DISTRIBUTION OF NITROGEN AND ACIDS OF FERMENTATION WITHIN UREA TREATED AND UNTREATED CORN SILAGE

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
RICHARD S. AUSTIN
1967

THESIS

ABSTRACT

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by Richard S. Austin

The effects of adding urea to whole plant corn silage on distribution of nitrogen and acids of fermentation was studied by comparing samples from each quarter (depth) of eleven urea-treated (average 10.4 pounds urea per ton) and eleven untreated farm silos. Results are means from 44 treated and 44 control samples.

Control silages averaged slightly dryer, 33.9 percent vs. 31.3 percent for urea treated silages. Dry matter content was directly related to the harvest date of corn. Mean dry matter percentage was lower near the bottom and higher at top of silos.

Dry matter was positively correlated with pH in control silage ($r = +0.40$) but much less so in treated silage ($r = +0.18$). Lactic, acetic and butyric acid production were negatively correlated with dry matter content of silage. The concentration of propionic acid was positively correlated with dry matter in control silage ($P < 0.05$) but not in treated silage ($P > 0.10$). Butyric acid levels were relatively low in both treated and control silages.

Urea-nitrogen levels were negatively correlated with dry matter content in treated silage and positively though nonsignificantly in control silage.

The mean pH for control silage was 3.80 and for treated silage 3.96 ($P < 0.10$). Urea treatment increased lactic, acetic and butyric acid concentrations ($P < 0.05$) but not propionic acid ($P < 0.10$). Mean values for lactic, acetic, propionic and butyric respectively in control silage were 4.22, 0.88, 0.07 and 0.03 percent of the dry matter. Comparable values for urea-treated silage were 5.44, 1.21, 0.06 and 0.119 percent of dry matter.

Lactic acid concentrations were greatest in the bottom quarters of all silos. Urea resulted in a nine fold increase in lactic acid production in top quarters of treated silos.

Urea addition (10.4 pounds per ton) increased mean nitrogen content 45.4 percent from 1.60 in control to 2.33 percent total Kjeldahl nitrogen of the dry matter in treated silage.

Ammoniacal nitrogen accounted for 20 percent and urea nitrogen 34 percent of total Kjeldahl nitrogen in urea treated silage but only 7 percent was ammoniacal and 15 percent urea nitrogen in control silages.

Ammoniacal nitrogen level was positively correlated ($r = +0.66$) with pH in treated silage but negatively correlated with pH in control silage ($r = -0.21$). Lactic acid levels were negatively ($r = -0.36$) correlated with levels of ammoniacal-nitrogen in urea treated silages but positively ($r = +0.21$) correlated with ammoniacal-nitrogen levels in control silages ($P > 0.05$). These treatment differences were significant ($P < 0.01$).

Urea loss from silage was measured by nitrogen recovery by silo quarters top to bottom amounted to 100.5, 94.6, 98.2 and 99.9 percent of the amount calculated to be present. The average recovery rate of added nitrogen for all urea treated silage was 98.4 percent.

Mean values of green chop corn when harvested were: pH 5.0, dry matter 36.2 percent, total Kjeldahl nitrogen 1.78 percent, urea-nitrogen 0.399 percent of dry matter. These values were compared to similar material from known locations in the silo after fermentation. Urea treatment increased crude protein-equivalent, acetic, lactic acids and ammoniacal-nitrogen as percent of total nitrogen present ($P < 0.05$). There was a decrease in pH, dry matter, percent of total Kjeldahl nitrogen and urea nitrogen in control silage, compared to the original green chop material ($P > 0.05$).

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A THESIS

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

MASTER OF SCIENCE

Department of Dairy

1967

ACKNOWLEDGMENTS

The author expresses his sincere appreciation to Dr. Donald Hillman for his counsel in progress of this study and in preparing this thesis to Dr. J. W. Thomas and Dr. L. D. Brown for help provided in Dairy department nutritional laboratories and suggestions in method of presenting the data; to Mr. W. Askew, Mr. G. N. Blank, Mr. J. Bell, Dr. Albert Gorrill all of whom helped in making chemical analysis and interpreting the results, to Dr. J. Gill and Mrs. J. Landis of dairy statistical section who guided the statistical analysis of results.

