and additional weathering (58 and 63% DM). Dry matter yield was 19 to 27% less when harvest was delayed 50 to 60 days. This loss in yield was attributed to plant lodging. Reduction in dry matter yield of late harvested corn in both the Illinois (Byers and Ormiston, 1964) and the USDA (Gordon et al., 1968) experiments should have been greater had their early harvested corn been physiologically mature. Huber et al. (1968) harvested corn for silage at 30, 36, and 44% dry matter and noted dry matter yields of 10.4, 12.2, and 10.2 metric tons per hectare, respectively. The lower yield at the earliest harvest was attributed to the plant not having reached physiological maturity, while the reduced yield at the last harvest was due primarily to field losses. South Dakota researchers (Owens et al., 1968) showed three to five-fold greater field losses due to leaf and ear droppage and whole plant lodging when corn was harvested for silage at 62 compared to 38% dry matter. Geasler (1970) reported decreasing dry matter yields on three harvest dates with dry matter contents of 28, 48 and 60%. Obviously, peak yields were again missed. However, the subsequent year there were no yield differences for three harvest dates ranging in dry matter percent from 31 to 43. Hoglund (1964) appraised field and harvest losses of corn harvested for silage to be only 4% when total plant dry matter is 30 to 35% at ensiling time.

Ensiling losses

Seepage losses should be negligible to nonexistent if corn is harvested for silage at the point of maximum dry matter yield and physiological maturity. Gordon (1967) reviewed seepage losses of grass and legume silages and concluded that seepage loss was practically eliminated when dry matter content reaches 30 to 32%. Georgia researchers (Miller and Clifton, 1965) report no dry matter lost as seepage from 38% DM corn silage. Seepage tends to be less in horizontal silos, because of less vertical pressure. However, in unsealed bunkers, leaching from rain and snow can increase seepage losses an additional eight to ten percentage units above that observed in sealed bunkers (Gordon, 1967).

Sprague and Leparulo (1965) stored corn silage of 24 and 33% DM in 1.8 metric ton plastic silos and reported DM losses of 5.6 and 3.8%, respectively. After summarizing several studies, Hoglund (1964) concluded that 30 and 35% DM corn silage at ensiling lost 11 and 8%, respectively, of the DM in a concrete vertical silo, but only half that amount in a gas-tight vertical silo. Huber et al. (1968) observed average DM losses of 7.0, 6.4 and 15.1% for corn silage harvested at 30, 36, and 44% DM and either ensiled as control or with .5% urea. This greater DM loss at the higher dry matter was attributed to less effective air exclusion which caused extended heating and aerobic oxidation. Byers and Ormiston (1964) suggest that fine chopping and packing to exclude air are more critical with 55% DM corn silage.

Spoilage losses are directly proportional to the amount of air permeating the silage mass and the extent of surface exposure to air and weather (Gordon, 1967). Gordon et al. (1968) reported spoiled silage of 2.3 and 4.3% for corn silage stored in gas-tight silos at 28 and 60% DM, respectively. Gaseous losses are also increased by increasing permeability of the silage structure and silage mass to air. This is particularly true in forages of low moisture content, which are less dense and readily oxidized (Gordon, 1967).

Voluntary silage dry matter intake

The interrelationship of corn plant maturity (% DM) at harvest and voluntary silage dry matter intake by lactating dairy cows from 19 experimental comparisons is summarized in Table 1. Pertinent details such as number of cows, length of experiment, and amount of feed in addition to silage for each comparison are also included. The first nine comparisons (Bechdel, 1926; Bryant et al., 1966; Buck et al., 1969; Huber et al., 1965; and Owens et al., 1968) show increased dry matter intake with increasing silage dry matter content. The response was greatest when comparing silages harvested in the 20% DM range with those harvested near 32 (Bechdel, 1926; and Bryant et al., 1966). Gas tight silos used in two of these first nine comparisons resulted in four to eight percent greater dry matter intake with advancing maturity when comparing silages of 38 to 39% DM with those of 61 to 64; however, the grinding of the latter silage in a hammermill may have accounted for this increased intake (Owens et al., 1968).

The remaining ten comparisons either showed an increase followed by a decrease of dry matter intake over the range of silage DM percentage studied (Montgomery et al., 1974; Huber et al., 1968; and Polan et al., 1968) or a slight (Huber et al., 1973c; Gordon et al., 1968; Huber, 1971) to a significant (Byers and Ormiston, 1966; Gordon et al., 1968; and Huber, 1971) decrease in dry matter intake with advancing maturity.

Data on intake and stage of maturity from Table 1 (excluding the two South Dakota gas tight silo comparisons of Owens et al., 1968) were subjected to covariance analysis with experimental trials serving as treatments and dry matter percent at harvest as the polynomial covariate. The multiple regression coefficients thus derived were used in the equation $Y = B_0 + B_1X + B_2X^2 + B_3X^3$ to best estimate silage dry matter intake (Y) over the dry matter (X) range of 15 to 63%. The polynomial regression curve in Figure 2 indicates maximum dry matter intake corresponding to corn silage maturity at 38% DM. However, intake would not differ appreciably within the silage dry matter range of 33 to 43%.

Depressed DM intake associated with silage dry matter percent above 43 is probably not due to maturity *per se*, but to an inability to exclude air from the silage mass. High DM

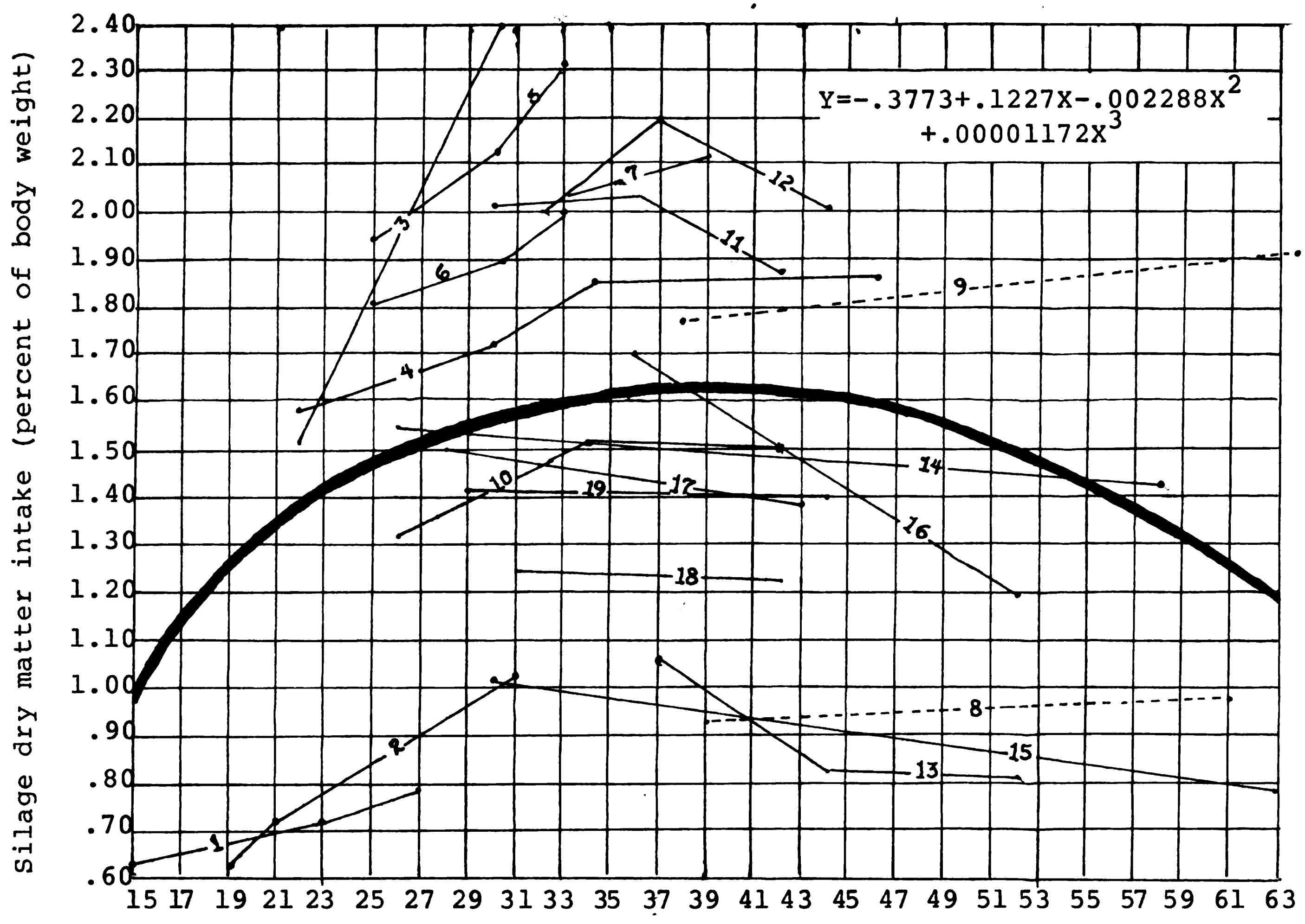
Table 1. Corn silage maturity (%DM) vs. voluntary silage intake by lactating dairy cows

	% DM	DM intake %of BW	
1.	15 23 27	.64 .72 .78	Bechdel, 1926. 12 to 16 cows/trt for 56 to 84 days. Corn silage + conc. @ 1:4 + 4 kg hay/day.
2.	19 21 31	.62 .71 1.04	Bechdel, 1926. 8 to 16 cows/trt for 84 days. Corn silage + conc. @ 1:4 + 3.2 kg hay/day.
3.	22 32	1.51 2.38	Bryant et al., 1966. 12 cows/trt for 105 days. Corn silage + 1.8 kg CSM or conc. @ 1:3 and 2.3 kg hay.
4.	22 30 34 46	1.58 1.73 1.85 1.86	Buck et al., 1969. 16 cows, (4 X 4 latin sq. with 5-wk periods). Corn silage + 4.2 kg conc./day.
5.	25 30 33	1.95 2.13 2.31	Huber et al., 1965. 6 cows/trt each of 2 years. Corn silage + SBM @ 1:7.6 for last 8 wks of 140 days in 1962 & SBM @1:9.6 for first 11 wks of 150 days in 1963.
6.	25 30 33	1.81 1.90 2.00	Huber et al., 1965. 6 cows/trt each of 2 years. Corn silage + conc. @ 1:3.5 for first 12 wk of 140 days in 1962 and last 4 wk of 105 days in 1963.
7.	33 39	2.04 2.12	Buck et al., 1969. 16 cows (4 X 4 latin sq. with 5-wk periods). Corn silage + 4.6 kg conc./day. Each silage normal-cut and recut.
8.	39 61	.93 .97	Owens et al., 1968. Gas tight silos. Silage ground in a hammermill. 10 cows for 119 days. Corn silage + 4.5 kg hay/day + conc. @ 1:2.5.
9.	38 64	1.78 1.92	Owens et al., 1968. Gas tight silos. Silage ground in a hammermill. 10 cows/trt in double reversal trial (5-wk periods). Corn silage + conc. @ 1:3.
10.	26 34 42	1.31 1.51 1.50	Montgomery et al., 1974. 6 Jersey cows/trt in a switchback trial (5-wk periods) each of 2 years. 8 cows/trt for 12 wks for 3rd year. Corn silage + conc. @ 1:3 all years.

Table 1. Continued.

	% DM	DM intake % of BW	
11.	30 36 44	2.02 2.04 1.88	Huber *et al.*, 1968. 18 cows/trt for 60 days. Corn silage + conc. @ 1:2.5 above 11.4 kg milk/day. One-third of cows from each group received urea-treated silage.
12.	32[a] 37[b] 44	2.00 2.20 2.10	Polan *et al.*, 1968. 6 cows/trt for 63 days. Corn silage ([a].85 or [b].6% urea) + conc. @ 1:3.
13.	37 44 52	1.06 .84 .81	Byers and Ormiston, 1966. 11 cows/trt for 56 days. Corn silage + conc. @ 1:3 + hay @ 1% of body weight.
14.	26 58	1.54 1.44	Gordon *et al.*, 1968. Gas tight silos. 7 cows/trt for 50-day *ad lib.* trial. Corn silage *ad lib.* + conc. @ 1:2.8 + hay @ .5% of body weight.
15.	30 63	1.02 .79	Gordon *et al.*, 1968. Gas tight silos. 10 cows/trt for 110 days. Corn silage + conc. @ 1:2.4 or 1:3.6 + hay @ .5% of body weight. Silages fed in summer with a tendency to heat.
16.	36 52	1.70 1.20	Huber, 1971. 6 cows/trt for 70 days. Corn silage (urea .5 or .75%, fresh basis) + conc. @ 1:3.
17.	28 43	1.50 1.38	Huber, 1971. 6 cows/trt for 56 days. Corn silage + conc. @ 1:3.
18.	31 42	1.25 1.24	Huber *et al.*, 1973c. 8 cows/trt for 84 days. Corn silage (2 or 2.5% NH_4-soln.) + 2.2 kg hay/day + conc. @ 1:3.

Figure 2. Silage dry matter intake as affected by corn silage maturity[a]



[a]Numbers 1-19 are trials in Table 1 (trials 8 & 9 omitted from polynomial regression line).

corn silage in the South Dakota study (Owens et al., 1968) was ground in a hammermill (2.5 to 3.8 cm screen) and ensiled in gas tight silos. Both operations would tend to exclude air resulting in a cooler more desirable fermentation thus improving intake.

Silage dry matter digestibility

Digestibility of corn silage dry matter is not appreciably nor consistently altered by maturity of the corn plant. Various researchers have reported modest declines in DM digestibility with advancing maturity (Noller et al., 1963; Owens et al., 1968), others noted no change (Buck et al., 1969; Huber et al., 1965; Perry et al., 1968; and Johnson and McClure, 1968), while others observed modest increases with advancing maturity from 20 to 30 (Bryant et al., 1966; Buck et al., 1969;) and from 20 to 40% dry matter (Buck et al., 1969).

Mean dry matter digestibilities taken from nine digestion trials by seven groups of researchers (Bryant et al., 1966; Buck et al., 1969; Huber et al., 1965; Johnson and McClure, 1968; Owens et al., 1968; Perry et al., 1968; and Sprague and Leparulo, 1965) were subjected to covariance analysis similar to the dry matter intake data. There were 42 individual dry matter digestibility (Y) observations ranging from 22 to 80% dry matter (X) (avg. 4.7 observations per experimental trial). A simple linear regression, Y=69.39 - .00136X, failed to have significant slope, i.e. the effect of maturity on dry matter (DM) digestibility was not significant.

Crude protein digestibility of the corn silage dry matter tended to decline an average of .16 percentage unit for each percentage unit increase in silage dry matter over a range of 21 to 71% (Bryant et al., 1966; Johnson and McClure, 1968; Owens et al., 1968; and Sprague and Leparulo, 1965).

Milk production

Milk yield of cows fed corn silage is either positively correlated with (Bechdel, 1926; Bryant et al., 1966; Buck et al., 1969; Byers and Ormiston, 1966; Huber et al., 1965; Huber, 1971; Owens et al., 1968; Polan et al., 1968) or not significantly affected by silage dry matter intake (Gordon et al., 1968; Huber et al., 1968; Huber et al., 1973c; Montgomery, et al., 1974). The polynomial regression curve of estimated silage dry matter intake in Figure 2 also approximates the milk production response to silage maturity (DM%). The stimulus being greatest up to 33% DM (Bechdel, 1926; Bryant et al., 1966; Buck et al., 1969; Huber et al., 1965; Montgomery et al., 1974), then stable from 33 to 42 (Buck et al., 1969; Montgomery et al., 1974; Polan et al., 1968; Huber et al., 1973c) and either increasing slightly (Owens et al., 1968) or slowly decreasing (Byers and Ormiston, 1966: Gordon et al., 1968; Huber et al., 1968; Huber, 1971) beyond 42% dry matter.

Non-Protein-Nitrogen Additives to Corn Silage

Corn silage is an excellent source of energy for dairy and beef animals but crude protein (8% of dry weight) is inadequate for most productive purposes. Ruminants can utilize NPN via microbial protein synthesis and corn silage is a convenient and uniform carrier for such additives. Distribution of silage intake over a greater part of the day compared to concentrate intake should permit more efficient utilization of NPN when added to corn silage as compared to addition to the concentrate.

Urea treatment of corn silage

Urea is the most widely used and accepted NPN source for addition to corn silage. Uniform distribution of urea at from .5 to 1% over the top of the load of silage before unloading or by metering onto silage at the blower should increase crude protein on a dry basis by four to eight percentage units.

Woodward and Shepherd (1944) and Wise *et al.* (1944) were among the first to add urea to corn silage in this country. Addition of .5% urea to 25 to 30% DM corn silage reduced voluntary intake but did not alter milk production. The latter researchers (Wise *et al.*, 1944) successfully fed two cows a diet of 1% urea treated corn silage in substantial amounts for 60 days. Conrad and Hibbs (1961) showed that .7% urea treated corn silage was unsatisfactory as the only feed for lactating cows. Feed intake, nitrogen utilization, and milk production were lower than comparable cows on alfalfa-hay and grain. However, this study was of short duration (3 wk), the corn silage of low DM% (26.6) with accompanying seepage, and milk production was low for both groups of cows.

Even so, it was concluded that corn silage contained insufficient energy to support NPN utilization in dairy cows and that urea should be supplemented via concentrate feeding. These same researchers, three years later (Conrad and Hibbs, 1964), suggested limiting urea-treatment of corn silage at .35 to .5%, and subsequently admitted (Conrad and Hibbs, 1967) that 32% DM corn silage ensiled with 1% urea provided sufficient supplemental nitrogen to maintain daily milk production at an average of 22 kg (highest 34 kg) during the third to sixth months of lactation provided that 50% of the ration DM came from cereal grains. Klosterman et al. (1965 and 1970) also at Ohio have reached similar conclusions for finishing beef cattle.

Schmutz et al. (1969) treated 29% DM corn silage with 0, .5, or .75% urea and compared these three silages as the only roughages for lactating cows. Rations were made isocaloric and isonitrogenous by additions of ground shelled corn and soybean meal. DM intake was reduced by the highest level of urea but milk production responses were similar for all treatments. Van Horn et al. (1967, 1969) found no intake or milk production differences when 33% DM corn silage was untreated or treated with .5% urea at ensiling, even though the latter was fed with or without urea in the concentrate.

Knott et al. (1972) compared corn silage with .5% urea added at ensiling to non-urea silage both fed with 13% crude protein concentrates containing either 0 or 1.5% urea. Intake of dry matter and persistency of milk production were both enhanced by urea in the corn silage.

Huber and Thomas (1971) compared 35% DM corn silages treated at ensiling with 0, .5, or .75% urea. Concentrates containing 8, 12, or 18% preformed crude protein were also fed with certain of the silages. Milk production and intake were lowest ($p < .05$) for cows receiving no supplemental nitrogen. Addition of .5% urea to corn silage resulted in a 4.5 kg increase in daily milk yields when compared to the negative control group. The highest and most economical milk yields were noted for the ration in which approximately 50% of the supplemental N came from .5% urea added to the corn silage and the remainder from concentrate with 12% crude protein.

Polan *et al.* (1968) added between .5 and .85% urea to corn silage which ranged in dry matter from 32 to 44%. Concentrates contained protein from natural sources. Milk production and silage intake were not significantly altered by urea-treatment. Cows receiving .85% urea-treated corn silage tended to have lowered silage intakes and milk production; however, silage dry matter for this treatment was lowest (32%) and nearly 40% of the total nitrogen intake was from NPN.

Ammonia treatment of corn silage

Anhydrous ammonia is a less expensive source of nitrogen than urea, and thus has appeal as an alternative silage NPN additive. Polish researchers (Abgarowicz *et al.*, 1963) treated corn silage of 17% dry matter with .5% urea, .5% diammonium sulfate and/or ammonia water (isonitrogenous with .5%

urea). Silages were stored for four months in cylindrical basalt vessels of 62 liter capacity. Silage quality was subjectively scored organoleptically and structurally. The ammonia water treated silage was rated inferior to the other silages, but all silages were considered to be representative of good silage.

Huber and Santana (1972) treated 35% DM corn silage with anhydrous ammonia dissolved in water (equivalent to .28 and 3% of NH_3 and H_2O on a fresh silage basis, respectively). Water and liquid NH_3 hoses were connected by means of a "Y" tube and the above solution was delivered to the silo blower. Comparable silage was either ensiled with .5% urea or no additive (control). Heifers weighing approximately 420 kg (10/ silage) were offered either control or ammonia treated corn silage _ad libitum_ for 16 days. Crude protein was increased (from 8.8 to 11.5% of silage DM) by ammonia treatment as was daily silage DM intake (2.08 _vs_. 2.33% of BW). Lactating cows, fed concentrates containing preformed protein at one kg per three kg milk and silages _ad libitum_, consumed control, urea-treated, and ammonia-treated silages equally well when offered iso-nitrogenous rations. Persistency of lactation was similar for all treatments. Corn silage (30% DM) treated with 1% aqua ammonia (22% N) prior to ensiling was compared in a 12-wk lactation experiment with silages treated with either .75% urea or no additive. There were no differences in silage intake or milk production (Huber and Santana, 1972).

In an experiment by Huber _et al._ (1973c), factors influencing utilization of ammonia-treated corn silage were studied in two trials. In trial 1, aqua ammonia, a commercial ammonia-mineral-molasses mixture (ProSil) or urea was added to 32% DM corn silage prior to ensiling. The ammoniated silage resulted in greater ($P < .025$) milk production but there were no differences in silage DM intake. ProSil additions to 31 and 42% DM corn silage gave similar results. In the second trial, ProSil and urea treated silages were compared at low (29-30%) and high (44-53%) dry matter. Milk production among NPN treatments favored the low dry matter silage ($P < .025$). Cows fed ProSil-treated corn silage outyielded those fed urea treated corn silage when dry matter of both silages was 30%. NPN treatment of silage significantly increased ($P < .025$) DM intakes compared to untreated control corn silage.

Anhydrous ammonia gas (.4 or .5%), ProSil (2 or 4%), and urea (.6%) were added to 33% DM corn silage (Huber _et al._, 1973c). The gaseous NH_3 was sprayed onto the silage from a perforated steel tube as the silage passed through an auger box en route to the blower. The ProSil was mixed with silage at the blower and the urea was distributed evenly on top of the load of silage prior to delivery to the blower from a self unloading wagon. There were no differences in nitrogen recoveries due to form of NH_3. Milk production (8 cows/silage for 77 days) was similar for all silages even though cows fed silages containing the two highest levels of NH_3 received no soybean meal. Silage intakes were highest for the high ammonia treatments.

Anhydrous ammonia (.38%), applied directly to corn silage at the forage harvester; ProSil (2%), applied at the silo blower; and urea (.6%) applied to the top of the load were added to 33% DM corn silage and compared to untreated silage (Lichtenwalner et al., 1972). Recovery of added nitrogen at feeding time was 48, 88, and 91% for the respective silages. Intake of the control and the anhydrous ammonia silages was significantly lower than for the ProSil or urea treatments. Milk yields (8 cows/silage for 9 wk) were not different between treatments. Farm application of anhydrous ammonia to corn silage (Hillman et al., 1973) at the forage harvester either directly as a gas or mixed with water increased silage crude protein 3.15 and 4.28 percentage units.

This review of literature identifies a real need to further investigate and evaluate the very intriguing observation that urea and ammonia treated corn silage have higher water-insoluble nitrogen levels than untreated silage. It has been theorized that this is due to: 1) enhanced microbial synthesis of protein during fermentation (Coppock and Stone, 1968); or 2) to a protein sparing action caused by reduced proteolysis (Coppock and Stone, 1968; and Bergen et al., 1974) during fermentation. If in fact, either or both of the above phenomena are true there exists a tremendous potential to increase the level of NPN in corn silage based rations. Further work needs to be done to clearly elucidate this principle.

PART A. NITROGEN FRACTIONS, PH, DRY MATTER

AND

ORGANIC ACIDS OF N-P-N, MINERAL AND DISACCHARIDE TREATED

CORN SILAGE

MATERIALS AND METHODS

Experiment 1. Pilot Silo Fermentation (1972-73)

Freshly chopped corn silage (35% DM) in 56 kg portions was thoroughly mixed with additives and stored in pilot silos. Each silo was two, 004 gauge, 1 X 1.7 m transparent polyethylene bags (one inside the other) positioned inside a 210 l metal drum. Table 2 describes silage treatments. Added nitrogen was .23, .46, or .92% of silage fresh weight. Additions were: .5, 1, or 2% urea; 1, 2, or 4% aqua-ammonia; or 1.8, 3.6, or 7.2% ProSil (NH_3-molasses-mineral suspension). Control received no additive.

Distribution of the silage additive(s) was achieved by spreading an even layer of the 56 kg of silage upon a large polyethylene sheet. The additive was then uniformly applied over the silage surface. The entire silage mass was stirred and blended with a shovel and then rolled and rotated several times in the polyethylene sheet to insure complete mixing. The treated material was then transferred to pilot silos, sampled (500g), evacuated of air with a vacuum cleaner and sealed by tying with a .3 mm cord. Containers were stored inside an enclosed barn on a concrete floor. Silos were again opened, sampled (500g) and re-evacuated of air on days 1, 3, 5, 10, 15, 20 and 40 and of fermentation. Temperatures were measured at the center of silage mass while the bags were open using mercury thermometers attached to wooden dowels.

Table 2. Description of pilot silo treatment combinations[a]

		Nitrogen additive								
		Urea(45%N)			Aqua-NH_3(22%N)			ProSil(13.6%N)		
	0	.5%	1%	2%	1%	2%	4%	1.8%	3.6%	7.2%
	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X
Disaccharide or mineral additive										
1% $CaCO_3$	X	X			X			X		
1% $NaHCO_3$	X	X			X			X		
1% Sucrose	X	X			X			X		
1% Lactose	X	X			X			X		
.5% NaCl	X	X			X			X		
1% $CaCO_3$+										
1% Sucrose		X			X			X		
1% Lactose		X			X			X		

[a]Additives were thoroughly mixed into silage mass as a percent of silage fresh weight. Each 'X' represents one pilot silo.

Samples were immediately frozen at -20° C to await chemical analysis.

Frozen silages were comminuted with a commercial food cutter[a] to insure representative sampling. Dry matter (in duplicate) was determined on approximately 40 g fresh silage by drying in a forced-air oven at 80° C for 24 hours. Total nitrogen was determined on 5 g wet silage by macro-Kjeldahl procedures. Silage extracts were prepared by homogenizing 20 g wet silage and 180 ml distilled H_2O in a Sorval Omni-mixer[b] for three minutes. The homogenizer cup was immersed in ice. The homogenate was strained through two layers of cheese-cloth and a 20 ml aliquot of strained liquor was centrifuged 10 minutes at 15,000 rpm. The supernatant was used for determination of pH[c] and soluble nitrogen by micro-Kjeldahl. A second 20 ml aliquot was deproteinized with 50% sulfosalicylic acid (1 part SSA to 10 parts extract). The deproteinized extract was then centrifuged at 15,000 rpm for ten minutes and the resulting supernatant was analyzed for lactic acid and VFA.

Colorimetric procedures of Barker and Summerson (1941) were used to determine lactic acid. The VFAs were analyzed by injecting 3 micro liters of the deproteinized sample into a Hewlett Packard F & M gas chromatograph[d] using a glass

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[a]Hobart Manufacturing Co., Troy, Ohio
[b]Ivan Sorvall Inc., Newton, Conn.
[c]Beckman Zeromatic SS3
[d]Hewlett Packard F & M Scientific Co., Model 402

column packed with chromasorb 101 (80/100 mesh)[e]. The injection-port temperature was set at 225° C, the column at 225° C., and the flame detector at 240° C. Nitrogen was used as the carrier gas and flow rate was 30-40 ml per minute which created a retention time of approximately seven minutes per sample. Sample VFA concentrations were calculated by comparing peak heights with those from a standard solution made with known weights of analystical grade acids in a stock solution diluted until concentrations comparable with samples were reached.

Water insoluble nitrogen in silages was calculated by the difference between the total nitrogen determined by macro-Kjeldahl and the soluble nitrogen determined by micro-Kjeldahl.

Experimental parameters from the various treatment combinations outlined in Table 2 were divided into three groups for statistical examination by analysis of variance and partition of single factor or treatment effects by orthogonal contrasts as outlined in Table 3.

The mean square error terms from the first statistical comparison (ten trts with 2 silos/trt) were used to test for single factor, main, and interaction effects for each experimental parameter of the remaining two comparisons because the latter had only one observation per treatment combination.

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[e]Johns-Manville, Celite Div., Denver, Colorado

Table 3. Statistical organization of parameters from Experiment 1 Part A

1 - 10 treatments, 2 silos/trt, 4 days* sampled

2 - 4 X 6 X 4* 4 N-sources X 6 additives X 4 days sampled (one silo per combination)

3 - 3 X 8 X 4* 3 N-sources X 8 additives X 4 day sample (one silo per combination)

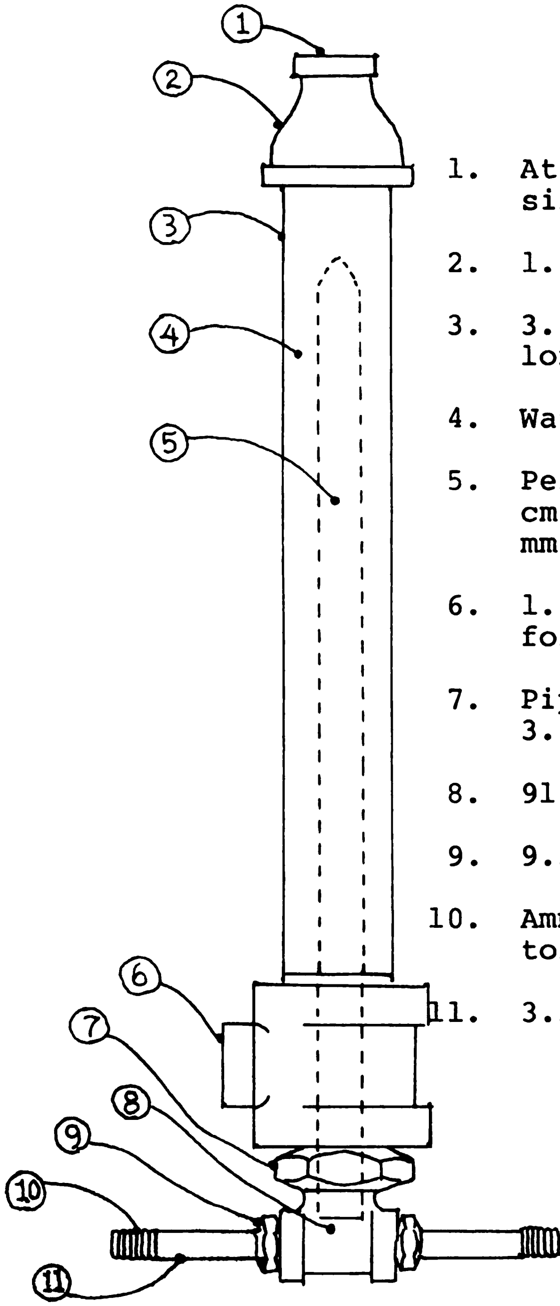
1		2		3	
Item	DF	Item	DF	Item	DF
Treatments	9	Nitrogen	3	Nitrogen	2
Nitrogen	(2)	Additive	5	Additives	7
N-level	(2)	N X A	15	N X A	14
N X level	(4)	Error a from 1		Error a from 1	
Control *vs.* others	(1)				
Error a = silo/ trt	10	Days	3	Days	3
		N X days	9	N X days	6
Days	3	A X days	15	A X days	21
Trt X days	27	N X A X days	45	N X A X days	42
Error b (silos X days)	30	Error b from 1		Error b from 1	
Total (n - 1)	79		95		95

*Temperatures were obtained on 7 days (1, 3, 5, 10, 15, 20 & 40) while all other parameters were analyzed on days 0, 3, 15 & 40.

Experiment 2. Pilot Silo Fermentation (1973-74)

Freshly chopped corn silage was ensiled in pilot silos as described in Experiment 1. Four harvests were made at estimated whole corn plant DM of 30, 35, 40 and 45%. Experimental design at each of the above DM was a 4 X 3 factorial replicated twice with the respective factors being: 1) anhydrous ammonia application of 0, .24, .48, and .72%; and 2) water addition at 0, .8, and 1.6% of fresh silage weight.

The anhydrous ammonia and water were added to silage via the mixing apparatus illustrated in Figure 3. Ammonia was metered into the mixing chamber using a regulator from a conventional anhydrous ammonia field applicator. Source of water was the farm tap water system. Silage was conveyed to blower at the rate of 500 kg/min, where the desired amounts of ammonia and water were added via the mixing apparatus. Thirty seconds were allowed for the ammonia-water mixture to equilibrate itself during which time the treated silage was discarded. After equilibration enough silage was diverted onto a large polyethylene sheet to fill two pilot silos and then the flow of silage was directed away to be discarded while the mixing procedure was shut down. Each pilot silo was filled with 56 kg of experimental silage. The air was evacuated, bags were sealed and silos transferred to an enclosed barn for storage. After 40 days, 1 kg samples were taken from the middle of each pilot silo and frozen (-20 C°) for subsequent chemical analysis. Nitrogen, VFA, lactic acid, pH, and DM determinations were identical



1. Attach 3 m water hose to silage blower
2. 1.9 cm pipe thread
3. 3.8 cm pipe about 40 cm long
4. Water jacket
5. Perforated 9.5 mm pipe 45 cm long with 32 holes 3.2 mm in diameter.
6. 1.9 cm hose pipe fitting for water hose
7. Pipe bushing, 915 mm to 3.8 cm
8. 915 mm pipe "T"
9. 9.5 mm to 3.2 mm busing
10. Ammonia gas from applicator meter manifold
11. 3.2 mm pipe niples

Figure 3. Anhydrous ammonia and water mixing apparatus (Standard black iron pipe and standard pipe fittings).

to those described in Experiment 1. Experimental parameters of this 4 X 4 X 3 factorially designed experiment were examined statistically by analysis of variance and partition of single factor or treatment effects by orthogonal contrasts.

RESULTS AND DISCUSSION

Experiment 1. Temperature of NPN treated corn silage as affected by nitrogen source and level on seven fermentation dates is presented in Table 4. Temperature was not changed by nitrogen source or level. Temperature decreased ($P < .01$) linearly from day one to day 15. Temperature for day 40 was lower ($P < .05$) than that for day 20. The low temperature on day 15 coincides with the lowest daily mean outside temperature and an extreme low the previous night of -8.9° C. Obviously silage temperature closely paralleled ambient temperature. There was no excessive heating in any of the silages. Temperatures were lower than those which normally occur in farm silos because of: 1) the relatively small volume (56 kg) allowed heat from fermentation to be readily dissipated; 2) anaerobic conditions created by promptly evacuating the air reduced aerobic fermentation (Federson, 1971); and 3) the low ambient temperatures during October and November. Geasler (1970) reported mean temperatures of 25° C for the first 12 days of fermentation of corn silage harvested at ten maturities from 22 to 48% DM and stored in experimental silos, but he harvested earlier in the year and his experimental silos were placed in a warmer building.

The pH values of the corn silages treated with three sources and levels of NPN and sampled on four different days are presented in Table 5. Control silage had a lower ($P < .05$) average pH than the nine NPN treatments; however, these

Table 4. Effect of source and level of nitrogen and days of fermentation on temperature (°C) of corn silage.

Nitrogen		Days of fermentation[a]							N-source	
Source	% added	1	3	5	10	15	20	40	mean	SEM
Control	0	20.8	18.5	15.0	11.9	7.9	9.4	7.8	13.04	
Urea									13.11	
	.2	20.8	19.0	14.2	12.0	8.3	9.7	7.5		
	.4	20.0	19.2	14.2	12.0	8.3	9.7	7.5		
	.8	20.5	19.0	15.0	12.2	8.6	9.9	7.8		
Aqua-NH_4									12.95	
	.2	20.5	18.6	14.5	12.2	8.2	9.8	7.5		
	.4	20.0	18.2	14.5	11.8	8.2	9.6	7.0		
	.8	20.4	17.4	14.0	12.4	9.6	10.6	7.5		
NH_4-sol									13.34	
	.2	21.0	20.2	15.8	12.0	8.3	9.6	7.2		
	.4	19.9	22.0	14.5	12.1	9.0	9.8	7.5		
	.8	20.2	16.5	15.2	13.2	9.0	9.7	7.5		
Day mean		20.4	18.9	14.7	12.2	8.5	9.8	7.5		.664
Outside daily mean		14.2	8.0	3.9	7.5	-2.2	2.5	1.7		
Nitrogen level mean		(0)13.04	(.2)13.19		(.4)13.10		(.8)13.15			
								(N-level & source)		.314

[a]Tabular values for days of fermentation represent averages from two experimental silos.

differences were least ($P < .25$) on day 40 of fermentation. Urea treatment lowered ($P < .05$) silage pH when compared to silage treated with aqua-NH_4 and NH_4-solution. The overall mean for pH within nitrogen sources increased as level of nitrogen addition increased with .4 and .8% added nitrogen being higher ($P < .01$) than the control, and .8 being higher ($P < .01$) than .2% added nitrogen. A significant ($P < .05$) source X level interaction due to the inconsistent effect of urea compared to the ammonia treatments at the different levels of N addition and a rather large standard error for means makes subtle differences difficult to detect. Control and silages treated with .2% added nitrogen had similar pH values on day 40 that were significantly lower ($P < .05$) than the two highest levels of the ammonia treatments and the intermediate level of aqua-NH_4. There was a general decrease in pH with fermentation time. Day zero values were higher ($P < .01$) than all others and day three values were higher ($P < .01$ than those on day 40. A significant ($P < .05$) day X treatment interaction was due to an inconsistent pattern noted for urea particularly on day three of fermentation. One would question the lower value for day three, but it coincided with a higher lactic acid (Table 6).

The pH values (Table 5) are in general agreement with those reported by other researchers (Geasler, 1970; Lopez et al., 1970a; and Cash, 1972) using similar experimental silos; however, such high levels of ammonia with the resultant high pH values have not been previously reported.

Table 5. Effect of source and level of nitrogen and days of fermentation on pH of corn silage.

Nitrogen		Days of fermentation[a]				Treatment	N-source	
Source	% added	0	3	15	40	mean	mean	SEM
Control	0	5.46	4.58	4.71	4.27	4.76	4.76	
Urea							5.07	
	.2	6.12	4.38	4.68	4.36	4.89		
	.4	6.61	4.89	4.96	4.52	5.24		
	.8	6.50	4.35	4.96	4.54	5.09		
Aqua-NH_4							6.85	
	.2	8.18	5.76	4.63	4.32	5.72		
	.4	8.40	7.12	5.76	5.39	6.67		
	.8	9.04	8.78	8.06	6.73	8.15		
NH_4-sol							6.64	
	.2	8.02	5.47	4.63	4.35	5.62		
	.4	8.38	7.14	6.02	4.92	6.62		
	.8	8.80	8.42	7.31	6.20	7.68		
Day mean		7.55	6.09	5.57	4.96			.283
Nitrogen level mean		(0)4.76	(.2)5.41		(.4)6.18	(.8)6.97	(N-level & source)	.368

[a]Tabular values for days of fermentation represent averages from two experimental silos.

Lactic acid content of corn silages treated with three sources and three levels of NPN is shown in Table 6. The tremendous variation in lactic acid content of silages in this experiment as evidenced by the large standard error of the mean, makes statistical interpretation difficult. Urea appears to elicite more of a stimulatory effect on lactic acid production than the NH_4-solution or aqua-NH_4. However, lactic acid content at .2% added nitrogen (the level recommended to farmers) on day 40 was substantially higher (6.11 and 5.52 vs. 4.81) for the ammonia treated silages. The important biological phenomenon starting at .4 and manifesting itself at the .8% added nitrogen was the marked inhibition of lactic acid production with the ammonia treatments. There are two explanations for this reduction of lactic acid: First, initial application of similar levels of ammonia has been shown to destroy fungi present in fresh-cut corn silage (Britt, 1973) and eliminate or significantly reduce all microorganisms in high moisture shelled corn (Bothast et al., 1973, 1975) and; Second, most rod shaped forms of lactic acid bacteria cannot grow in media with an initial pH greater than 6.0 (Stanier et al., 1970). The pH values (Table 5 observed for ammonia added at .4 and .8% N were initially above those for optimum growth of lactic acid bacteria (Langston et al., 1958). The low level (.2%) of nitrogen resulted in the highest ($P<.05$) concentrations of silage lactic acid. Lactic acid at day zero was lower ($P<.01$) than at days three, 15 and 40.

Table 6. Effect of source and level of nitrogen and days of fermentation on lactic acid of corn silage.

Nitrogen		Days of fermentation[a]				Treatment	N-source	
Source	% added	0	3	15	40	mean	mean	SEM
Control	0	.32	4.62	3.48	3.88	3.08	3.08	
Urea							3.82	
	.2	.05	9.20	5.08	4.81	4.78		
	.4	.04	3.34	3.77	5.22	3.09		
	.8	.16	5.48	3.88	4.77	3.58		
Aqua-NH_4							1.62	
	.2	.04	.85	5.02	6.11	3.01		
	.4	.12	.00	2.08	3.02	1.30		
	.8	.03	.00	2.19	.00	.56		
NH_4-sol							2.11	
	.2	.12	4.01	9.42	5.52	4.77		
	.4	.05	.00	1.82	3.65	1.38		
	.8	.02	.00	.62	.14	.19		
Day mean		.10	2.75	3.74	3.71			1.33
Nitrogen level mean		(1)3.08	(.2)4.19	(.4)1.83	(.8)2.05			
						(N-level & source)		1.80

[a]Tabular values for days of fermentation are expressed on a dry basis and represent averages from two experimental silos.

Lactic acid values (Table 6) for control and urea treated silages are similar to those reported by Lopez et al. (1970a), Thomas et al. (1975), Cash (1972), and Geasler (1970) at equivalent maturities.