He also wishes to express a most deserved thank you to Dr. E. J. Benne for his advice and guidance, offered always when needed most and to include his able staff, Mrs. E. Linden and Mr. F. Hoag for analysis conducted on essential silage samples.

The author is especially grateful to have had an understanding wife, Catherine, and family who were patient in awaiting the completion of this study.

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INTRODUCTION

The major concern of this research was to measure by chemical response the changes in composition which did occur from the addition of urea to corn silage. Man is faced with complex problems of feeding an increasing population and at the same time providing an adequate diet for animals, which convert unusable human feed materials into savory foods for his needs. Animals to survive in the future must be chiefly forage users and protein suppliers.

Most critical is the growing competition of man and animals for protein sources. Humanity depends heavily on animal protein sources with limited competition with animals. Man competes directly with animals for plant-cereal proteins and energy sources.

Animal nutritionists have been aware of growing demands on all known sources of protein available in the world. W. H. Pfander of the University of Missouri, Animal Husbandry Department, was quoted by Takheim (89) as saying, "In the light of the world food situation non-protein-nitrogen must eventually furnish all or a great part of the protein for animals." Human competition for natural proteins commonly fed to livestock tends to cause a scarcity of such feeds and could price the oil meals out of the market for livestock producers.

Scientists have been attempting to learn more about how to utilize non-protein-nitrogen for many years. Extensive studies have been made in duplicating natural processes in laboratories as well as with animals. DuPont's review of urea research (104), stated that the use of urea as a protein replacer started in Germany during World War I. Feeding urea to cattle, in the United States, did not come until early 1937, Reid (76). Urea and Biuret are the two major sources of non-protein-nitrogen now in use in cattle feeding. Scientists are learning more about effects of non-protein-nitrogen in cattle feeding. They are learning more about metabolism of nitrogen products of urea. Indications are that urea can make a much greater contribution to the world economy of protein than it is now making.

Urea and similar forms of non-protein-nitrogen have been extensively studied because they are relatively simple to manufacture and widely available as nitrogen fertilizers. Urea was first used commercially as 42 percent nitrogen fertilizer and is now available as 42 or 45 percent nitrogen, purified, feed grade urea.

Urea and other non-protein-nitrogen compounds are of great interest to animal nutritionists. Ruminant animals by presence of microorganisms break down cellulose in the rumen into usable organic acids. Microorganisms also have the ability to synthesis amino acid. The essential amino acids are reunited in cellular bodies of bacteria, forming proteins. The liver and other tissues synthesize non-essential amino acids. As microorganisms are carried through the digestive tract the bacterial proteins are utilized by ruminants.

Rumen bacteria can use a wide variety of nitrogen sources to form proteins. These can include amides, ammonium salts, urea, nitrates or other proteins. The use of a non-protein-nitrogen source is a means of economizing on formation of protein by use of the natural microbacterial route. Protein formed from non-protein-nitrogen sources are essentially equal to other sources of protein used in growth, maintenance, production, and reproduction of the ruminant.

Urea is generally fed as additive to dry grain, and as a part of the protein supplement. Large quantities of urea in dry grain are unpalatable. It is difficult to get cattle to consume adequate amounts of urea in grain mixtures, or when urea is added in dry form to the top of forage, such as corn silage. Much research has been directed to improving the palatability of urea with molasses and other chemical ingredients, primarily flavor compounds.

Whitehair et al. (95) and other researchers have worked at establishing toxicity levels of urea. Working with unconditioned feeder steers, and wide levels of urea in grain, they studied toxic effects of urea. Their work established safe feeding levels which are being followed today. These commonly accepted rules are:

1. Urea should not make up more than 1 percent of the entire ration.

2. Urea should not supply more than 1/3 of total nitrogen in ration.

3. Urea should not make up more than 3 percent of the concentrate ration.

Corn silage offers a number of advantages for incorporating urea into the diet of ruminants. Corn silage is a widely accepted feed for dairy and beef cattle in the United States, and is gaining in use wherever corn plant production can be assured. Corn silage provides energy in the form of starch, which is a prime essential in the conversion of urea to bacterial protein. Adding urea at ensiling time allows the necessary time interval for breakdown of urea to ammonia and possibly newly formed nitrogen compounds. Full or partial feeding of corn silage along with high corn grain, or cereal rations is logically ideal for urea supplementation. Corn silage, of all forage crops, provides the most total digestible nutrients per acre on good land and is equal to hay on poorer agricultural lands. Corn silage, if properly fed in balanced rations, also provides the greatest amount of milk or animal growth at lowest cost per acre. Corn silage is notably low in both protein and calcium content. Corn silage is uniquely easily adapted to automation in harvesting and feeding. It has been used successfully as the basic forage in formulating all-in-one rations.

Urea needs the energy of corn silage or similar feeds to make it equal to oil-meal products in feeding value. Urea could possibly increase the palatability and digestibility of treated corn silage. Urea, a concentrated nitrogen source, is best utilized if spread uniformly in the silage mass.

Corn silage can provide urea with an abundance of easily digestible starch in the silo as well as within the rumen. Corn silage provides usable carbohydrates for bacterial activity needed to form new amino acids in the rumen. The various components of corn silage provide rapid, then delayed carbohydrate digestion. Silage, a carbonatious forage, is retained in rumen for further synthesis of bacterial protein by recycling of blood and salivary urea. Because corn silage is a forage and usually available ad libitum it is consumed by cattle over a longer period of time which results in slower urea intake, and theoretically reduces the amount of ammonia released in the rumen thus improving utilization of urea nitrogen.

Urea has also worked well in the presence of low protein, high fiber feeds; where the diet was formerly lacking protein; and in comination with other protein provided by cereal grains, oil meals, forages and other feed materials.

Extensive research of adding urea to corn silage is relatively new starting in Ohio in 1958, with beef cattle.

It was first adapted to dairy cattle feeding in Michigan in 1963. The amount of urea to add for best results is still open to question but the favorable range appears to be 10 to 20 pounds per ton of green chopped forage. The extent to which nitrogen may be lost from silage during storage, and management or in-silo environmental factors affecting such losses has not been established over a wide range of conditions. In view of the theoretical and apparent advantages cited above for corn silage to be a logical carrier of urea in ruminant feeding many of these concepts have neither been refuted nor confirmed with research evidence. The final form in which nitrogen occurs, the degree of nitrogen recovery, and the distribution and concentration of the major acids of fermentation in corn silage containing added urea compared to untreated corn silage and fresh, chopped corn, are the major concerns of this study.

REVIEW OF LITERATURE

Increased Use of Urea

Takheim (89) in a 1965 survey of nineteen national protein feed suppliers found: (1) urea now supplies 40 percent of total nitrogen in conventional protein supplements and (2) protein supplement levels are increasing as demand for high protein concentrates has increased. Seventy-five percent of total nitrogen in eighteen high protein supplements was supplied by urea, the range being 59 percent to 94 percent of the total nitrogen. Reid *et al*. (77) in discussion of new energy and protein requirement for milk production, recently published by National Research Council "Nutrient Requirements of Dairy Cattle" (105) points out, total digestible nutrients requirements increased with increased feed intake or milk output. New requirements for producing dairy cattle recommend a crude protein increase of 12 percent on cows producing 44 to 77 pounds of milk per day and 26 percent increase in crude protein for cows producing 77 pounds or over. Total digestible nutrients allowances have been increased by 15 to 30 percent respectively. Percentages were acquired by figuring requirements necessary for Holstein cows milking 50 or 80 pounds daily, testing 3.5 percent butterfat. Reid *et al*. (77) reported on nitrogen balance data, of 265 individual cow trials with 7 diets at intake levels 1 to 5 times maintenance level that average utilization of digestible protein was 65 percent. Digestibility of proteins decreased with volume intake; 24.4 g of digestible protein were required per pound of 4 percent fat corrected milk. It was advised to maintain digestible protein to total digestible nutrient ratio of 1 to 5.7, a much higher level than previously recommended for both protein and total digestible nutrients, in high producing cattle.

Archibold (2) in 1943 concluded in his study of urea compared to cottonseed meal, soybean oil-meal and corn gluten feed, that urea was well utilized though not quite as effective as standard protein-concentrate used. Bell et al. (9) in 1953 found when added to dry grain feeds urea increased protein content only with no effect on digestibility of ration nutrients other than protein. Brown et al. (16), Davis et al. (25), and Parham et al. (71) agreed that urea can effectively replace natural protein in these feeds for growth, gains, and milk yields, provided the protein replacement with urea does not exceed one-third of the total protein of the ration.