Acetic acid values for control silage were consistently lower ($P < .05$) than those for the NPN treated silages (Table 7). Values for urea treatment were generally lower than for the NPN treated silages (Table 7). Acetic acid values for urea treatment were lower than for the NH_4-solution and aqua-NH_4 treated silages at the two highest levels (.4 and .8%) of nitrogen addition on day 40 ($P < .05$). In fact this elevation of acetic acid concentration coincides with the depression of lactic acid as is graphically portrayed in Figure 4. Acetic acid concentration increased linearly as fermentation progressed with day 15 values higher ($P < .01$) than day zero and day 40 values higher ($P < .01$) than all others. Level of added nitrogen and acetic acid concentration of silage were positively correlated, i.e. the .4 and .8% levels of added nitrogen stimulated the highest ($P < .05$) levels of acetic acid in silage.

Total organic acid content of corn silage (Table 8) paralleled that of lactic acid. There was a tendency toward higher ($P < .10$) total organic acids with urea compared to aqua-NH_4 and NH_4-solution. The .2% level of nitrogen addition tended toward higher total organic acids than at .4 and .8%. This was particularly true for the NH_4-solution and aqua-NH_4 treatments since lactic was so markedly reduced

Table 7. Effect of source and level of nitrogen and days of fermentation on acetic acid of corn silage.

Nitrogen		Days of fermentation[a]				Treatment	N-source	
Source	% added	0	3	15	40	mean	mean	SEM
Control	0	.40	.46	1.40	1.76	1.00	1.00	
Urea							1.22	
	.2	.42	1.08	1.30	2.08	1.22		
	.4	.40	1.04	1.12	2.07	1.16		
	.8	.40	.90	2.18	1.68	1.29		
Aqua-NH_4							1.55	
	.2	.52	.82	1.52	2.20	1.27		
	.4	1.00	.86	2.48	2.59	1.73		
	.8	1.22	1.32	1.47	2.58	1.65		
NH_4-sol							1.62	
	.2	.58	.74	1.55	2.52	1.35		
	.4	.68	1.07	2.12	2.92	1.70		
	.8	1.20	1.20	1.72	3.18	1.83		
Day mean		.68	.96	1.69	2.36			.305
Nitrogen level mean		(0)1.00	(.2)1.28	(.4)1.53	(.8)1.59			
						(N-level & source)		.214

[a]Tabular values for days of fermentation are expressed on a dry basis and represent averages from two experimental silos.

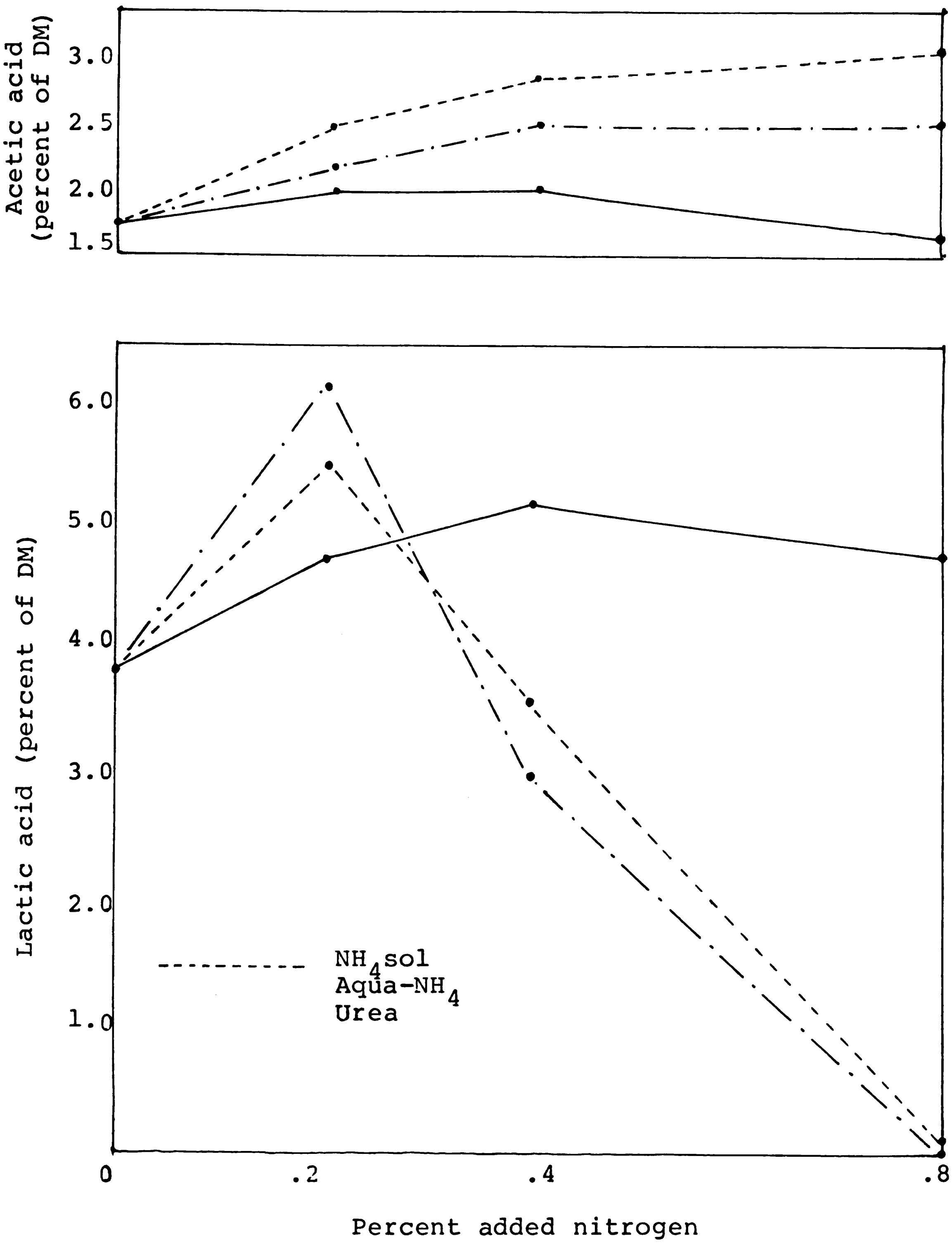


Figure 4. Effect of source and level of nitrogen upon lactic and acetic acids after 40 days of fermentation.

Table 8. Effect of source and level of nitrogen and days of fermentation on total organic acids.

Nitrogen		Days of fermentation[a]				Treatment	N-source	
Source	% added	0	3	15	40	mean	mean	SEM
Control	0	.94	5.09	4.94	5.71	4.17	4.17	
Urea							5.09	
	.2	.46	10.28	6.37	6.98	6.02		
	.4	.54	4.38	4.88	7.29	4.28		
	.8	.68	6.50	5.24	6.44	4.96		
$Aqua-NH_4$							3.28	
	.2	.56	2.03	6.60	8.32	4.38		
	.4	1.18	.86	4.70	5.62	3.09		
	.8	1.31	1.66	3.83	2.63	2.36		
NH_4-sol							3.99	
	.2	.74	6.82	10.96	8.10	6.65		
	.4	.73	1.36	4.25	6.56	3.23		
	.8	1.41	1.30	2.34	3.32	2.09		
Day mean		.86	4.03	5.51	6.10			1.81
Nitrogen level mean		(0)4.17	(2)5.68	(.4)3.54	(.8)3.14			
						(N-level & source)		1.95

[a]Tabular values for days of fermentation expressed on a dry basis and represent averages from two experimental silos.

(Table 6 and Figure 4). Total organic acids were higher ($P<.05$) on day 40 than day zero. Other differences were not detected because of the large standard errors for means which is indicative of the unusually large amount of variation in treatment combination response.

Dry matter content of all silages decreased slightly as fermentation progressed (Table 9). Values on day 40 tended to be lower ($P<.25$) than those on days zero and three. Similar results were reported by Lopez _et al._ (1970b). This phenomenon was possibly due to a loss of volatile organic acids during oven drying coupled with the evolution of CO_2 and the production of H_2O in the silos during fermentation.

Total nitrogen of corn silage (Table 10) was increased ($P<.01$) by each incremental addition of nitrogen at ensiling. Increases due to urea were greater ($P<.05$) than for aqua-NH_4 and NH_4-solution. Nitrogen addition was calculated to be equal from each source. An explanation for these differences is that less nitrogen from urea was volatilized during application and mixing than from the aqua-NH_4 and the NH_4-solution. The NH_4-solution lost less NH_3 during application and mixing than the aqua-NH_4 probably because of a lower NH_4^+ concentration or a possible binding by minerals and molasses. TN at the .8% N level on day 40 was actually lower for the urea silage. This could have been due to sampling error or a reflection of more stability of ammonia compared to urea treated silages (Britt, 1973). These barrels had been opened seven times for sampling by day 40 and secondary fermentation

Table 9. Effect of source and level of nitrogen and days of fermentation on dry matter content of corn silage.

Nitrogen		Days of fermentation[a]				Treatment	N-source	
Source	% added	0	3	15	40	mean	mean	SEM
Control	0	35.4	35.6	34.9	33.8	34.9	34.9	
Urea							34.7	
	.2	33.6	34.8	34.8	32.4	33.9		
	.4	34.7	36.2	34.2	33.8	34.7		
	.8	35.6	36.0	35.4	34.6	35.4		
Aqua-NH_4							35.0	
	.2	36.0	34.7	35.1	33.8	34.9		
	.4	36.2	37.2	33.5	34.1	35.2		
	.8	36.0	34.5	34.7	34.6	34.9		
NH_4-sol							34.8	
	.2	37.0	34.8	33.4	34.4	35.0		
	.4	34.2	36.0	35.7	33.4	34.8		
	.8	36.1	34.5	35.3	33.0	34.7		
Day mean		35.5	35.4	34.7	33.8			.926
Nitrogen level mean		(0)34.9	(.2)34.6		(.4)34.9	(.8)35.0		
						(N-level & source		.517

[a]Tabular values for days of fermentation represent averages from two experimental silos.

Table 10. Effect of source and level of nitrogen and days of fermentation on total nitrogen of corn silage.

Nitrogen Source	Nitrogen % added	Days of fermentation[a] 0	3	15	40	Treatment mean	N-source mean	SEM
Control	0	1.540	1.474	1.528	1.614	1.539	1.539	
Urea							2.810	
	.2	2.285	2.164	2.252	2.502	2.300		
	.4	2.730	2.667	2.712	2.870	2.745		
	.8	3.596	3.349	3.421	3.167	3.384		
Aqua-NH_4							2.467	
	.2	1.970	2.001	1.974	2.046	1.998		
	.4	2.356	2.208	2.314	2.558	2.359		
	.8	3.207	2.729	2.906	3.340	3.045		
NH_4-sol							2.565	
	.2	1.988	1.979	1.934	1.952	1.964		
	.4	2.668	2.318	2.410	2.551	2.486		
	.8	3.050	3.080	3.339	3.518	3.246		
Day mean		2.539	2.397	2.479	2.612			.182
Nitrogen level mean	(0)1.539		(.2)2.087		(.4)2.530	(.8)3.225		
						(N-level & source)		.252

[a]Tabular values for days of fermentation are expressed on a dry basis and represent averages from two experimental silos.

could have caused a loss of nitrogen. The total nitrogen content at .8% added nitrogen was higher ($P < .01$) than .2 and .4 and .4 higher ($P < .01$) than .2. The only other difference was that for day three total nitrogen values tended to be lower than for days zero, 15 and 40. Lopez et al. (1970b) reported slightly lower total nitrogen at urea additions equivalent to the .2 and .4% added N of this study, but ground shelled corn was also added at either 3.5 of 7.0% of silage fresh weight which would dilute the total nitrogen concentration.

Total soluble nitrogen (TSN) of control silage was lower ($P < .01$) than the other nine treatments (Table 11). TSN values for all levels of urea were higher ($P < .05$) than those for aqua-NH_4 and NH_4-solution because of greater initial incorporation of total nitrogen (Table 10). Likewise, when all N sources were averaged, .4 and .8% added N resulted in higher ($P < .01$) levels of TSN than .2, with .8 being higher ($P < .01$) than .4%. TSN values on day zero of fermentation were not different ($P < .25$) from the other days. Day 15 values tended to be lower than days zero, three and 40 but there was a significant ($P < .05$) day X treatment interaction and the difference between duplicate silos was also significant ($P < .05$), i.e., the observed difference was not statistically significant.

Control silage TSN was unchanged as fermentation proceeded from day zero to 40. This is not in agreement with the bulk of published research. Johnson et al. (1967), Lopez

Table 11. Effect of source and level of nitrogen and days of fermentation on total soluble nitrogen of corn silage.

Nitrogen		Days of fermentation[a]				Treatment	N-source	
Source	% added	0	3	15	40	mean	mean	SEM
Control	0	.438	.396	.348	.454	.409	.409	
Urea							1.605	
	.2	1.250	1.102	.928	1.008	1.072		
	.4	1.603	1.636	1.436	1.574	1.562		
	.8	2.122	2.475	2.170	1.960	2.182		
Aqua NH_4							1.268	
	.2	.816	.892	.684	.769	.790		
	.4	1.252	1.304	1.158	1.152	1.217		
	.8	1.627	1.918	1.683	1.960	1.797		
NH_4-sol							1.412	
	.2	.742	.888	.756	.814	.800		
	.4	1.370	1.537	1.132	1.435	1.368		
	.8	1.620	2.122	2.058	2.474	2.090		
Day mean		1.284	1.427	1.235	1.360			.105
Nitrogen level mean		(0).409	(0.2).887	(0.4)1.382		(0.8)2.016		
						(N-level & source		.205

[a]Tabular values for days of fermentation are expressed on a dry basis and represent averages from two experimental silos.

et al. (1970b), Geasler (1970) and Cash (1972) have all observed substantial increases in TSN during fermentation as a result of proteolysis. Bergen *et al.* (1974) showed that the TSN content of control corn silage accounts for about 35% of the total nitrogen after 12 hours of fermentation and remains rather constant thereafter. At 0 hours fermentation it accounted for only 13% of the TN. It is possible that some proteolysis occurred in all silages on day zero before the samples were frozen and thus the initial TSN value remained constant. Cool temperatures coupled with anaerobic conditions in the experimental silos may have further minimized protein degradation.

The effect of source and level of nitrogen on water insoluble nitrogen (WIN) was negligible (Table 12). The only observed differences were that day three values appeared lower than the values for 0, 15 and 40 days; however, the differences between duplicate silos was significant ($P < .05$) making statistical interpretation of results extremely difficult.

The control silage WIN values were not decreased by proteolysis during fermentation as is usually the case (Lopez *et al.*, 1970b; Johnson *et al.*, 1967; Cash, 1972; and Geasler, 1970). There is normally an increase in water insoluble nitrogen with ammonia additions (Huber and Santana, 1972; Huber, 1973c; Beattie, 1970; and Cash, 1972). Explanations for this abnormality are: 1) the corn silages may have already undergone proteolysis before being treated with NPN, or 2) the

Table 12. Effect of source and level of nitrogen and days of fermentation on water insoluble nitrogen.

Nitrogen Source	Nitrogen % added	Days of fermentation[a] 0	3	15	40	Treatment mean	N-source mean	SEM
Control	0	1.102	1.077	1.179	1.160	1.130	1.130	
Urea							1.204	
	.2	1.035	1.062	1.324	1.493	1.229		
	.4	1.126	1.030	1.275	1.296	1.182		
	.8	1.474	.874	1.252	1.207	1.202		
Aqua-NH_4							1.266	
	.2	1.154	1.110	1.291	1.276	1.208		
	.4	1.605	.903	1.156	1.405	1.267		
	.8	1.580	1.104	1.224	1.380	1.322		
NH_4-sol							1.153	
	.2	1.246	1.091	1.178	1.138	1.163		
	.4	1.298	.780	1.278	1.116	1.118		
	.8	1.430	.958	1.282	1.043	1.178		
Day mean		1.305	.999	1.244	1.251			.182
Nitrogen level mean		(0)1.130		(.2)1.200	(.4)1.189	(.8)1.234		
						(N-level & source)		.336

[a]Tabular values for days of fermentation are expressed on a dry basis and represent averages from two experimental silos.

cool temperatures in the barrels and the evacuation of air (creating rapid anaerobic conditions) may have inhibited proteolysis.

The mean values for control and nitrogen source, disaccharide or mineral additive, and day of fermentation on temperature of corn silage are shown in Table 13. Temperatures for individual treatment combinations are presented in Appendix Table 1. Standard errors of means were calculated from the mean square error terms outlined in Table 3. They are larger than those reported in Tables 4 through 12 because of only one observation per treatment combination, i.e. SEM = the square root of MS_E instead of the square root of $MS_E/2$. Data were analyzed statistically as two separate factorials but are presented together in the same table for brevity and clarity. Nitrogen source had no significant affect on temperature. In the 3 X 8 X 7 factorial mean temperature of control and $CaCO_3$ silages, averaged across days and N-sources tended to be lower than those for sucrose alone and the two $CaCO_3$ and disaccharide combinations; however, the standard error of the mean was too large to allow one to say that these differences were statistically significant. The day effect on silage temperature was similar to that reported for level and source of nitrogen in Table 4, i.e. temperature decreased ($P < .05$) linearly from days 1 and 3 to day 15 where they plateaued near 8°C for the remainder of the experiment.

Silage pH values in the 4 X 6 X 4 factorial (Table 14) were generally lower for control than the N-sources and lower

Table 13. Corn silage temperature (°C) main effects during fermentation.

N-source[a]	Mean	Additive[b]	Mean	Day	Mean	SEM
		4 X 6 X 7 Factorial				
Control	13.32	Control	13.15	1	20.88	
Urea	13.36	$NaHCO_3$	13.34	3	19.61	
Aqua-NH_4	13.27	$CaCO_3$	13.31	5	15.30	
NA_4-sol	13.40	Sucrose	13.51	10	11.95	
		Lactose	13.34	15	8.29	
		NaCl	13.39	20	9.61	
				40	7.74	
		3 X 8 X 7 Factorial				
Urea	13.40	Control	13.19	1	20.95	
Aqua-NH_4	13.32	$NaHCO_3$	13.37	3	19.59	
NH_4-sol	13.47	$CaCO_3$	13.20	5	15.57	
		Sucrose	13.51	10	12.09	
		Lactose	13.38	15	8.35	
		NaCl	13.40	20	9.68	
		$CaCO_3$		40	7.74	
		Sucrose	13.53			
		Lactose	13.64			
					(day)	.939
					(N-source & additive)	.444

[a]Nitrogen added at .2% of fresh silage.
[b]Each disaccharide or mineral added at 1% of fresh silage, except NaCl at .5%.

Table 14. Effect of nitrogen source, additive,[a] and day of fermentation on pH of corn silage.

Additive[b]	Day of fermentation	N-source				Day avg.	Additive main effects	SEM
		Control	Urea	Aqua-NH_4	NH_4-Sol			
Control	0	5.46	6.12	8.18	8.02	6.94	5.24	
	3	4.58	4.38	5.76	5.47	5.05		
	15	4.71	4.68	4.63	4.63	4.66		
	40	4.27	4.36	4.32	4.35	4.32		
	N-source avg	4.76	4.89	5.72	5.62			
$CaCO_3$	0	5.95	6.35	8.08	7.97	7.09	5.81	
	3	5.18	5.38	6.31	6.02	5.72		
	15	5.34	5.65	5.33	5.35	5.42		
	40	5.05	5.00	4.96	5.00	5.00		
	N-source avg	5.38	5.60	6.17	6.08			
$NaHCO_3$	0	8.30	8.93	9.22	9.08	8.88	6.23	
	3	5.11	6.94	3.68	6.60	5.58		
	15	4.97	6.48	5.45	5.34	5.56		
	40	4.36	5.43	4.82	5.02	4.91		
	N-source avg	5.68	6.94	5.79	6.51			
Sucrose	0	5.60	5.90	8.15	8.00	6.91	5.15	
	3	3.92	3.78	5.92	5.72	4.84		
	15	4.26	4.45	4.71	4.48	4.48		
	40	4.26	4.50	4.65	4.15	4.39		
	N-source avg	4.51	4.66	5.86	5.59			

(Table continued on next page)

Table 14. Continued

Additive[b]	Day of fermentation	N-source: Control	Urea	Aqua-NH_4	NH_4-Sol	avg.	Additive main effects	SEM
Lactose	0	5.53	5.97	8.29	7.95	6.94	5.08	
	3	3.98	4.58	5.93	4.39	4.72		
	15	4.22	4.36	4.68	4.52	4.44		
	40	4.00	4.13	4.47	4.20	4.20		
	N-source avg	4.43	4.76	5.84	5.26			
NaCl	0	5.55	6.55	8.40	7.92	7.10	5.16	
	3	4.47	4.36	5.82	3.87	4.63		
	15	4.36	4.48	4.86	4.60	4.58		
	40	4.56	4.26	4.22	4.28	4.33		
	N-source avg	4.74	4.91	5.82	5.17			
N-source main effects		4.92	5.29	5.87	5.71		(& additive)	.400
Day main effects		(0)7.31	(3)5.09	(15)4.86	(40)4.53			.520

[a]Nitrogen from urea, aqua-NH_4 and NH_4-solution added at .2% and other additives at 1% (except NaCl at .5%) of fresh silage.

[b]Treatment combination values are means from duplicate silos for control (none) and single observations for all other disaccharide or mineral additives.

for urea than aqua-NH_4 and NH_4-solution; however, these apparent differences disappeared by day 40. Within dissacharide or mineral additives, $CaCO_3$ and $NaHCO_3$ tended to increase overall pH more than the other additives. This apparent difference was still evident on day 40. There was a consistent drop ($P < .05$) in pH as fermentation progressed with values on days 3, 15 and 40 being lower than those on day zero.

The 3 X 8 X 4 factorial design includes all of the treatment combinations of Table 14 except the six control silages to which no nitrogen was added. The additional two additives ($CaCO_3$ + sucrose and $CaCO_3$ + lactose) and their treatment combinations with the three NPN treatments are presented in Appendix Table 2. The general effect on pH was the same as discussed for Table 14, i.e. $NaHCO_3$ and $CaCO_3$ alone and in combination with sucrose and lactose tended to elicite slightly higher average and final pH values than the remaining mineral or disaccharide additives.

The intitial increase in pH with ammonia treatments was similar to that previously observed in Table 5 at the .2% level of N addition, but by day 40 any differences due to NPN treatment had disappeared. The mean effect of the mineral and disaccharide additions was to increase final pH by 0.2 units compared to the control and NPN treated silages. Lactose alone (lowest pH value, 4.0) and in combination with NPN additives caused the lowest pH values. It is theorized that lactose was a preferred substrate for lactic acid bacteria in the silage and hence the low pH value. The ammonia _per se_ increases silage pH and the NH_4^+ ion combines with acetate and lactate to thus neutralize these acids and extend fermentation. $CaCO_3$ and $NaHCO_3$ contribute their respective anions to the silage media and raise the pH. Limestone (consisting mainly of $CaCO_3$) has long been shown to increase silage pH (Owen, 1971; Johnson _et al._, 1967).

Silage lactic acid values even on day 40 were quite erratic (Table 15). Generally higher mean values were noted for those treatment combinations containing the NH_4-solution. The final lactic acid concentration on day 40 was higher for NH_4-solution (6.34) than for aqua-HN_4 (4.74), urea (3.80) and control (3.41) silages. Sucrose and $NaHCO_3$ additions to silages tended to lower ($P < .25$) lactic acid especially on day 40. There was a significant increase ($P < .05$) in lactic acid of all silages from day zero to 15.

The same general trends for silage lactic acid as seen in Table 15 were manifest in the 3 X 8 X 4 comparison (Appendix Table 2). Lactic acid concentration was lowest on day 40 for the sucrose and lactose treatments and highest for $CaCO_3$ alone and for $CaCO_3$ in combination with sucrose and lactose. $CaCO_3$ has been shown by other workers (Owen, 1971; Johnson _et al._, 1967) to stimulate silage lactic acid. $NaHCO_3$ and sucrose both lowered lactic acid compared to $CaCO_3$, but the depression was much less than for the high ammonia additions (Table 6).

The general trend for increased lactic acid with time

Table 15. Effect of nitrogen source, additive, and day of fermentation on lactic acid content of corn silage.[a]

Additive[b]	Day of fermentation	N-source: Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Control	0	.34	.05	.04	.12	.14	3.91	
	3	4.62	9.20	.85	4.01	4.67		
	15	3.48	5.08	5.02	9.42	5.75		
	40	3.88	4.81	6.11	5.52	5.08		
	N-source avg	3.08	4.78	3.00	4.77			
$CaCO_3$	0	.00	.00	.00	.16	.04	5.26	
	3	4.20	2.22	1.09	3.19	2.68		
	15	3.98	4.77	4.81	5.82	4.84		
	40	.84	4.05	6.05	10.91	5.46		
	N-source avg	2.56	2.76	2.99	5.02			
$NaHCO_3$	0	.00	.00	.00	.00	.00	2.84	
	3	2.65	2.15	3.45	3.28	2.88		
	15	4.45	4.36	4.56	4.90	4.57		
	40	5.66	2.94	5.22	1.77	3.90		
	N-source avg	3.19	2.36	3.31	2.49			
Sucrose	0	.00	.00	.00	.00	.00	2.48	
	3	3.07	1.65	1.47	2.28	2.12		
	15	1.91	3.58	5.20	6.28	4.24		
	40	3.25	3.18	1.68	6.06	3.54		
	N-source avg	2.06	2.10	2.09	3.66			

(Table continued on next page)

Table 15. Continued.

Additive[b]	Day of fermentation	N-source Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Lactose	0	.00	.00	.00	.00	.00	3.44	
	3	2.82	7.88	1.50	2.14	3.58		
	15	4.41	5.44	5.63	5.84	5.33		
	40	5.39	3.24	4.35	6.36	4.82		
	N-source avg	3.16	4.13	2.87	3.58			
NaCl	0	1.60	.00	.00	.00	.40	3.22	
	3	1.72	8.10	1.06	1.61	3.12		
	15	4.23	3.02	5.60	6.11	4.74		
	40	1.44	4.57	5.05	7.43	4.62		
	N-source avg	2.25	3.92	2.93	3.79			
N-source main effects		2.66	3.34	2.86	3.88		(& additive)	2.54
Day main effects	(0).10	(3)3.18		(15)4.91	(40)4.57			1.88

[a]Nitrogen from urea, aqua-NH_4 and NH_4-solution added at .2% and other additives at 1% (except NaCl at .5%) of fresh silage.

[b]Treatment combination values (percent of DM) are means from duplicate silos for control and single observations for all other disaccharide or mineral.

(Table 6 and 15) and concurrent decreases in pH (Table 5 and 14) is logical; however, pH on day 40 of the silage treated with NH_4-solution was not different from control, urea or Aqua-NH_4 but lactic acid was increased. This indicates that ammonia does extend fermentation by neutralizing organic acid (Huber and Santana, 1972; Huber, 1973a and c).

Acetic acid levels (Table 16) tended to be lower for control than for N-treated silages. Values on day 40 were lower ($P<.05$) for control compared to the NH_4-solution treated silage. $CaCO_3$ and $NaHCO_3$ additions generally resulted in elevated acetic acid compared to sucrose and lactose and these differences were significant ($P<.05$) on day 40. Trends in acetic acid were similar for the 3 X 8 X 4 factorial (Appendix Table 3 and Table 16). There was a tendency for higher acetic acid levels on day 40 for NH_4-solution than for urea and aqua-NH_4. $NaHCO_3$ and $CaCO_3$ elicited higher ($P<.05$) levels of acetic acid on day 40 than sucrose and lactose alone or sucrose in combination with $CaCO_3$. The increase in acetic acid was consistent from day zero to 40 with day 40 values higher than ($P<.05$) days 0, 3 and 15. In contrast, lactic acid reached a plateau after 15 days.

Acetic acid levels seemed to parallel those of lactic acid (Table 15) and were increased most (.4 percentage unit) by NH_4-solution treatment. However, the extremely low lactic values for the treatment combinations $CaCO_3$ + control and $NaHCO_3$ + NH_4-solution on day 40 correspond to unsually high acetic acid values. This same inverse relationship was

Table 16. Effect of nitrogen source, additive, and day of fermentation on acetic acid content of corn silage[a].

Additive[b]	Day of fermentation	N-source: Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Control	0	.40	.42	.52	.58	.48	1.21	
	3	.46	1.08	.82	.74	.78		
	15	1.40	1.30	1.52	1.55	1.44		
	40	1.76	2.08	2.20	2.52	2.14		
	N-source avg	1.00	1.22	1.26	1.35			
$CaCO_3$	0	.38	.34	.50	.49	.43	1.67	
	3	1.06	1.21	.96	1.04	1.07		
	15	1.65	2.28	1.33	1.53	1.70		
	40	4.17	3.07	3.15	3.51	3.48		
	N-source avg	1.82	1.72	1.48	1.64			
$NaHCO_3$	0	.43	.43	.52	.62	.50	1.76	
	3	1.61	1.67	1.40	.87	1.39		
	15	.79	2.97	1.89	1.67	1.83		
	40	2.36	3.94	2.44	4.60	3.34		
	N-source avg	1.30	2.25	1.56	1.94			
Sucrose	0	.39	.30	.63	.54	.46	.82	
	3	.32	.66	.56	.83	.59		
	15	.46	.84	1.11	.96	.84		
	40	1.54	.98	1.00	1.99	1.38		
	N-source avg	.68	.70	.82	1.08			

(Table continued on next page)

Table 16. Continued.

Additive[b]	Day of fermentation	N-source Control	Urea	$Aqua-NH_4$	NH_4-Sol	Day avg.	Additive main effects	SEM
Lactose	0	.44	.38	.57	.68	.52	1.01	
	3	.63	.48	.63	.60	.58		
	15	.55	1.22	1.61	1.17	1.14		
	40	1.60	1.71	1.36	2.56	1.81		
	N-source avg	.81	.95	1.04	1.25			
NaCl	0	.29	.31	.72	.62	.48	1.17	
	3	.47	.72	.58	.64	.60		
	15	1.37	.93	.90	1.68	1.22		
	40	1.02	2.33	3.33	2.77	2.36		
	N-source avg	.79	1.07	1.38	1.43			
N-source main effects		1.06	1.32	1.26	1.45		(& additive)	.303
Day main effects	(0).48	(3).84		(15)1.36	(40)2.42			.431

[a]Nitrogen from urea, $aqua-NH_4$ and NH_4-solution added at .2% and other additives at 1% (except NaCl at .5%) of fresh silage.

[b]Treatment combination values (percent of DM) are means from duplicate silos for control and single observations for all other mineral or disaccharide additives.

noted when lactic was depressed by high levels of ammonia (Figure 4). Nevertheless, lactic acid values were normal on day 15, suggesting that secondary fermentation may have occurred due to air leakage into the plastic bags and may have resulted in conversion of lactic to acetic acid. Britt (1973) observed a threefold reduction in lactic acid content of corn silage seven days after bi-daily aeration; however, acetic acid values were not reported.

Total organic acids (TOA) tended to increase more ($P < .25$) in silages treated with NH_4-solution than control, urea or aqua-NH_4 (Table 17). Day 40 control silage levels of TOA were lower ($P < .05$) than all others, with urea and aqua-NH_4 lower ($P < .05$) than the NH_4-solution treated silage. There was a tendency for sucrose treated silages to be lower ($P < .25$) in TOA than all other disaccharide or mineral additives. Day 40 TOA levels were highest for $CaCO_3$ treated silages and lower ($P < .10$) for sucrose than all other disaccharide or mineral additives. Total organic acids increased with fermentation time but differences were slight between day 15 and day 40.

The trends in TOA levels described for Table 17 were similar to those noted for the 3 X 8 X 4 factorial analysis (Appendix Table 3). Day 40 TOA levels for silages treated with $CaCO_3$ alone or in combination with sucrose and lactose were not different; however, sucrose and lactose silages resulted in lower ($P < .10$) TOA than when added in combination with $CaCO_3$.

Table 17. Effect of nitrogen source, additive, and day of fermentation on total organic acid content of corn silage[a].

Additive[b]	Day of fermentation	N-source: Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Control	0	.94	.46	.56	.74	.68	5.31	
	3	5.09	10.28	2.03	6.82	6.06		
	15	4.94	6.37	6.60	10.96	7.22		
	40	5.71	6.98	8.32	8.10	7.28		
	N-source avg	4.17	6.02	4.38	6.66			
$CaCO_3$	0	.38	.34	.50	.49	.43	5.01	
	3	5.46	3.65	2.43	4.56	4.02		
	15	5.77	7.05	6.14	7.35	6.58		
	40	5.01	7.12	9.38	14.48	9.00		
	N-source avg	4.16	4.54	4.61	6.72			
$NaHCO_3$	0	.53	.43	.52	.62	.52	4.64	
	3	4.43	3.82	4.85	4.36	4.36		
	15	5.24	7.33	6.45	6.57	6.40		
	40	8.02	6.88	7.80	6.37	7.27		
	N-source avg	4.56	4.62	4.90	4.48			
Sucrose	0	.49	.38	.63	.54	.51	3.36	
	3	3.39	2.49	2.18	3.48	2.88		
	15	2.37	4.42	6.31	7.24	5.08		
	40	4.79	4.16	2.80	8.05			
	N-source avg	2.76	2.86	2.98	4.83			

(Table continued on next page)

Table 17. Continued.

Additive[b]	Day of fermentation	N-source Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Lactose	0	.44	.38	.57	.68	.52	4.50	
	3	3.45	8.36	2.51	2.74	4.26		
	15	4.96	6.82	7.42	7.01	6.55		
	40	6.99	4.91	5.86	8.92	6.67		
	N-source avg	3.96	5.12	4.09	4.84			
NaCl	0	1.89	.31	.72	.62	.88	4.46	
	3	2.46	9.03	1.64	2.52	3.91		
	15	5.81	3.95	6.50	8.03	6.07		
	40	2.47	6.90	8.38	10.20	6.99		
	N-source avg	3.16	5.05	4.31	5.34			
N-source main effects		3.79	4.70	4.21	5.48		(& additive)	2.76
Day main effects	(0).59	(3)4.25		(15)6.32	(40)7.02			2.56

[a]Nitrogen from urea, aqua-NH_4 and NH_4-solution added at .2% and other additives at 1% (except NaCl at .5%) of fresh silage.
[b]Treatment combination values (percent of DM) are means from duplicate silos for control and single observations for all other disaccharide or mineral additives.

Silage dry matter content (Table 18) was not altered by the addition of .2% nitrogen in the form of urea, aqua-NH_4 or NH_4-solution. The disaccharide and mineral additives generally increased ($P < .10$) silage DM compared to no additive. This was probably a reflection of the amount of additive incorporated into the silage since they were added at approximately 3% of silage dry weight. Lopez et al. (1970b) found similar increases in silage DM when they added corn at similar levels. $CaCO_3$ and $NaHCO_3$ treated silages tended to be lower ($P < .10$) in dry matter and higher ($P < .05$) in acetic acid than silages with added sucrose, lactose, or NaCl. It is probable that acetic acid was volatilized during oven drying and resulted in a lower apparent silage dry matter. Day zero dry matter values tended to be higher than all others.

Trends in DM content as affected by N-source and day of fermentation for the 3 X 8 X 4 factorial analysis (Appendix Table 4) were similar to those reported above. All treatments containing sucrose and lactose tended to be higher ($P < .10$) in DM than those without disaccharide. Here again, the dry matter of the additive incorporated into the silage was most likely responsible for this increase. $CaCO_3$ in combination with disaccharide amounted to about 6% of the silage dry weight.

Silage total nitrogen (TN) as affected by N-source, mineral or disaccharide additive, and day of fermentation are presented in Table 19. As expected, control silage (TN) was lower ($P < .05$) than that treated with nitrogen. Urea

Table 18. Effect of nitrogen source, additive, and day of fermentation on dry matter content of corn silage.[a]

Additive[b]	Day of fermentation	N-source: Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Control	0	35.40	33.60	36.00	37.00	35.50	34.65	
	3	35.58	34.82	34.66	34.80	34.96		
	15	34.91	34.75	35.08	33.37	34.53		
	40	33.80	32.40	33.80	34.40	33.60		
	N-source avg	34.92	33.89	34.88	34.89			
$CaCO_3$	0	34.30	35.80	36.30	37.60	36.00	35.36	
	3	34.06	34.73	35.30	34.50	34.65		
	15	34.50	34.61	35.44	35.90	35.11		
	40	38.00	36.30	35.70	32.80	35.70		
	N-source avg	35.22	35.36	35.68	35.20			
$NaHCO_3$	0	39.00	37.30	34.60	36.90	36.95	36.05	
	3	35.32	35.85	35.08	36.89	35.78		
	15	34.59	34.02	34.95	35.91	35.12		
	40	37.30	36.00	36.00	36.10	36.35		
	N-source avg	36.55	35.79	35.41	36.45			
Sucrose	0	38.70	40.00	37.90	36.70	38.32	36.34	
	3	33.99	36.59	37.38	36.26	36.06		
	15	35.72	36.86	37.01	37.65	36.81		
	40	31.10	34.60	36.00	35.00	34.18		
	N-source avg	34.88	37.01	37.07	36.40			

(Table continued on next page)

Table 18. Continued.

Additive[b]	Day of fermentation	N-source: Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Lactose	0	36.00	39.60	36.80	36.80	37.30	36.80	
	3	34.65	37.67	38.08	38.53	37.23		
	15	36.18	35.39	36.12	35.81	35.88		
	40	37.50	38.70	37.90	33.20	36.82		
	N-source avg	36.08	37.84	37.22	36.08			
NaCl	0	37.70	38.20	38.70	38.40	38.25	36.98	
	3	35.65	35.92	36.17	37.52	36.32		
	15	36.40	36.43	35.38	36.91	36.28		
	40	38.20	36.10	38.20	35.80	37.08		
	N-source avg	36.99	36.66	37.11	37.16			
N-source main effects		35.77	36.09	36.23	36.03		(& additive)	.731
Day main effects	(0) 37.05	(3) 35.83		(15) 35.62	(40) 35.16			1.31

[a]Nitrogen from urea, aqua-NH_4 and NH_4-solution added at .2% and other additives at 1% (except NaCl at .5%) of fresh silage.

[b]Treatment combination values are means from duplicate silos for control and single observations for all other disaccharide or mineral additives.

Table 19. Effect of source of nitrogen, additive, and day of fermentation on total nitrogen content of corn silage[a].

Additive[b]	Day of fermentation	N-source Control	N-source Urea	N-source Aqua-NH_4	N-source NH_4-Sol	Day avg.	Additive main effects	SEM
Control	0	1.540	2.285	1.970	1.988	1.946	1.950	
	3	1.474	2.164	2.001	1.979	1.904		
	15	1.528	2.252	1.970	1.934	1.922		
	40	1.614	2.502	2.046	1.952	2.028		
	N-source avg	1.539	2.301	1.998	1.963			
$CaCO_3$	0	1.083	2.183	1.979	1.825	1.768	1.843	
	3	1.387	2.096	1.894	1.924	1.825		
	15	1.513	2.250	1.808	1.888	1.865		
	40	1.378	2.194	2.038	2.043	1.913		
	N-source avg	1.340	2.181	1.930	1.920			
$NaHCO_3$	0	1.369	2.286	2.155	1.730	1.885	1.859	
	3	1.431	2.031	1.892	1.877	1.808		
	15	1.457	2.157	1.739	1.888	1.810		
	40	1.642	2.171	1.941	1.983	1.934		
	N-source avg	1.475	2.161	1.932	1.870			
Sucrose	0	1.423	1.652	1.871	1.770	1.679	1.721	
	3	1.414	1.898	1.697	1.873	1.720		
	15	1.436	1.882	1.648	1.644	1.652		
	40	1.692	2.044	1.891	1.695	1.830		
	N-source avg	1.491	1.869	1.777	1.746			

(Table continued on next page)

Table 19. Continued.

Additive[b]	Day of fermentation	N-source Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Lactose	0	1.452	1.806	1.746	1.942	1.736	1.740	
	3	1.382	1.858	1.630	1.779	1.662		
	15	1.431	1.921	1.752	1.946	1.762		
	40	1.509	1.826	1.777	2.084	1.799		
	N-source avg	1.444	1.853	1.726	1.938			
NaCl	0	1.154	1.838	1.723	1.901	1.654	1.729	
	3	1.331	1.872	1.784	1.791	1.694		
	15	1.453	1.874	1.797	1.986	1.778		
	40	1.379	2.076	1.740	1.959	1.788		
	N-source avg	1.329	1.915	1.761	1.909			
N-source main effects		1.436	2.047	1.854	1.891		(& additive)	.356
Day main effects	(0)1.778	(3)1.769	(15)1.798	(14)1.882				.257

[a]Nitrogen from urea, aqua-NH_4 and NH_4-solution added at .2% and other additives at 1% (except NaCl at .5%) of fresh silage.

[b]Treatment combination values (percent of DM) are means from duplicate silos for control and single observations for all other disaccharide or mineral additives.

additions tended toward higher ($P<.10$) TN than aqua-NH_4 or NH_4-solution. A similar observation was noted in Table 10. Among disaccharide and mineral additives, the control resulted in slightly higher ($P<.25$) silage TN than all others. This subtlety is attributed to a dilution of nitrogen concentration since the various additives tended to increase ($P<.10$) silage DM. Consistent with this idea is the observation in the 3 X 8 X 4 comparison (Appendix Table 4 and Table 19) that control silage with no additive tended toward slightly higher ($P<.25$) TN than those silages to which additives were incorporated.

Silage total soluble nitrogen (TSN) values are presented in Table 20 and Appendix Table 5. Control silage TSN was lower ($P<.10$) than all other N-sources, while urea values tended to be higher ($P<.25$) than those for aqua-NH_4 and NH_4-solution for both factorial comparisons. Values on day 40 were similar to those mentioned above. There were no differences due to disaccharide or mineral additive and no significant interactions. The lack of TSN increase for control as fermentation time increased was similar to that observed and discussed with Table 11. Bergen _et al._ (1974) observed that TSN of control corn silage accounted for 36% of the total nitrogen after only 12 hours of fermentation, but then changed slowly to 42% by day 20. TSN of control silage in this trial was 28% of the total nitrogen on day zero and also on day 40. As discussed earlier, it is possible that some proteolysis may have occurred before samples were frozen. The cool

Table 20. Effect of nitrogen source, additive, and day of fermentation on total soluble nitrogen content of corn silage[a].

Additive[b]	Day of fermentation	N-source Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Control	0	.438	1.250	.816	.748	.812	.767	
	3	.396	1.102	.892	.888	.820		
	15	.348	.928	.684	.756	.679		
	40	.454	1.008	.769	.814	.761		
	N-source avg	.409	1.072	.790	.800			
$CaCO_3$	0	.092	.748	.564	.691	.524	.698	
	3	.390	1.035	.917	.809	.788		
	15	.296	1.330	.638	.638	.726		
	40	.363	.900	.849	.901	.753		
	N-source avg	.285	1.003	.742	.760			
$NaHCO_3$	0	.222	.802	.728	.555	.577	.666	
	3	.333	1.001	.965	.887	.796		
	15	.240	.897	.592	.699	.607		
	40	.528	1.039	.700	.469	.684		
	N-source avg	.330	.935	.746	.652			
Sucrose	0	.285	1.024	.873	.734	.729	.705	
	3	.348	.780	.812	1.001	.735		
	15	.266	.730	.662	.762	.605		
	40	.443	1.036	.779	.746	.751		
	N-source avg	.336	.892	.782	.811			

(Table continued on next page)

Table 20. Continued.

Additive[b]	Day of fermentation	N-source: Control	Urea	Aqua-NH_4	NH_4-Sol	Day avg.	Additive main effects	SEM
Lactose	0	.317	.915	.738	.818	.697	.662	
	3	.258	.841	.726	.850	.669		
	15	.301	.709	.612	.693	.579		
	40	.242	.967	.623	.980	.703		
	N-source avg	.280	.858	.675	.835			
NaCl	0	.293	.855	.763	.738	.662	.660	
	3	.309	1.043	.837	.826	.754		
	15	.272	.747	.520	.673	.556		
	40	.309	.840	.598	.935	.670		
	N-source avg	.296	.871	.680	.796			
N-source main effects		.323	.939	.736	.776	(& additive)		.290
Day main effects		(0).667	(3).760	(15).625	(40).720			.149

[a]Nitrogen from urea, aqua-NH_4 and NH_4-solution added at .2% and other additives at 1% (except NaCl at .5%) of fresh silage.

[b]Treatment combination values (percent of DM) are means from duplicate silos for control and single observations for all other mineral or disaccharide additives.

temperatures and the anaerobic conditions established early by rapid evacuation of air from the experimental silos may have inhibited any further proteolysis.

Water insoluble nitrogen (WIN) content of control corn silage (Table 21) was identical to that treated with urea, aqua-NH_4 and NH_4-solution. There is usually a higher final WIN content of NPN and especially ammonia treated corn silages when compared to the same silage ensiled without NPN additives. Bergen et al. (1974) reported that WIN accounted for 58% of the total corn silage nitrogen after 20 days of fermentation. The WIN for control corn silage in the present experiment (Table 21) accounted for 72% of the total nitrogen after 40 days of fermentation. Fermentation over a 40-day period failed to appreciably alter WIN content of the various corn silage treatments. A slight increase in WIN content for control corn silage and the $NaHCO_3$ treatment combinations was noted (Table 21 and Appendix Table 5); but, rather large standard errors of the treatment means due to lack of sufficient replication precludes any declaration of significance of differences.

Table 21. Effect of nitrogen source, additive, and day of fermentation on water insoluble nitrogen content of corn silage.[a]

Additive[b]	Day of fermentation	N-source				Day avg	Additive main effects	SEM
		Control	Urea	Aqua-NH_4	NH_4-Sol			
Control	0	1.102	1.035	1.154	1.246	1.134	1.182	
	3	1.077	1.062	1.110	1.091	1.085		
	15	1.179	1.324	1.291	1.178	1.243		
	40	1.160	1.493	1.276	1.138	1.267		
	N-source avg	1.130	1.228	1.208	1.163			
$CaCO_3$	0	.991	1.435	1.415	1.134	1.244	1.136	
	3	.996	.920	.977	1.114	1.002		
	15	1.217	.920	1.170	1.250	1.139		
	40	1.015	1.294	1.189	1.142	1.160		
	N-source avg	1.055	1.142	1.188	1.160			
$NaHCO_3$	0	1.147	1.484	1.427	1.175	1.308	1.193	
	3	1.099	1.030	.926	.990	1.011		
	15	1.217	1.260	1.147	1.189	1.203		
	40	1.114	1.132	1.241	1.514	1.250		
	N-source avg	1.144	1.226	1.185	1.217			
Sucrose	0	1.138	.628	.998	1.036	.950	1.014	
	3	1.067	1.118	.884	.871	.985		
	15	1.170	1.152	.968	.882	1.043		
	40	1.249	1.008	1.112	.949	1.080		
	N-source avg	1.156	.976	.990	.934			

(Table continued on next page)

Table 21. Continued

Additive[b]	Day of fermentation	N-source Control	Urea	Aqua-NH_4	NH_4-Sol	avg.	Additive main effects	SEM
Lactose	0	1.135	.891	1.008	1.124	1.040	1.078	
	3	1.124	1.017	.904	.929	.994		
	15	1.130	1.212	1.140	1.253	1.184		
	40	1.267	.859	1.154	1.104	1.096		
	N-source avg	1.164	.995	1.052	1.102			
NaCl	0	.861	.983	.960	1.163	.992	1.068	
	3	1.022	.830	.947	.965	.941		
	15	1.18	1.127	1.277	1.303	1.222		
	40	1.070	1.236	1.142	1.024	1.118		
	N-source avg	1.034	1.044	1.082	1.114			
N-source main effects		1.114	1.102	1.117	1.115		(& additive)	.475
Day main effects	(0)1.111	(3)1.003		(15)1.172	(40)1.162			.257

[a]Nitrogen from urea, aqua-NH_4 and NH_4-solution added at .2% and other additives at 1% (except NaCl at .5%) of fresh silage.

[b]Treatment combination values (percent of DM) are means from duplicate silos for control and single observations for all disaccharide or mineral additives.

<u>Experiment 2</u>. The pH values of corn silage of four maturities treated with four levels of ammonia and three levels of water are presented in Table 22. Increasing dry matter percent (DM%) at harvest had the general effect of also increasing silage pH. The 28% DM maturity level resulted in lower pH ($P<.05$) than 39 and 46, and 36 was lower ($P<.05$) than 46% DM corn silage. Ammonia additions also increased silage pH with the control being lower ($P<.05$) than .72% added ammonia and the latter level being higher than ($P<.05$) the two lowest levels of added ammonia. A consistently higher pH was noted at both levels of added water (.8 and 1.6%) than for the control (no added water). A significant DM X NH_3 interaction ($P<.05$) suggests that ammonia affects silage pH differently at the various stages of maturity (DM%). The DM and NH_3 effects appear to be additive at 39 and 46% DM, slightly additive at 36% DM, and at 28% DM the added NH_3 had no affect. The DM X H_2O interaction ($P<.05$) can be explained by the differences of pH within the 1.6 H_2O level with a low mean of 3.71 at 28% DM and the high mean of 4.34 at 46%. The NH_3 X H_2O interaction ($P<.05$) is mainly due to the marked increase in pH observed for the three levels of ammonia addition to the 46% DM silage. The significant ($P<.05$) three-factor interaction was probably due to the abnormally high pH values of the 39 and 46% DM silage at .72% NH_3 and .8 added H_2O.

The change in pH of the 36% DM corn silage resulting from added ammonia was negligible and far different from that

Table 22. Ammonia treated corn silage pH after 40 days fermentation.[a]

Dry matter	H_2O%	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	3.48	3.70	3.85	3.85	3.72	
	.8	3.60	3.78	3.85	3.88	3.78	
	1.6	3.52	3.70	3.80	3.82	3.71	
							3.74
	NH_3 avg	3.53	3.73	3.83	3.85		
36%	0	3.78	3.78	3.84	3.98	3.84	
	.8	3.85	3.88	3.88	3.90	3.84	
	1.6	3.88	3.85	3.82	3.99	3.86	
							3.85
	NH_3 avg	3.84	3.81	3.84	3.92		
39%	0	3.75	3.99	3.85	4.14	3.93	
	.8	3.74	3.90	4.00	4.66	4.08	
	1.6	3.71	3.85	4.07	4.62	4.07	
							4.02
	NH_3 avg	3.73	3.91	3.98	4.48		
46%	0	3.85	3.95	4.08	4.15	4.01	
	.8	3.89	4.13	4.08	4.22	4.06	
	1.6	3.99	4.12	4.22	5.02	4.34	
							4.13
	NH_3 avg	3.88	4.07	4.13	4.47		
NH_3 (%) main effects		3.74	3.88	3.94	4.17		
							SEM = .081

H_2O (%) main effects		
	0	3.87
	.8	3.84
	1.6	3.99

[a]Treatment combination means are from duplicate silos.

of Experiment 1 (Table 5). However, the method of application was different, the levels of addition were somewhat lower and the experimental silos were opened once in this trial while those of Experiment 1 were opened and resealed several times.

Huber et al. (1973c) have observed that increased silage dry matter % and addition of ammonia raised silage pH values. Other workers (Huber et al., 1974; and Huber and Santana, 1972) have observed pH values of 4.0 and above for ammoniated corn silage.

The lactic acid content of ammoniated corn silage is presented in Table 23. Neither ammonia level nor level of added water had any overall significant effects on lactic acid concentration of corn silage. Maturity exerted an inhibitory influence on silage lactate with 28% DM higher ($P<.01$) than 36, 39 and 46. There was a consistent but statistically non-significant decline with each incremental increase in maturity. There was a significant DM X NH_3 interaction ($P<.05$) due to the large difference in response to NH_3 at the different dry matter levels. Within the 36% DM silage the three levels of ammonia each increased lactic acid compared to controls, with maximum concentrations at .48% NH_3. Even though there was a consistent decline in lactic acid content from .48 to .72% NH_3, there was no marked decrease in lactic acid production at the slightly higher levels of NH_3 addition as observed in Experiment 1 (Table 6) at 40 days fermentation. However, concentrations of total N were not high enough to

Table 23. Lactic acid of ammonia treated corn silage after 40 days fermentation.[a]

Dry matter	H_2O%	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	12.66	11.88	9.79	11.02	11.34	
	.8	12.63	14.18	12.22	12.82	12.97	
	1.6	13.26	9.70	14.84	19.95	14.44	
							12.91
	NH_3 avg	12.85	11.92	12.28	14.60		
36%	0	4.74	9.18	9.44	8.35	7.93	
	.8	6.47	10.04	9.88	9.70	9.02	
	1.6	6.31	8.66	14.36	8.54	9.47	
							8.81
	NH_3 avg	5.84	9.29	11.23	8.86		
39%	0	12.48	6.50	8.50	7.18	8.67	
	.8	9.77	8.41	4.84	6.16	7.29	
	1.6	10.51	8.97	5.02	4.39	7.22	
							7.73
	NH_3 avg	10.92	7.96	6.12	5.91		
46%	0	6.04	6.53	6.44	8.56	6.90	
	.8	5.84	6.98	5.04	5.02	5.72	
	1.6	6.13	7.04	7.02	4.75	6.23	
							6.28
	NH_3 avg	6.00	6.85	6.16	6.11		
NH_3 main effects (%)		8.90	9.00	8.95	8.87		
						SEM =	1.60
H_2O (%) main effects			0		8.71		
			.8		8.75		
			1.6		9.34		

[a]Treatment combination means expressed as a percent of DM are from duplicate silos.

show severe decreases in lactate. Also, the method of application and frequency of sampling were different and may have influenced fermentation patterns differently.

Various researchers (Beattie, 1970; Huber *et al.*, 1973c; Huber *et al.*, 1974; and Huber and Santana, 1972) have noted modest to marked increases in corn silage lactate with additions of ammonia at ensiling time. Huber *et al.* (1973c) also showed decreased lactic acid (5.78%) in 35% DM corn silage treated with .66% added NH_3 compared to 30% DM corn silage treated with .33% added NH_3 (10.54% lactic acid). Lopez *et al.* (1970a), Geasler (1970), Johnson *et al.* (1967) and Huber *et al.* (1973c) have all shown that lactic acid concentrations decrease with advancing silage maturity.

The acetic acid content of the ammoniated silages at four stages of maturity is presented in Table 24. There was a consistent decrease in acetic acid with increasing maturity. The 28% DM silage tended to be higher ($P<.10$) than 36, 39 and 46. Ammonia tended to increase ($P<.25$) silage acetic acid compared to the control. The effect of H_20 level was negligible on acetic acid concentration. In the face of strong two and three factor interactions and a rather large SEM it is difficult to make definitive conclusions about the influince of maturity and level of NH_3 and H_20 on corn silage acetic acid content. One can rather safely draw the conclusion, however, that acetic acid levels in corn silage are decreased with advancing maturity and elevated with incremental levels of ammonia up to .48 or .72%. Huber *et al.* (1973c) showed

Table 24. Acetic acid of ammonia treated corn silage after 40 days fermentation.[a]

Dry matter	H_2O%	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	2.83	3.07	4.60	3.40	3.47	
	.8	2.79	3.55	3.63	3.74	3.43	
	1.6	2.30	2.36	3.00	2.95	2.65	
							3.18
	NH_3 avg	2.64	3.00	3.74	3.36		
36%	0	1.64	2.26	2.16	2.71	2.19	
	.8	1.83	2.62	2.08	2.24	2.19	
	1.6	2.92	2.46	2.86	3.10	2.83	
							2.40
	NH_3 avg	2.13	2.45	2.36	2.68		
39%	0	.88	1.48	2.04	2.24	1.66	
	.8	1.22	1.68	2.62	2.45	2.00	
	1.6	1.31	1.38	2.04	2.30	1.76	
							1.80
	NH_3 avg	1.14	1.52	2.24	2.33		
46%	0	1.21	1.16	1.72	1.80	1.47	
	.8	1.20	1.62	1.67	1.88	1.59	
	1.6	1.21	1.87	1.37	1.90	1.59	
							1.55
	NH_3 avg	1.20	1.55	1.59	1.86		
NH_3 (%) main effects		1.78	2.13	2.48	2.55		
						SEM = 1.90	

H_2O (%) main effects		
	0	2.20
	.8	2.30
	1.6	2.21

[a]Treatment combination means expressed as a percent of DM are from duplicate silos.

increased silage acetate with addition of ammonia and decreases with advancing maturity.

Total organic acid (TOA) content of ammoniated silage was reduced with advancing maturity of the corn plant (Table 25). Silage at 28% DM had higher ($P<.05$) TOA content than the other silages harvested at more mature stages of growth. Levels of NH_3 and H_2O had no consistent influence on TOA content of corn silage harvested over the range of 28 to 46% dry matter. In general, NH_3 caused an increase in TOA of corn silage at 28 and 36% DM and caused a decrease or no change in 39 and 46% DM corn silages.

High acidity has been shown to depress intakes and growth of young calves offered corn silage _ad libitum_ (Thomas and Wilkinson, 1973; Wilkinson _et al._, 1975). The lower pH values (Table 22) and the higher concentrations of lactic, acetic, and total organic acids (Tables 23, 24 and 25) associated with 28% DM corn silage would suggest that reduced voluntary consumption would have occurred compared to higher dry matter and less acid silages. The reduced silage dry matter intakes reported in Table 1 and Figure 2 for lactating dairy cows of silages below 33% DM would support this hypothesis.

The dry matter content of the ammonia treated corn silages is shown in Table 26. An attempt was made to equalize the dry matter content within the four maturity groups. Because of the small amount of silage in each experimental silo, and since duplicate silos were processed conjointly, it was

Table 25. Total organic acids of ammonia treated corn silage after 40 days fermentation.[a]

Dry matter	H_2O%	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	15.92	15.51	14.91	14.78	15.28	
	.8	15.80	18.18	16.28	16.96	16.80	
	1.6	15.98	12.61	18.17	23.35	17.53	
							16.54
	NH_3 avg	15.90	15.44	16.45	18.36		
36%	0	6.74	11.98	11.85	11.28	10.46	
	.8	8.58	12.92	21.27	12.18	11.49	
	1.6	9.55	11.53	17.56	11.88	12.63	
							11.53
	NH_3 avg	8.29	12.14	13.90	11.78		
39%	0	13.74	8.54	10.78	9.76	10.70	
	.8	11.88	10.38	7.74	9.12	9.78	
	1.6	12.14	10.67	7.35	7.04	9.30	
							9.93
	NH_3 avg	12.58	9.86	8.62	8.64		
46%	0	7.54	8.06	8.28	10.54	8.60	
	.8	7.04	8.99	6.90	7.14	7.52	
	1.6	7.48	9.11	8.74	6.88	8.05	
							8.06
	NH_3 avg	7.35	8.72	7.96	8.18		
NH_3 (%) main effects		11.03	11.54	11.74	11.74		
							SEM = 1.68

H_2O (%) main effects		
	0	11.26
	.8	11.40
	1.6	11.88

[a]Treatment combination means expressed as a percent of DM are from duplicate silos.

Table 26. Dry matter content of ammonia treated corn silage after 40 days fermentation.[a]

Dry matter	H_2O%	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	25.40	26.88	26.48	27.12	26.47	
	.8	26.14	26.39	26.08	27.02	26.41	
	1.6	29.22	30.32	30.95	31.02	30.38	
							27.75
	NH_3 avg	26.92	27.86	27.83	28.38		
36%	0	36.50	35.98	36.44	36.20	36.28	
	.8	36.58	35.87	36.54	37.10	36.52	
	1.6	34.00	35.59	36.24	26.76	35.65	
							36.15
	NH_3 avg	35.70	35.81	36.40	36.69		
39%	0	39.64	40.17	40.14	40.33	40.07	
	.8	38.84	38.61	38.15	40.60	39.05	
	1.6	39.00	38.65	37.37	40.60	38.90	
							39.34
	NH_3 avg	39.16	39.14	38.55	40.51		
46%	0	42.92	46.76	48.29	48.74	46.68	
	.8	43.30	46.82	50.18	47.18	46.87	
	1.6	43.80	47.68	45.44	46.53	45.86	
							46.47
	NH_3 avg	43.34	47.09	47.97	47.48		
NH_3(%) main effects		36.28	37.48	37.69	38.27		
							SEM = .418

H_2O (%) main effects		
	0	37.37
	.8	37.21
	1.6	37.70

[a]Treatment combination means are from duplicate silos.

difficult to obtain a completely uniform DM content within each maturity. It would have been most beneficial to have been able to mix the entire load of silage at each maturity before processing the individual silos. The differences in DM ($P < .01$) between maturities were intentional. There appeared to be a true difference in DM content within NH_3 level with zero NH_3 lower ($P < .05$) than .24, .48 and .72%. However, there were significant DM X NH_3 ($P < .01$), NH_3 X H_2O ($P < .05$), and DM X NH_3 X H_2O ($P < .01$) interactions suggesting that the response to added NH_3 was different at the various maturities, that the effects NH_3 and H_2O were additive at the lowest and highest but not at the intermediate maturities, and that the effect of NH_3 upon maturity was different at each of the H_2O levels.

Total nitrogen (TN) content of corn silage was intentionally increased ($P < .01$) with increasing levels of added NH_3 (Table 27). There was a general effect of lowered TN with advancing maturity. This difference was significant ($P < .01$) when 28% DM was compare with 36, 39 and 46. Ammonia was applied on a fresh silage basis at a constant rate within levels of addition. Silage flow rate was not that different and thus lower DM silages received more NH_3 per unit of dry silage. Also the less mature silages contain more natural plant H_2O to capture the NH_3 initially and produce greater amounts of organic acids. These organic acids combine with the NH_3 to form ammonium salts and thus retain the nitrogen after fermentation is complete. The general effect of added

Table 27. Total nitrogen of ammonia treated corn silage after 40 days fermentation.[a]

Dry matter	H_2O	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	1.580	2.209	2.718	3.010	2.379	
	.8	1.580	2.336	2.881	3.024	2.455	
	1.6	1.486	2.004	2.556	2.712	2.189	
							2.341
	NH_3 avg	1.548	2.183	2.718	2.916		
36%	0	1.598	1.894	2.172	2.403	2.024	
	.8	1.500	2.077	2.181	2.350	2.040	
	1.6	1.627	2.120	2.202	2.580	2.132	
							2.065
	NH_3 avg	1.591	2.030	2.185	2.455		
39%	0	1.514	1.750	2.078	2.206	1.887	
	.8	1.548	1.962	2.342	2.512	2.091	
	1.6	1.588	1.800	2.346	2.658	2.098	
							2.025
	NH_3 avg	1.550	1.837	2.255	2.459		
46%	0	1.564	1.838	1.809	2.016	1.807	
	.8	1.641	1.792	1.921	2.050	1.851	
	1.6	1.444	1.992	2.433	2.440	2.077	
							1.912
	NH_3 avg	1.550	1.874	2.054	2.168		
NH_3 (%) main effects		1.560	1.981	2.303	2.499		
							SEM = .054

H_2O (%) main effects		
	0	2.024
	.8	2.109
	1.6	2.124

[a]Treatment combination means expressed as a percent of DM are from duplicate silos.

water (.8) and 1.6%) was to increase silage TN via greater nitrogen uptake. This increased uptake of nitrogen was manifest at 36, 39 and 46 but not at 28% DM content of the corn silage. All possible interactions were significant ($P < .05$). The DM X NH_3 interaction is indicative of level of NH_3 affecting silage TN differently at the various maturities. The magnitude of increase being greatest at 28, least at 46, and intermediate at 36 and 39% DM. The DM X H_2O interaction suggests an inconsistent response of TN to added H_2O at the various maturities. The NH_3 X H_2O interaction is clearly due to a substantial increase in TN at 1.6% H_2O with .48 and .72% NH_3 but a slight decrease at zero and .24% NH_3. The three-way interaction suggests that the effect of NH_3 upon DM was different at 1.6 than for zero and .8% added H_2O.

Nitrogen recovery (Appendix Table 6) was consistently lower as rate of application increased. Overall recovery was 78, 69 and 59% for the respective ammonia additions of .24, .48 and .72% NH_3 but a slight decrease at zero and .24% NH_3. The three-way interaction suggests that the effect of NH_3 upon DM was different at 1.6 than for zero and .8% added H_2O.

Nitrogen recovery (Appendix Table 6) was consistently lower as rate of application increased. Overall recovery was 78, 69 and 59% for the respective ammonia additions of .24, .48 and .72%. Water added to the 28% DM silage did nothing to enhance NH_3 uptake. However, as maturity increased from 36 to 46% DM the addition of water did increase N incorporation into silage. The overall effect of added water was 58, 69 and 78% recovery at the zero, .8 and 1.6% added water levels, respectively. The overall effect of stage of maturity at harvest (% DM) was for recovery of added NH_3 to decrease with increasing plant maturity, i.e. recovery was 91, 68, 60 and 54% for the respective silage dry matters of 28, 36, 39 and 46%. Huber and Santana (1972) reported recovery of 79% of added nitrogen from aqua ammonia added to 31% DM corn silage.

Total soluble nitrogen (TSN) of ammonia treated corn silage is shown in Table 28. There was a linear decrease ($P<.01$) in the amount of total soluble nitrogen with increasing maturity of the corn silage. This would be expected since less total NH_3 was added (on a dry basis) with increasing maturity (Table 27) and less fermentation and proteolysis occurs as silage maturity increases (Geasler, 1970). The general effect of adding NH_3 was to increase TSN with each added increment of NH_3. The zero level of added NH_3 was lower ($P<.05$) than .24, .48, and .72% NH_3. The average differences in TSN between zero and .24, and between .24 and .48 % were equal (.27) while the increase between .48 and .72% added NH_3 was much less (.10). The overall effect of H_2O level on TSN was negligible. The significant DM X NH_3 interaction ($P<.05$) was mainly due to the greater TSN increase from NH_3 on the 28 than 46% DM silage. The significant DM X H_2O interaction ($P<.05$) can be explained by the fact that added water decreased TSN at 28 but increased TSN at 46% dry matter. The TSN content of control silage was nearly twice that observed

Table 28. Total soluble nitrogen of ammonia treated corn silage after 40 days fermentation.[a]

Dry matter	H_2O%	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	.923	1.364	1.794	2.300	1.595	
	.8	.840	1.642	1.692	1.785	1.490	
	1.6	.833	1.288	1.760	1.687	1.389	
							1.492
	NH_3 avg	.866	1.428	1.748	1.924		
36%	0	.823	1.170	1.316	1.555	1.216	
	.8	.869	1.210	1.310	1.528	1.229	
	1.6	.870	1.247	1.334	1.526	1.244	
							1.230
	NH_3 avg	.854	1.209	1.320	1.536		
39%	0	.762	.960	1.250	1.135	1.011	
	.8	.794	1.060	1.400	.993	1.210	
	1.6	.804	.906	1.544	1.120	1.206	
							1.142
	NH_3 avg	.787	.975	1.398	1.408		
46%	0	.779	.814	.866	.909	.842	
	.8	.808	.623	1.010	1.024	.866	
	1.6	.816	.937	1.194	1.070	1.004	
							.904
	NH_3 avg	.801	.791	1.023	1.001		
NH_3 (%) main effects		.827	1.101	1.372	1.467		
							SEM = .109

H_2O (%) main effects		
	0	1.166
	.8	1.199
	1.6	1.211

[a]Treatment combination means expressed as a percent of DM are from duplicate silos.

in Experiment 1 (Table 11 and 20) and is representative of most published values (Bergen _et al._, 1974; Huber _et al._, 1973c).

Water insoluble nitrogen (WIN) was increased ($P<.05$) at 46% DM compared to the earlier maturities and by NH_3 addition compared to control (Table 29). The zero level was lower ($P<.01$) than .24, .48, and .72% added NH_3. Added H_2O (.8 and 1.6%) slightly increased ($P<.25$) WIN compared to no added water. None of the interactions were significant.

Geasler (1970), Cash (1972), and Huber _et al._ (1973c) have all shown increased water insoluble nitrogen (WIN) with increased maturity (% DM) of corn silage. Addition of ammonia to corn silage prior to ensiling has also increased the final level of WIN (Cash, 1972; Beattie, 1970) by as much as 40% (Huber _et al._, 1973c) when compared to control corn silage. Bergen _et al._ (1974) found proteolytic activity of ammonia treated silage extracts was lower than extracts from control silage. This observation implies that NH_3 additions to corn silage may spare plant protein breakdown. Ammonia may initially bind to the insoluble nitrogen fraction of corn silage. This binding could initially inhibit proteolysis of the natural plant protein and also provide protection against plant protein breakdown as fermentation progresses.

.able 29. Water insoluble nitrogen of ammonia treated corn silage after 40 days fermentation.[a]

Dry matter	H_2O%	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	.657	.844	.924	.710	.784	
	.8	.740	.694	1.188	1.239	.965	
	1.6	.652	.726	.796	1.026	.800	
							.850
	NH_3 avg	.683	.755	.970	.992		
36%	0	.774	.724	.856	.879	.808	
	.8	.681	.868	.870	.823	.810	
	1.6	.756	.873	.868	1.054	.888	
							.835
	NH_3 avg	.737	.822	.865	.919		
39%	0	.752	.777	.824	1.135	.872	
	.8	.801	.902	.942	.993	.910	
	1.6	.784	.894	.801	1.120	.900	
							.894
	NH_3 avg	.779	.858	.856	1.082		
46%	0	.785	1.024	.943	1.106	.965	
	.8	.832	1.172	.912	1.027	.986	
	1.6	.628	1.054	1.239	1.370	1.073	
							1.008
	NH_3 avg	.749	1.084	1.031	1.168		
NH_3 (%) main effects		.737	.879	.930	1.040		
							SEM = .128
H_2O (%) main effects			0	.857			
			.8	.918			
			1.6	.915			

[a]Treatment combination means expressed as a percent of DM are from duplicate silos.

SUMMARY AND CONCLUSIONS

Total nitrogen content of corn silage stored in 210 liter metal drums was increased ($P < .05$) by each addition level of urea, or the various forms of ammonia. Total soluble nitrogen was increased in direct relation to the amount of NPN added. Water insoluble nitrogen (WIN) was not increased by NPN addition in Experiment 1, but there was a general increase ($P < .05$) of WIN with each increment of added ammonia and with increased maturity of the corn plant in Experiment 2. The increased WIN is believed to be due to reduced proteolysis during fermentation. More mature corn silage has less proteolytic enzymes of plant origin and also enduces less bacterial fermentation. Ammonia could act in a similar manner by: 1) Inhibition of plant enzymes by absorption into cellular and extruded cell contents; and 2) Adsorption to insoluble portions of the corn plant to protect against bacterial proteolysis either by direct inhibition of certain bacteria or by serving as a more readily available substrate for bacterial proliferation.

Recovery of ammonia added at the silo blower decreased with advanced silage maturity and increasing amounts of ammonia. Mixing ammonia with water increased uptake when silage dry matter content was in excess of 30%. In practice farmers should mix water with anhydrous ammonia before adding the latter to corn silage above 30% dry matter.

In Experiment 1, lactic acid content of corn silage was dramatically reduced ($P < .01$) by addition of ammonia at .92% of the fresh silage weight. This trend was not as obvious when ammonia was added at the blower in Experiment 2, possibly because of lowered retention of high levels of ammonia when added in this manner. In contrast to the depressing effect of high ammonia levels on lactate production, acetate production was increased suggesting a selective inhibition of lactic acid producing bacteria at this high ammonia concentration. Ammonia at .2 and .4% increased silage lactic acid and in practice would thus enhance preservation of silage. The lactate depressing effect caused by excessive ammonia additions (above .5% of silage fresh weight) would result in a poor preservation of silage and should be avoided in field conditions. Ammonia at lower levels should increase WIN and also reduce spoilage losses (refermentation) especially in silage fed during warm periods of the year.

Mineral and/or disaccharide additives had no consistent influence on silage nitrogen fractions. Final silage lactic acid concentration was elevated by $CaCO_3$ additions especially in combination with ammonia. These results should be considered preliminary because of limited replication. However, it is stimulating to conjecture that $CaCO_3$ and lactose, in certain combinations might be desirable ammendments for ammonia additions to corn silage. $CaCO_3$ might buffer and extend fermentation while lactose and ammonia might serve as preferential substrates to spare plant carbohydrate and protein respectively from degradation during fermentation. More research needs to be conducted in this area with appropriate replication.

PART B. LACTATION AND METABOLISM TRIALS

WITH

NPN-TREATED CORN SILAGES

MATERIALS AND METHODS

Experiment 1. Lactation Trial

Corn silage (36% DM) was ensiled with: 1) no additive; 2), 3) and 4) anhydrous ammonia at .3, .6 and .9%; or 5) and 6) urea at .5 or 1.% of silage fresh weight. Silos were 3.5 X 9.1 m and each held 35 metric tons. Anhydrous ammonia and water were mixed in the mixing apparatus shown in Figure 3. Silage was unloaded into a blower at 572 kg/min, water flowed through the mixing apparatus at 4.6, 9.2 or 13.7 liters/min and ammonia application was 1.7, 3.4, or 5.1 kg/min. Urea was spread over the top of each silage load before unloading at the blower. Silages were stored for 150 days before feeding.

Experimental rations consisted of control corn silage with either 9 or 20% crude protein (CP) concentrate, serving respectively as negative and positive controls compared with the five NPN-treated corn silages fed in conjunction with the 9% CP concentrate. The concentrates described in Table 30 were fed at .4 kg/kg daily milk and uniformly decreased 10% after five weeks of experiment.

Thirty-five lactating Holsteins were blocked into five groups according to milk production during a 3-wk standardization, at which time all cows were fed similarly. Cows from each block were randomly assigned to one of seven different treatment groups which were also balanced for breeding

Table 30. Composition of concentrates[a] and description of rations fed to lactating cows.

Concentrate ingredient	9% CP Percent	20% CP Percent
Ground shelled corn	62.0	43.5
Oats	31.0	21.5
Soybean meal (50% CP)	-	28.0
Dried molasses	4.0	4.0
Dicalcium phosphate	1.0	1.0
Ground limestone	1.0	1.0
Trace-mineral salt	1.0	1.0

Experimental Ration

Silage	Concentrate Crude Protein %
Control	9
Control	20
Ammonia (.3%)	9
Ammonia (.6%)	9
Ammonia (.9%)	9
Urea (.5%)	9
Urea (1%)	9

[a]Concentrates were also fortified with 4,400 and 2,200 IU/kg of vitamins A and D, respectively.

groups. Cows were individually fed silage once daily at 10% in excess of consumption and concentrates were fed twice daily on top of silage. Orts were determined each day, but milk weights were obtained only Monday through Friday. Body weights were taken on two consecutive days after adjustment to experimental ration and at the end of the 10-wk experimental feeding period. Milk fat, protein, and total solids were determined by the Babcock method, micro-Kjeldahl, and oven drying (2 hr at 100° C), respectively.

Blood and rumen samples, taken twice during the last week of experiment, were obtained by caudal puncture and stomach pump, respectively. Blood was collected in vacutainers with anticoagulant, centrifuged at 2,000 X g and the plasma urea determined by Conway microdiffusion (Conway, 1950). Rumen contents were strained through four layers of cheese cloth, and pH[a] of resulting liquor immediately determined. Concurrently, 10 ml of rumen liquor were transferred to a 20 ml plastic vial containing one ml each of 9N H_2SO_4 and a saturated solution of mercuric chloride, and frozen (-20° C) to await, chemical analysis. Rumen ammonia (mg/100 ml strained liquor) was determined by Conway microdiffusion (Conway 1950) and rumen VFA (mg/100 ml) by gas chromatograph as described for Experiment 1, Part A.

Silages were sampled three times each week. Five weekly composites were later combined for dry matter, pH, N

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[a]Beckman Zeromatic SS3

fractionation, lactic acid, and VFA determinations as described for Experiment 1, Part A. Concentrates were sampled periodically as new batches were mixed and dry matter and total nitrogen levels were determined.

Experimental parameters were statistically compared by least squares analysis of variance and partition of individual treatment means by orthogonal contrasts.

Experiment 2. Metabolism Trial

Corn silages treated with .5% urea or .3% anhydrous ammonia from the lactation feeding trial were compared with control corn silage to which equivalent amounts of nitrogen from urea or aqua ammonia were added just prior to feeding. Eight rumen cannulated Holstein steers weighing an average of 245 kg and housed in metabolism stalls at the Beef Cattle Research Center were used for the above comparisons. The experimental design employed two 4 X 4 latin squares with 11-day periods. Steers were weighed at the beginning of the experiment and at the end of each period. Silage dry matter intake was limited to 1.8% of body weight to eliminate orts. All silages were weighed, mixed with minerals at 1% of silage dry weight (Table 31) and where appropriate with either urea or aqua ammonia in a 75 liter horizontal feed mixer[a] before being fed. Water was available to steers at all times.

Urine was collected daily in 19 liter polyethylene bottles containing 50 ml of 6N H_2SO_4. Daily urine volume was

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[a]H.C. Davis Sons Manufacturing Co., Bonner Springs, Kansas

Table 31. Mineral supplement for Holstein steers fed NPN treated corn silage.

Ingredient	Percent
Sodium sulfate (22.5% S)	11.2
Trace mineral salt (high Zn)	21.0
Limestone (38% Ca)	35.0
Defluorinated rock phosphate (32% Ca, 18% P)	31.6
Vitamin A (30,000 IU/g)	.6
Vitamin D (9,000 IU/g)	.6

measured and a 10% aliquot composited for nitrogen determination by macro-Kjeldahl. All feces voided were collected daily, weighed, thoroughly mixed and a 5% aliquot was composited for dry matter, total nitrogen, acid detergent fiber (ADF) and ADF-nitrogen determinations. The latter two analyses were determined after drying at room temperature by the Van Soest method (Van Soest, 1963).

Blood and rumen samples were taken immediately before feeding (0 hr) and at two and four hours after feeding on the last two days of each period. Ammonia, pH and VFA of rumen liquor and blood urea were determined using methods described in Experiment 1, Part B. Silages were composited for each collection period and subjected to analysis for DM content, N fractions, pH, lactic acid, and VFA as described for Experiment 1, Part A.

Experimental parameters were statistically compared by least squares analysis of variance and individual treatment mean differences were partitioned by orthogonal contrasts.

RESULTS AND DISCUSSION

Experiment 1. Chemical composition of corn silages used in the lactation trial are presented in Table 32. Silage pH was increased in direct proportion to the amount of nitrogen added. The increase being greatest ($P<.05$) for .9% added ammonia compared to the control silage. The total nitrogen content of the NH_3 treated silages was not as high as what was calculated to have been added. Possible reasons are: 1) the amount of ammonia added may have been overestimated; and 2) some ammonia delivered to the blower was vaporized and lost, especially at the .9% level of NH_3 addition. However, ammonia at .6 and .9% and urea at 1% did substantially increase ($P<.05$) silage total nitrogen content. There was a trend toward higher water insoluble nitrogen (WIN) for the .6% NH_3 addition similar to that reported in Experiment 2, Part A. Huber (1975) has consistently observed increased WIN with NH_3 additions to corn silage. Lactic acid was increased ($P<.05$) by all NPN additions except the highest level (.9%) of added ammonia. This latter observation is similar to but not as marked as the inhibition of lactic acid production by high levels of ammonia reported in Experiment 1, Part A. Acetic acid was elevated by all nitrogen additions when compared to control silage.

Milk yield (Table 33) was maintained at higher levels ($P<.05$) for cows consuming the positive control ration (no

Table 32. Chemical composition of corn silages fed in lactation trial.[a]

	Percent of N-source addition						
		Urea		Ammonia			
Constituent	Control	.5%	1%	.3%	.6%	.9%	SEM
Dry matter percent	37.0	34.9	34.5	35.5	35.6	36.8	1.26
Silage pH	3.97	4.30	4.55	4.21	4.49	5.14	.29
Nitrogen (% of DM)							
Total	1.47	2.14	2.85	1.90	2.53	2.83	.38
Water soluble	.75	1.50	2.24	1.14	1.53	2.05	.41
Water insoluble	.72	.64	.61	.76	1.00	.78	.16
Lactic acid (% of DM)	2.96	6.86	7.97	7.90	6.72	3.59	1.09
Acetic acid (1% of DM)	1.18	1.98	1.46	1.48	2.07	1.68	.41

[a]Tabular values are means of two 5-wk silage composites.

Table 33. Milk and milk constituent yield and body weight changes of cows fed urea and ammonia treated corn silages.[a]

	Corn Silage Ration							
	Control		Urea		Ammonia			
Milk	Positive	Negative	.5%	1%	.3%	.6%	.9%	SEM
kg/day	20.7	18.6	19.2	19.6	18.7	19.9	18.1	
Persistency (%)	91.1^{c}	81.2^{d}	83.3^{d}	86.5^{d}	86.0^{d}	87.6^{d}	83.6^{d}	2.04
4% FCM								
kg/day	18.8	17.6	17.3	17.4	17.8	17.2	17.0	
Persistency (%)	87.6	85.5	35.2	84.5	89.5	86.8	85.9	3.19
Milk protein								
kg/day	744	585	638	672	639	661	610	
Persistency (%)	98.5^{c}	79.9^{d}	84.5^{d}	92.8^{cd}	84.5^{d}	85.7^{d}	88.4^{d}	2.79
Body weight								
Change (g/day)	212^{c}	122^{c}	472^{d}	201^{c}	232^{c}	302^{c}	472^{d}	47.0

[a]Tabular values represent means of five cows per treatment for 70 days.
[b]Persistency is equal to treatment/pre-treatment X 100.
[c,d]Means not sharing a common superscript are different ($P < .05$).

added NPN) than for cows receiving the negative control, .5% added urea and .9% added ammonia rations. The remaining three silage treatments (1% urea, .3 and .6% NH_3) tended toward ($P < .10$) increased persistency of lactation when compared to the negative control. Milk fat percentages during the 70-day treatment period were higher for those silages with lower milk production, therefore yields of 4% fat-corrected-milk were similar for all treatments. Daily production of milk protein (Table 33) was maintained at higher ($P < .05$) levels for the positive control ration followed by the two highest levels of NPN addition (1% urea and .9% NH_3) to corn silage than for the remaining four silage treatments. Silage and concentrate dry matter intakes were not different (Table 34); therefore, the increased milk protein cannot be explained on the basis of higher energy intake _per se_. Closer examination of Table 34 reveals that more than 50% of the total daily crude protein for the positive control ration came from concentrates. This increase in the proportion of concentrate crude protein could explain the increased milk and protein production on this ration. Possible reasons are: 1) more concentrate protein could bypass the rumen to be digested in the abomasum; and 2) an improvement of protein nutriment of rumen microorganism _via_ increased essential amino acids and/or carbon skeletons for microbial protein synthesis. The greater concentration ($P < .05$) of ruminal acetic, butyric and total VFA for cows on the positive control ration, Table 35, suggests enhanced rumen microbial production. It is of

Table 34. Intake of dry matter and crude protein equivalent as affected by NPN treatment of corn silage.[a]

	Corn silage ration							
	Control		Urea		Ammonia			
	Positive	Negative	.5%	1%	.3%	.6%	.9%	SEM
DM intake (kg/day)								
Silage	11.73	10.42	11.63	10.82	11.49	10.66	11.15	.35
Concentrate	6.26	6.44	.631	6.28	6.13	6.38	6.01	
Total	17.99	16.86	17.94	17.10	17.62	17.04	17.16	
(% of body weight)								
Silage	1.90	1.75	1.90	1.69	1.87	1.80	1.87	.033
Concentrate	1.02	1.08	1.03	.99	1.01	1.10	1.01	
Total	2.92	2.83	2.93	2.68	2.88	2.90	2.88	
Crude protein intake (kg/day)								
Silage	1.07	.95	1.53	1.92	1.36	1.67	1.98	.061
Concentrate	1.19	.63	.61	.61	.60	.62	.59	
Total	2.26	1.58	2.14	2.53	1.96	2.29	2.57	

[a]Tabular values represent means from five cows for 70 days.

interest to note that blood urea-N values (Table 35) were highest ($P < .01$) for the three rations which elicited the highest milk protein production. The crude protein of these same rations was also higher. The NPN in milk was not analyzed separately so it is possible that some of the increase in milk crude protein was due a higher NPN uptake by the mammary gland. Another explanation might be greater recycling of urea back into the rumen to provide a more constant and adequate supply of nitrogen for the rumen microbes.

The negative control ration contained 9.4% crude protein and was obviously inadequate to sustain high levels of milk production. It did provide a "bench mark" for assessing the value of added NPN to corn silage rations in this experiment. Milk and milk protein yields were higher at all levels of NPN addition than those of the negative control. There were no detectable differences in milk yield due to source of NPN; however, cows consuming the higher levels of added nitrogen tended toward higher milk yields. One might be concerned about the relatively high pH value for silage treated with .9% NH_3 (5.14); however voluntary silage consumption was not adversely affected (Table 34). Other workers (Huber _et al._, 1973c; Huber, 1974; Huber and Santana, 1972) have demonstrated milk yields from ammonia treated silages comparable to cows consuming iso-nitrogenous corn silage rations that were supplemented with preformed protein. Also ammonia has nearly always been superior to urea as a NPN additive to corn silage.

Body weight gains (Table 33) were highest ($P < .01$)

Table 35. Effect of urea and ammonia additions to corn silage on rumen and blood parameters.[a]

Parameter	Corn silage ration							SEM
	Control		Urea		Ammonia			
	Positive	Negative	.5%	1%	.3%	.6%	.9%	
Rumen liquor								
pH	6.60[b]	6.86[c]	7.04[c]	6.74[c]	7.00[c]	6.88[c]	6.97[c]	.11
Acetic acid, mg %	335[b]	295[c]	296[c]	293[c]	291[c]	318[c]	268[c]	14
Propionic acid, mg %	120	117	111	111	115	133	99	8.1
Butyric acid, mg %	106[b]	88[c]	77[c]	83[c]	77[c]	83[c]	81[c]	5.8
Total acid, mg %	577[b]	517[c]	493[c]	496[c]	490[c]	545[c]	468[c]	26
Blood urea-N mg %	16.0[b]	8.3[c]	9.9[c]	15.5[b]	9.94[c]	11.7[c]	15.7[b]	1.7

[a]Tabular values represent means from five cows per silage, three hr postprandial on two different days of last week of experiment.
[b,c]Means not sharing a common superscript are significantly different ($P < .05$).

for cows consuming corn silages treated with .5% urea and .9% NH_3, than for other rations. Persistency of milk production for these two rations was lower than for the other N-supplemented rations, suggesting that cows consuming these rations were utilizing more energy for growth and fattening than for milk production. The lowest weight gain and the lowest milk production for cows consuming the negative control ration suggests that 9% crude protein is too low for optimum digestibility and/or utilization of the digested energy.

Rumen fermentation parameters are presented in Table 35. The pH of the rumen liquor and ruminal concentrations of acetic, butyric and total volatile fatty acids (VFA) were greater ($P < .05$) for cows on the positive control ration than for the other rations. This ration contained higher levels of natural or preformed protein which probably enhance overall rumen fermentation. Propionic acid levels were higher ($P < .05$) for .6% NH_3 than for cows consuming silage with .9% added NH_3. There is no apparent explanation for this higher level except that acetic was also higher for .6% than .9% added NH_3 suggesting enhanced fermentation within ammonia addition for the .6% level. Total ruminal VFA was high for the .6% NH_3 silage ration. This silage contained the highest content of water insoluble nitrogen and consequently more natural protein than other NPN treatments and this may have stimulated VFA production.

Ruminal ammonia concentration for cows fed the various control, urea and ammonia treated corn silage rations cannot be reported. Unfortunately, ammonia levels were either not detectable in the laboratory or they were extremely erratic. This was true for both days of sampling. Apparently, rumen samples may have been contaminated with saliva during the process of obtaining them by stomach pump. A ten ml aliquot of strain rumen liquor had been immediately mix with one ml each of 9 N H_2SO_4 and a saturated solution of mercuric choride, but ammonia losses still occurred. This failure in ammonia analysis could also have been caused by faulty sample handling or improperly mixed or weak reagents.

Blood plasma urea nitrogen concentrations for the positive control and the two highest levels of silage NPN supplementation were greater ($P<.05$) than for the other treatments. A positive linear relationship was shown between the amount of NPN added to silage and plasma urea nitrogen. Also, the positive control ration (12.6% crude protein) caused similar elevations in plasma urea nitrogen to rations containing corn silage treated with 1% urea or .9% ammonia (14.8 or 15.0% crude protein).

Experiment 2. Chemical composition of the four silages fed in the metabolism trial are presented in Table 36. Total N tended to be lower ($P<.10$) and WIN was lower ($P<.05$) for urea than for NH_3 added at ensiling to corn silage. Ammonia added just prior to feeding did significantly increase ($P<.05$) the pH of corn silage. This increase in pH was similar to what has been reported by Huber et al. (1974) but not as marked as that reported by Wilkinson and Huber (1975).

Table 36. Chemical composition of silages fed to fistulated steers (Two 4 X 4 latin Squares).

	Corn silage				
	N added at ensiling		N added at feeding		
Constituent[a]	NH_3	Urea	NH_3	Urea	SEM
Dry matter	38.34	38.81	36.63	36.60	1.27
pH	4.42	4.23	5.20	3.98	.23
Lactic acid	6.96	8.69	6.89	8.60	.86
Total nitrogen	2.136	1.940	2.218	2.029	.086
Total soluble nitrogen	.946	1.239	1.434	1.224	.113
Water insoluble nitrogen	1.190	.701	.785	.805	.062
Acid detergent fiber	26.68	26.95	25.38	24.64	.64

[a]Tabular values are means for one silage composite per period and are expressed as a percent of dry silage (except pH and dry matter).

Silage dry matter intake was held constant at 1.8% of body weight and was by design equal for all treatments (Table 37). The ration usually was completely consumed by 8-10 hr postprandial. The silage to which ammonia was added at feeding was generally consumed more slowly than the other silages. Nitrogen intake was calculated to be equal but chemical analysis (Table 36) revealed lower average nitrogen contents for corn ensiled with ammonia and that treated with urea at feeding. Hence, somewhat lower ($P < .25$) nitrogen, and ADF digestibilities were lower ($P < .05$) for silages ensiled with ammonia and urea than for silage to which NPN was added at feeding. The reason for this difference is not readily apparent since intakes were not different. Dry matter digestibilities were lower than those reported by Wilkinson and Huber (1975) and those generally expected for corn silage (Bryant _et al._, 1966; Huber _et al._, 1965; Johnson and McClure, 1968). The range in N digestibility for this experiment is similar to that reported by other researchers (Cash, 1972; Wilkinson and Huber, 1975) using fistulated steers. Huber (1975) observed lower ADF digestibility of NPN treated corn silage compared to control silage. Body weight changes were considered to be of little significance since the periods were of short duration and silage intake was also restricted.

Nitrogen balance values tended to be lower ($P < .25$) for silages ensiled with NPN than for NPN added at feeding time. Urinary nitrogen as a percent of ingested nitrogen was lowest ($P < .10$) for corn silage ensiled with ammonia, while

Table 37. Intake digestibility and nitrogen balance obtained from fistulated steers fed corn silage treated at ensiling or at feeding with either ammonia or urea.[a]

	Corn silage				
	N added at ensiling		N added at feeding		
	NH_3	Urea	NH_3	Urea	SEM
Intake					
Dry matter (kg/d)	4.6	4.7	4.6	4.5	.92
Nitrogen (g/d)	89.2	100.2	101.0	91.5	23
Digestibility (%)					
Dry matter	56.5	55.5	61.0	61.6	1.71
Nitrogen	48.3	54.7	61.7	60.4	2.91
ADF	39.2	36.6	43.0	42.7	2.56
Body weight change (g/d)	496	131	220	287	228
N-balance (g/d)	14.8	15.7	20.2	20.5	3.34
Urinary N/feed N (%)	33.5	39.3	39.7	38.0	1.94
Fecal N/feed N (%)	51.7	45.3	38.3	39.6	2.86
Fecal N/excreted N (%)	60.5	53.5	48.9	51.0	2.12
Retained B/absorbed N (%)	28.2	27.8	31.0	36.5	5.82

[a]Tabular values are means from eight steers.

fecal nitrogen as a percent of ingested nitrogen was highest ($P<.05$) for silages ensiled with NPN. Route of nitrogen excretion was different for time of NPN addition. Urinary nitrogen as a percent of excreted nitrogen tended to be lower ($P<.10$) and fecal nitrogen as a percent of excreted nitrogen was higher ($P<.05$) for silages ensiled with NPN. The nitrogen retained as a percent of that absorbed was not different ($P<.25$) even though there was a tendency for more N-retention from the silages treated with NPN at feeding.

Rumen parameters are presented in Table 38. Strained rumen liquor pH was unaltered by silage treatment. There was a consistent decrease in pH with time after feeding corresponding to the general increase in ruminal VFA concentration. Acetic acid content of rumen liquor seemed to be lower ($P<.25$) on all samplings for corn silage to which ammonia or urea were added at feeding. This may have been due to steers not consuming those silages as rapidly as the silages ensiled with NPN. Compared to zero hr, overall ruminal acetic acid levels were 46 and 60% higher two and four hr postprandial, respectively. Ruminal butyric acid tended to be higher at two hr postprandial ($P<.25$) for silages treated with ammonia or urea at feeding, and ammonia added at feeding did elicite somewhat higher ($P<.10$) ruminal butyrate than urea. Ruminal valerate levels were increased with time after feeding, but even at four hr postprandial valerate accounted for less than three percent of the total ruminal VFA. There was a consistent increase in all of the individual VFA of ruminal liquor

Table 38. Rumen liquor and blood plasma parameters of fistulated steers fed corn silage treated at ensiling or at feeding with either ammonia or urea.

		Corn silage				
	Hours	N added at ensiling		N added at feeding		
Rumen liquor[a]	Postprandial	NH_3	Urea	NH_3	Urea	SEM
pH	0	7.13	7.06	7.08	7.15	.077
	2	6.34	6.45	6.50	6.41	.069
	4	5.80	5.92	5.92	5.87	.087
Acetic acid	0	263	284	233	233	18.5
	2	401	393	348	340	21.8
	4	417	447	361	394	24.0
Propionic	0	78	95	70	73	8.3
	2	173	172	158	144	14.1
	4	185	202	193	171	13.9
Butyric acid	0	34	43	41	33	6.3
	2	104	93	129	102	15.6
	4	158	156	206	185	24.9
Valeric acid	0	1	3	1	2	1.59
	2	9	10	13	11	4.23
	4	11	19	19	33	8.39
NH_3	0	-	-	-	-	-
	2	14.9	16.2	23.8	21.4	3.56
	4	8.5	8.8	23.4	14.1	3.11
Plasma Urea-N[a]	0	6.7	7.9	6.5	6.7	.58
	2	10.6	13.4	10.4	11.1	.86
	4	11.0	13.6	13.7	12.8	.92

[a]All tabular values except pH are expressed as mg/100 ml and are means from eight steers.

with time after feeding. The total VFA concentration at four hr postprandial was twice that at zero hr. Rumen acetate at all samplings was higher ($P<.05$) and rumen butyrate at four hr postprandial tended to be lower ($P<.25$) for silages treated with NH_3 or urea at ensiling compared to addition at time of feeding.

Rumen NH_3-N was too low to be detected just prior to feeding. This might be due to the feeding of steers only once daily and to limiting DM intake to 1.8% of body weight. Both ammonia and urea ensiled with corn silage elicited less ($P<.01$) ruminal ammonia at two and four hr postprandial than NPN additions to control silage at feeding. This lowering of rumen NH_3 should benefit the host animal by allowing a more constant supply of NH_3 to be released for rumen microbial protein sythesis. However, with only two samplings postprandial it is difficult to draw definitive conclusions concerning possible N utilization. Blood urea-N was lowest ($P<.05$) four hr postprandial for corn silage treated with NH_3 at ensiling which might indicate better utilization of NPN or may simply be a reflection of lower nitrogen intake.

Obviously, the digestibility and nitrogen balance data indicate no advantage of treating silage with NPN at ensiling, or any advantage of NH_3 compared to urea. In fact, the data suggest the reverse is true. On the ensiled treatments lower protein and ADF digestibilities accounted for about 50% of the decreased dry matter digestibility. Hence, a decrease in the digestibility of the starch fraction must have also occurred. Silages were harvested from the same field but there may have been differences in ensiling conditions and rate of removal from the silo. Unfortunately, the supply of control silage was exhausted during the third period and a different control silage was used for the remainder of the experiment. The supply of corn silage treated with NH_3 and urea was also limited and had to be frozen prior to the beginning of period four and thawed at feeding. These procedural modifications did not seem to influence dry matter digestibility, because results for the fourth period were similar to the previous periods.

Even though digestibility was depressed by ensiling with NPN, utilization of digested energy is obviously not changed as demonstrated, by Wilkinson and Huber (1975). These researchers added NH_3 at ensiling or at feeding in a growth study with 235 kg male Holstein calves. Live weight gain tended to be greater and plasma urea-N at four hr postprandial lower ($P < .01$) for NH_3 added at ensiling.

SUMMARY AND CONCLUSIONS

Corn silage pH and lactic acid concentrations were simultaneously increased by NPN additions in the lactation trial. This observation strengthens the hypothesis that ammonia (and urea) extends fermentation by formation of the ammonium salts of lactic and acetic acid. Water insoluble nitrogen was higher for the .6% added NH_3 than for the control and urea treated silages. The practical significance of this recurring phenomenon has not been fully exploited but it should allow for greater utilization of NPN in dairy cattle rations by: 1) reducing proteolysis of the natural corn plant protein; and 2) permitting higher levels of NPN to be fed, i.e. more urea might be included in the concentrate with ammonia than with urea treated silages before the threshold of excessive ration NPN were reached.

Milk yield, expressed as persistency of lactation, was highest for the positive control ration, lowest for the negative control and intermediate for the NPN supplemented rations. When expressed as 4% fat-corrected milk there were no differences in yield. In practice, therefore, to reduce feed costs, ammonia should be more extensively used to augment crude protein and water-insoluble nitrogen levels of corn silage rations for dairy cattle. Rumen fermentation parameters suggest enhanced VFA production for the positive (soybean meal) control and .6% NH_3 supplemented corn silage rations. Plasma urea nitrogen levels were highest and similar (15.7 mg%) for the positive control and the highest levels of urea and ammonia silage supplementation, even though the latter rations contained 20% more crude protein.

Silages fed in the metabolism trial were the control, .5% urea and .3% NH_3 silages of the lactation trial. Chemical analysis of four composited samples per silage revealed 60% more ($P < .05$) water insoluble nitrogen for NH_3 than urea treatment at ensiling. Nitrogen balance and digestibility parameters tended to be lower for those silages ensiled with NPN. This observation does not agree with previous studies and more research is needed in this are. Experiments that do not restrict silage intake and that are of longer duration are necessary.

APPENDICES

Appendix Table 1. Effect of source of nitrogen, disaccharide or mineral additive and day of fermentation on temperature (°C) of corn silage.

Nitrogen[a] source	Disaccharide[b] or mineral	Days of fermentation[c]							SEM
		1	3	5	10	15	20	40	
Control	Control	20.8	18.5	15.0	11.9	7.9	9.4	7.8	
	$CaCO_3$	20.0	23.0	15.0	11.8	7.8	9.5	8.3	
	$NaHCO_3$	20.0	19.0	15.5	12.0	8.2	9.5	8.5	
	Sucrose	22.0	19.5	15.0	11.8	8.8	9.8	7.5	
	Lactose	22.0	19.0	15.5	11.7	7.8	9.4	7.0	
	NaCl	22.0	18.2	15.5	11.6	8.8	9.4	8.0	
	$CaCO_3$:								
	Sucrose	-	-	-	-	-	-	-	
	Lactose	-	-	-	-	-	-	-	
Urea	Control	20.8	19.0	14.2	12.0	8.3	9.7	7.5	
	$CaCO_3$	20.0	21.0	15.0	12.0	8.0	9.5	8.5	
	$NaHCO_3$	20.5	20.0	15.5	11.5	8.0	9.5	8.0	
	Sucrose	23.0	19.2	16.0	11.6	8.5	9.9	7.0	
	Lactose	22.0	19.0	15.3	11.8	8.6	9.8	8.0	
	NaCl	22.0	18.5	15.5	11.8	7.8	9.7	7.5	
	$CaCO_3$:								
	Sucrose	23.0	19.0	16.0	12.2	8.0	9.8	8.0	
	Lactose	22.0	20.0	16.0	11.6	8.1	9.2	6.5	

(Table continued on next page)

Appendix Table 1. Continued.

Nitrogen source[a]	Disaccharide or mineral[b]	Days of fermentation[c] 1	3	5	10	15	20	40	SEM
Aqua NH_4	Control	20.5	18.6	14.5	12.2	8.2	9.8	7.5	
	$CaCO_3$	19.5	18.0	15.0	12.0	8.2	9.2	8.5	
	$NaHCO_3$	19.5	22.0	15.5	12.5	8.4	9.6	8.0	
	Sucrose	20.0	20.0	15.0	12.0	8.2	9.7	7.0	
	Lactose	21.0	19.0	15.0	12.5	8.6	10.0	7.5	
	NaCl	22.0	21.0	14.8	12.0	8.0	9.4	7.5	
	$CaCO_3$								
	Sucrose	20.0	19.5	17.0	12.8	8.8	10.0	7.5	
	Lactose	20.0	19.5	16.5	12.0	8.6	9.4	7.0	
NH_4-Sol	Control	21.0	19.5	15.0	12.5	9.1	9.4	7.5	
	$CaCO_3$	20.0	19.5	15.0	12.5	9.1	9.4	7.5	
	$NaHCO_3$	19.5	19.5	15.0	11.6	8.1	9.6	9.0	
	Sucrose	21.5	20.0	16.5	12.2	8.8	10.2	7.5	
	Lactose	21.0	19.5	16.0	12.0	8.0	9.4	7.0	
	NaCl	20.5	19.5	16.0	11.8	8.4	9.8	8.0	
	$CaCO_3$:								
	Sucrose	21.5	19.0	16.0	11.8	8.0	9.2	7.0	
	Lactose	20.0	19.5	16.5	13.8	9.3	11.0	8.0	
								(Day)	.939
								(N-source & additive)	.444

[a]Nitrogen from the three NPN sources was added at .2% of silage fresh weight.
[b]Each mineral or disaccharide additive included at 1% of silage fresh weight (except NaCl at .5%).
[c]Each treatment combination value represents one observation (except values for contraols are means from two silos).

Appendix Table 2. Effect of $CaCO_3$ plus sucrose or lactose and three sources of nitrogen on pH and lactic acid content of corn silage.

Experimental parameter[b]	$CaCO_3$+[a]	Day of fermentation	Nitrogen source: Urea	Aqua NH_4	NH_4-sol	SEM
pH	Sucrose	0	6.05	8.26	7.70	
		3	5.51	6.22	5.26	
		15	5.07	5.17	5.09	
		40	4.90	4.80	4.55	
	Lactose	0	6.20	8.23	7.80	
		3	5.32	6.18	5.77	
		15	5.12	5.62	5.13	
		40	4.74	4.64	4.61	
					(day)	.520
					(N-source & additive)	.400
Lactic acid	Sucrose	0	.00	.00	.00	
		3	2.42	1.83	1.90	
		15	.00	4.31	8.44	
		40	6.07	6.71	5.84	
	Lactose	0	.00	.00	.00	
		3	2.37	1.44	2.06	
		15	2.43	8.74	6.76	
		40	6.77	7.92	7.76	
					(day)	1.88
					(N-source & addition)	2.54

[a]Each additive incorporated at .1% of silage fresh weight (nitrogen at .2%)
[b]Tabular values are from one experimental silo and all except pH are expressed as a percent of silage dry matter.

Appendix Table 3. Effect of $CaCO_3$ plus sucrose or lactose and three sources of nitrogen on acetic and total organic acid content of corn silage.

Experimental parameter[b]	$CaCO_3$+[a]	Day of fermentation	Nitrogen source: Urea	Nitrogen source: Aqua NH_4	Nitrogen source: NH_4-sol	SEM
Acetic acid	Sucrose	0	.33	.61	.50	
		3	.85	.68	.61	
		15	1.37	1.26	1.40	
		40	2.84	3.09	2.35	
	Lactose	0	.31	.65	.61	
		3	.81	.78	.75	
		15	.86	1.78	1.44	
		40	2.34	2.75	2.59	
					(day)	.431
					(N-source & addition)	.303
Total organic acids	Sucrose	0	.33	.61	.50	
		3	3.27	2.83	2.51	
		15	1.52	5.57	9.84	
		40	8.91	9.80	8.19	
	Lactose	0	.31	.65	.61	
		3	3.18	2.22	3.11	
		15	3.29	10.70	8.20	
		40	9.11	10.67	10.35	
					(day)	2.56
					(N-source & addition)	2.76

[a]Each additive incorporated at .1% of silage fresh weight (nitrogen at .2%).
[b]Tabular values are from one experimental silo and all except pH are expressed as a percent of silage dry matter.

Appendix Table 4. Effect of $CaCO_3$ plus sucrose or lactose and three nitrogen sources on dry matter percent and total nitrogen content of corn silage.

Experimental parameter[b]	$CaCO_3$+[a]	Day of fermentation	Nitrogen source: Urea	Aqua NH_4	NH_4-sol	SEM
Dry matter (percent	Sucrose	0	38.90	40.30	36.30	
		3	36.29	36.09	37.43	
		15	36.48	35.89	37.45	
		40	39.40	35.60	35.30	
	Lactose	0	39.20	39.40	38.20	
		3	37.16	38.22	37.62	
		15	36.17	38.24	34.52	
		40	37.20	37.10	37.90	
						(day) 1.31
						(N-source & additive) .731
Total nitrogen	Sucrose	0	1.781	1.589	1.801	
		3	1.914	1.798	1.798	
		15	1.880	1.772	1.839	
		40	1.954	1.705	1.841	
	Lactose	0	1.612	1.683	1.713	
		3	1.805	1.692	1.712	
		15	1.827	2.080	1.726	
		40	1.855	1.814	1.651	
						(day) .257
						(N-source & addition).356

[a]Each additive incorporated at 1% of silage fresh weight (nitrogen at .2%).
[b]Tabular values are from one experimental silo and all except dry matter (%) are expressed as a percent of silage dry matter.

Appendix Table 5. Effect of $CaCO_3$ plus sucrose or lactose and three nitrogen sources on total soluble and water insoluble nitrogen content of corn silage.

Experimental parameter[b]	$CaCO_3$+[a]	Day of fermentation	Nitrogen source: Urea	Aqua NH_4	NH_4-sol	SEM
Total soluble N	Sucrose	0	.972	.762	1.115	
		3	.936	.788	.928	
		15	.828	.674	.603	
		40	.810	.774	.692	
	Lactose	0	.954	.810	.783	
		3	.824	.794	.754	
		15	.708	.800	.684	
		40	.910	.807	.707	
					(day)	.149
					(N-source & addition)	.290
Water insoluble N	Sucrose	0	.809	.827	.686	
		3	.978	1.010	.870	
		15	1.060	1.098	1.236	
		40	1.144	.930	1.149	
	Lactose	0	.658	.873	.930	
		3	.980	.898	.958	
		15	1.119	1.280	1.042	
		40	.945	1.007	.944	
					(day)	.257
					(N-source & addition)	.475

[a]Each additive incorporated at 1% of silage fresh weight (nitrogen at .2%).
[b]Tabular values are from one experimental silo and all except dry matter (%) are expressed as a percent of silage dry matter.

Appendix Table 6. Percent ammonia recovery as affected by silage dry matter and levels of added NH_3 and H_2O.[a]

Dry matter	H_2O%	Ammonia level (%) 0	.24	.48	.72	H_2O avg	DM% main effects
28%	0	-	99.0	88.2	75.6	87.6	
	.8	-	116.9	99.3	76.2	97.5	
	1.6	-	95.2	96.9	74.2	88.8	
							91.3
	NH_3 avg	-	103.7	94.8	75.3		
36%	0	-	57.1	56.2	52.2	55.2	
	.8	-	110.9	66.7	56.5	78.0	
	1.6	-	94.2	55.9	627	70.9	
							68.0
	NH_3 avg	-	87.4	59.6	57.1		
39%	0	-	46.1	55.3	45.4	48.9	
	.8	-	78.1	73.9	63.3	71.9	
	1.6	-	40.0	69.1	70.7	59.9	
							60.2
	NH_3 avg	-	54.7	66.1	59.9		
46%	0	-	56.2	35.0	32.3	41.2	
	.8	-	26.8	30.8	28.3	28.6	
	1.6	-	114.9	98.5	67.9	93.8	
							54.5
	NH_3 avg	-	66.0	54.8	42.8		
NH_3 (%) main effects		-	78.0	68.6	58.8		

H_2O (%) main effects		
	0	58.2
	.8	69.0
	1.6	78.4

[a]Tabular values for each treatment combination (t.c.) were estimated from Tables 26 and 27 using the formula O/E X 100: where, O = the observed t.c. increase in nitrogen and E = the expected t.c. increase in nitrogen assuming 100% recovery of the added N.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abfarowicz, F., U. Grzeszczak-Swietlikowska and A. Truszynski. 1963. An attempt to appraise the value of maize silage with addition of nonprotein nitrogen substances - urea, ammonium sulfate and ammonia water. Agr. Sci. Ann. 81-8: 695 (Translated from Polish).

Annett, H.E. and E.J. Russell. 1908. The composition of green maize and of the silage produced therefrom. J. Agr. Sci. 2:392.

Baker, R.F. 1971. Black layer development - one way to tell when your corn is mature. Crops Soils 24:8.

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

Barnett, A.J.G. 1954. Silage fermentation. Academic Press, Inc., New York.

Beattie, D. 1970. The effect of adding a liquid suspension of anhydrous ammonia, minerals and molasses to corn silage at ensiling, on fermentation parameters and beef cattle performance. M.S. Thesis. Michigan State University, East Lansing.

Bechdel, S.I. 1926. Quality of silage for milk production as affected by stage of maturity of corn. Penn. Agr. Exp. Sta. Bull. 207.

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

Bothast, R.J., G.H. Adams, E.E. Hatfield, and E.B. Lancaster. 1975. Preservation of high-moisture corn: A microbial evaluation. J. Dairy Sci. 58:386.

Bothast, R.J., E.B. Lancaster, and C.W. Hesseltine. 1973. Ammonia kills spoilage mold in corn. J. Dairy Sci. 56:241.

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

Britt, D.G. 1973. Effect of organic acids and non-protein-nitrogen on fungal growth, nutritive value, fermentation, and refermentation of corn silage and high moisture corn. Ph.D. Thesis. Michigan State University, East Lansing.

Bryant, H.T., R.E. Blaser, R.C. Hammes and J.T. Huber. 1966. Evaluation of corn silage harvested at two stages of maturity. Agronomy J. 58:253.

Buck, G.R., W.G. Merrill, C.E. Coppock and S.F. slack. 1969. Effect of recutting and plant maturity on kernel passage and feeding value of corn silage. J. Dairy Sci. 52:1617.

Byers, J.H. and E.E. Ormiston. 1964. Feeding value of mature corn silage. J. Dairy Sci. 47:707 (Abstr.).

Byers, J.R. and E.E. Ormiston. 1966. Feeding value of corn silage made at three stages of development. J. Dairy Sci. 49:741 (abstr.).

Cash, E.H. 1972. Relationship of silage fermentation and additives to dry matter consumption by ruminants. Ph.D. Thesis. Michigan State University. East Lansing.

Conrad, H.R. and J.W. Hibbs. 1961. Urea treatment affects utilization of corn silage. Ohio Farm and Home Res. 45:13.

Conrad, H.R. and J.W. Hibbs. 1964. Use care when feeding urea. Ohio Farm and Home Res. 49:52.

Conrad, H.R. and J.W. Hibbs. 1967. Urea-treated silage for dairy cows. Ohio Rep. Res. Div. 52:59.

Coppock, C.E. 1969. Symposium: Dairy cattle feeding. Problems associated with all corn silage feeding. J. Dairy Sci. 52:848.

Coppock, C.E. and J.B. Stone. 1968. Corn silage in the ration of dairy cattle: A review. N.Y. State College of Agr. Misc. Bul. 89.

Conway, E.I. 1950. Microdiffusion analysis and volumetric error. Crosby Lockwood and Son Ltd., London.

Cullison, A.E. 1944. The use of urea in making silage from sweet sorghum. J. Anim. Sci. 3:59.

Daynard, T.B. 1972. Relationships among black layer formation, grain moisture percentage, and heat unit accumulation in corn. Agron. J. 64:716.

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

Dexter, S.T., C.F. Huffman and E.J. Benne. 1959. Physical, chemical and biological principles in silage making. Proc. Michigan State University Silage Conference. p. 31.

Dox, A.W. and R.E. Neidig. 1913. Lactic acid in corn silage. Iowa Agr. Exp. Sta. Res. Bul. 10.

Dox, A.W. and R.E. Neidig. 1912. The volatile aliphatic acid of corn silage. Iowa Agr. Exp. Sta. Res. Bul. 7.

Geasler, M.R. 1970. The effect of corn silage maturity, harvesting techniques and storage factors on fermentation parameters and cattle performance. Ph.D. Thesis. Michigan State University, East Lansing.

Goffart, M.A. 1877. The ensilage of maize, and other green fodder crops. Trans. and Pub. by J.B. Brown. New York.

Gordon, C.H. 1967. Symposium: Storage losses in silage as affected by moisture content and structure. J. Dairy Sci. 50:397.

Gordon, C.H., J.C. Derbyshire and P.J. Van Soest. 1968. Normal and late harvesting of corn for silage. J. Dairy Sci. 51:1259.

Hanway, J.J. 1966. How a corn plant develops. Iowa State Univ. Spec. Rep. No. 48.

Hawkins, D.R. 1969. The effect of dry matter levels of alfalfa silage on intake and metabolism in the ruminant. Ph.D. Thesis. Michigan State University, East Lansing.

Hillman, D., C.N. Hansen, E. Linden, T. Bowerman, P. Thompson, L. Thompson and K. Sowerby. 1973. Protein content of corn silage treated with ammonia on Michigan farms. Dairy Notes: July, Cooperative Ext. Ser. Michigan State University, East Lansing.

Hooper, T.H. 1925. Composition and maturity of corn. N. Dakota Agr. Exp. Sta. Bul. 192.

Huber, J.T. 1975. Feeding NPN and NPN-treated silage to dairy cows. 2nd Annual Silage Symposium. Ann Arbor, Mich.

Huber, J.T. 1971. The nutritive value for lactating dairy cows of corn silage harvested at different maturities and treated with urea. Proc. 6th Internatl. Zootec. Sum. Milan, Italy.

Huber, J.T., N.A. Dutrow, D.G. Britt, R. Ledebuhr and C.M. Hansen 1973a. Addition of anhydrous ammonia to corn silage. Dairy Notes: Aug. Cooperative Ext. Er. Mich. State Univ., East Lansing.

Huber, J.T., G.C. Graf, and R.W. Engel. 1965. Effect of maturity on nutritive value of corn silage for lactating cows. J. Dairy Sci. 48:1121.

Huber, J.T., R.E. Lichtenwalner and D.G. Britt. 1973b. Acid and urea additions to high dry matter corn silage for lactating cows. J. Anim. Sci. 37:296.

Huber, J.T., R.E. Lichtenwalner, and H.E. Henderson. 1974. Direct addition of ammonia solutions to corn silage fed to dairy cattle. J. Dairy Sci. 57:263.

Huber, J.T., R.E. Lichtenwalner, D.D. Makdani, and H.E. Henderson. 1972. Influence of various organic acids on silage fermentation. J. Anim. Sci. 35:230.

Huber, J.T., R.E. Lichtenwalner and J.W. Thomas. 1973c. factors affecting response of lactating cows to ammonia-treated corn silage. J. Dairy Sci. 56:1283.

Huber, J.T. and O.P. Santana. 1972. Ammonia-treated corn silage for dairy cattle. J. Dairy Sci. 55:489.

Huber, J.T. and J.W. Thomas. 1971. Urea-treated corn silage in low protein rations for lactating cows. J. Dairy Sci. 54:224.

Huber, J.T., J.W. Thomas and R.S. Emery. 1968. Response of lactating cows fed urea-treated corn silage harvested at varying stages of maturity. J. Dairy Sci. 51:1806-1810.

Huffman, C.F. and C.W. Duncan. 1954a. Corn silage as a source of the unidentified grain factor(s) needed for milk production. Mich. Agr. Exp. Sta. Quart. Bul. 37:23.

Huffman, C.F. and C.W. Duncan. 1954b. The nutritive value of corn silage for milking cows. J. Dairy Sci. 37:957.

Huffman, C.F. and C.W. Duncan. 1956. Comparison of silages made from field corn (Ohio M 15) and silage corn (Eureka) for milk production. J. Dairy Sci. 39:998.

Huffman, C.F. and C.W. Duncan. 1959. Corn kernels in feces of dairy cattle fed corn silage. Mich. Agr. Exp. Sta. Quart. Bul. 41:539.

Huffman, C.F. and C.W. Duncan. 1960. Chemical composition, coefficients of digestibility, and total digestible nutrient content of corn silages. Mich. Agr. Exp. Sta. Quart. Bul. 43:261.

Hughes, A.D. 1970. The non-protein nitrogen composition of grass silages. II The changes occurring during the storage of silage. J. Agr. Sci. 75:421.

Hunter, C.A. 1921. Bacteriological and chemical studies of different kinds of silage. J. Agr. Res. 21:767.

Jenkins, M.R. 1941. Influence of climate and weather on the growth of corn in USDA Yearbook of Agriculture. U.S. Govt. Printing Office. Washington, D.C.

Johnston, J.F.W. 1843. On the feeding qualities of the natural and artificial grasses in different states of dryness. High. Agr. Soc. Scotland. Trans. L:57.

Johnson, R.R., T.L. Bolwani, L.J. Johnson, K.E. McClure and B.A. Dehority. 1966. Corn plant maturity. II Effect on in vitro cellulose digestibility and soluble carbohydrate content. J. Anim. Sci. 25:612.

Johnson, R.R. and K.E. McClure 1968. Corn plant maturity. IV. Effects on digestibility of corn silage in sheep. J. Animal Sci. 27:535-540.

Johnson, R.R., K.E. McClure, L.J. Johnson, E.W. Klosterman and G.B. Triplett. 1966. Corn plant maturity. I. Changes in dry matter and protein distribution in corn plants. Agron. J. 58:151.

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

Jorgensen, N.A. and J.W. Crowley. 1972. Corn silage for Wisconsin cattle. Production, harvesting, storage, use in dairy rations. Coop. Ext. Programs A1178, University of Wisconsin, Madison.

Kempton, A.G. 1958. Bacterial, biochemical and environmental interrelations in fresh and ensiled forages. Ph.D. Thesis. Michigan State University, East Lansing.

Klosterman, E.W. 1970. Urea for beef cattle. Ohio Report Res. Dev. 55:111.

Klosterman, E.W., L.J. Johnson and R.R. Johnson. 1965. Complete silage makes economical beef ration. Ohio Report Res. Dev. 50:75.

Knott, F.N., C.E. Polan and J.T. Huber. 1972. Further observations on utilization of urea by lactating cows. J. Dairy Sci. 55:466.

Lichtenwalner, R.E., J.T. Huber and C.N. Hansen. 1972. Effect of form of ammonia addition to corn silage. J. Dairy Sci. 55:709 Abst.

Lopez, J., N.A. Jorgensen, H.J. Larsen, and R.P. Niedermeier. 1970a. Effect of nitrogen source, stage of maturity, and fermentation time of pH and organic acid production in corn silage. J. Dairy Sce. 53:1225.

Lopez, J., N.A. Jorgensen, R.P. Niedermeier, and H.J. Larsen. 1970b. Redistribution of nitrogen in urea-treated and soybean meal-treated corn silage. J. Dairy Sci. 53:1215.

Mabbit, L.A. 1951. The role of plant cells in the ensilage process: an approach to the problem. Proc. Soc. Appl Bact. 14:147.

Michigan Agricultural Statistics. 1974. Michigan Department of Agriculture, Lansing.

Miles, M. 1918. Silos, ensilage and silage. A practical treatise on the ensilage of fodder corn. Orange Judd Co., N.Y.

Miller, W.J. and C.M. Clifton. 1965. Relation of dry matter content in ensiled material and other factors to nutrient losses by seepage. J. Dairy Sci. 48:917.

Montgomery, M.J., H.A. Fribourg, J.R. Overton and W.M. Hopper. 1974. Effect of maturity of corn on silage quality and milk production. J. Dairy Sci. 57:698.

Neidig, R.E. 1914. Chemical changes during silage fermentation. Iowa Agr. Exp. Sta. Res. Bul. 16.

Nesbit, D.M. 1882. Silos and insilage. A record of practical tests in several states and Canada. USDA. Spec. Rpt. 48.

Noller, C.H., J.E. Warner, R.S. Rumsey and D.L. Hill. 1963. Comparative digestibilities and intakes of green corn and corn silages with advancing maturity. J. Animal Sci. 22: 1135 (Abstr).

Owen, F.G. 1971. Silage additives and their influences on silage fermentation. Int. Silage and Res. Conf. p. 79.

Owens, M.J., N.A. Jorgensen and H.H. Voelker. 1968. Feeding value of high dry matter corn silage for dairy cattle. J. Dairy Sci. 51:1942.

Perry, R.W., D.M. Caldwell, J.R. Reedal and C.N. Knodt. 1968. Stage of maturity of corn at time of harvest for silage and silage and yield of digestible nutrients. J. Dairy Sci. 51:799.

Peterson, W.H., E.G. Hastings and E.B. Fred. 1925. A study of the principal changes which take place in the making of silage. Univ. Wisc. Exp. Sta. Bull. 61.

Polan, C.E., J.T. Huber, R.A. Sandy, J.W. Hall, Jr. and C.N. Miller. 1968. Urea-treated corn silage as the only forage for lactating cows. J. Dairy Sci. 51:1445.

Rench, W.E. and R.H. Shaw. 1971. Black layer development in corn. Agron. J. 63:303.

Russell, E.J. 1908. The chemical changes taking place during the ensilage of maize. J. Agr. Sci. 2:392.

Salas, F.J. 1971 Urea, ammonia and m-analog additions to corn silage rations for feedlot cattle. Ph.D. Thesis. Michigan State University, East Lansing.

Schmutz, W.G., L.D. Brown, and J.W. Thomas. 1969. Nutritive value of corn silages treated with chemical additives for lactation. J. Dairy Sci. 52:1408.

Sprague, M.A. and L. Leparulo. 1965. Losses during storage and digestibility of different crops as silage. Agron. J. 57:425.

Stallcup, O.T., G.V. Davis, and D.A. Ward. 1964. Factors influencing the nutritive value of forages utilized by cattle. Ark. Agr. Exp. Sta. Bul. 684.

Stanier, R.Y., M. Doudoroff and E.A. Adelberg. 1970. The Microbial World (3rd Ed.) Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Thomas, C. and J.M. Wilkinson. 1973. Nitrogen and acidity as factors affecting the voluntary intake of corn silage. Proc. Brit. Soc. Anim. Prod. 2:67.

Thomas, C, R.E. Wilson, R.J. Wilkins and J.M. Wilkinson. 1975. The utilization of maize silage for intensive beef production. II. The effect of urea on silage fermentation and on the voluntary intake and performance of young cattle fed maize silage-based diets. J. Agric. Sci. 84:365.

U.S.D.A. 1972. Agricultural Statistics. U.S. Gcvn. Printing Office., Washington, D.C.

U.S.D.A. 1974. Agricultural Statistics. U.S. Govn. Printing Office, Washington, D.C.

U.S.D.C. 1969. Census of Agriculture. Graphic Summary. Vol. 5, part 15. U.S. Govn. Printing Office, Washington, D.C.

Van Horn, H.H., C.F. Foreman and J.E. Rodriguez. 1967. Effect of high-urea supplementation on feed intake and milk production of dairy cows. J. Dairy Sci. 50:709.

Van Horn, H.H., D.R. Jacobson and A.P. Graden. 1969. Influence of level and source of nitrogen on milk production and blood components. J. Dairy Sci. 52:1395.

Van Royen, W. 1954. The Agricultural Resource of the World. Prentice-Hall, Inc., New York.

Van Soest, P.J. 1964. Use of detergents in the analysis of librous fibrous feeds. II. A rapid method for the determination of fiber and lignin. J.A.O.A.C. 46:829.

Virtanen, A.I. 1933. (From Watson and Nash, 1960) Emp. J. Exp. Agric. 1:143.

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

Wilkinson, J.M. and J.T. Huber. 1975. Addition of ammonia to corn silage: Effects on distribution of nitrogen, voluntary feed intake, animal growth and efficiency of feed use. J. Dairy Sci. In press.

Wilkinson, J.N., J.T. Huber and H.E. Henderson. 1975. Acidity and proteolysis as factors affecting the nutritive value of corn silage. J. Anim. Sci. In press.

Wise, G.H., J.H. Mitchell, J.P. LaMaster and D.B. Roderick. 1944. Urea-treated corn silage vs. untreated corn silage as a feed for lactating dairy cows. J. Dairy Sci. 27:649. Abstr.

Wittwer, S.H. 1974. Maximum production capacity of food crops. Bio-Science 24:216.

Woodward, R.E. and J.B. Shepherd. 1944. Corn silage made with the addition of urea and its feeding value. J. Dairy Sci. 27:648. Abstr.