Factors Which Have Effected Use of Urea

DuPont, 1958 review of urea research (104) points out six factors which influence the use of urea as protein source in ruminant nutrition.

1. Early use of urea in Germany was made during World War I to relieve protein shortage for cattle feeds. It was not seriously considered in United States until 1936.

2. Urea from natural fodder source has always been a constituent of ruminants dietary intake, the metabolism of urea is quite well understood by nutritionists by *in vitro* and *in vivo* tests.

3. Ruminants survived evolution change because of beneficial relationship between rumen microorganisms and host animals. To man this ruminant-bacterial relationship has become increasingly economically important.

4. The concept of feeding chemical urea to ruminant rations resulted in major contribution to world economy and is important to human diet.

5. Successful management of the ruminant animal depends upon proper nutrients to rumen bacteria. Bacteria reduce feed to acceptable state to host animal and in the process synthesize vitamins, bacteria convert feed to usable protein and absorbs it, bacteria then becomes digested and releases new formed proteins to animal use.

The importance of protein synthesis was demonstrated by Conrad *et al*. (24) in 1965, who concluded that rumen synthesis of essential amino acids via microbial growth apparently provides the major source of these nutrients for dairy cows. Urea and biuret are two major sources of non-protein-nitrogen used to replace natural protein in cattle rations.

Directing Future Research Efforts

Reid (76) in 1953, review of urea research, dispensed many misconceptions regarding use of urea and laid out principles under which future research studies would follow. He listed fourteen summary points worthy of consideration here:

1. Urea ability to share responsibility of providing protein was recognized but not demonstrated prior to 1937 in the United States. The nature of diet influence to degree urea was utilized needs further study.

2. Experiments in nitrogen balance, ruminal-ingesta composition, body composition and isotopic tracers have been employed to demonstrate that urea nitrogen is converted into and stored as protein nitrogen. Conversion of urea nitrogen to protein is mediated by microorganisms of rumen and reticulum consequent digestion of bacteria and protozoa containing this new protein.

3. Nature of diet effects urea utilization. Low-level intake of true protein plus high level of starch is favorable. Sugar and celluloses are less favorable than starch for rumen microorganism. Urea, and ready source of carbohydrates, must be present or readily available to support a satisfactory conversion of urea nitrogen to protein.

4. _In vitro_ studies may be misleading as to _in vivo_ results. _In vitro_ studies only tend to support what is found to be true _in vivo_.

5. Age affects urea usage.

    a. Calves as young as two months old can use some urea.
    b. Faster growth rates are reported for older calves.
    c. Slightly inferior to other high protein feeds for older cattle.
    d. Less difference in utilization by milk cows and older steers.
    e. In sheep better used when fed to mature ewes than by growing lambs.

6. Addition of methionine (sulfur containing amino acid) or sulfur have improved nitrogen retention. Sulfur content of diets depends on soil fertility, some crops are deficient in sulfur.

7. Other minerals not needed beyond normal levels.

8. Problems of urea rapidly hydrolyzed--gives too quick a release. Ways of delaying release of ammonia should be studied.

9. Calf studies conclude that urea may be somewhat inferior to conventional supplements as source of nitrogen for growth.

10. Urea for fattening cattle not clearly defined. Can presently replace 25 percent of required nitrogen for fattening steer rations.

11. Urea is not a good substitute for fattening lambs. Up to 25 percent of 12 percent crude protein was well used by over 12 percent resulted in poor utilization of urea. Urea is a satisfactory substitute for about one-third of nitrogen in pregnant or lactating ewes.

12. Milking cows can handle up to 3 percent of the concentrate ration or up to 1 percent of total ration if fed in grain.

13. Small concentrated quantities of urea in feed or supplements can be toxic, however high levels can be safely fed if well mixed with other feeds. Molasses and/or cobalt salt have improved palatability.

14. Urea has no energy source, it must draw on some other ingredient for energy. Fourteen pounds of urea plus 86 pounds of shelled corn is equal to 100 pounds of soybean or cotton seed oil-meal, 115 pounds of linseed oil-meal, 145 pounds of distiller dried corn grains. This should be figured in economical grain supplementation.

Economics of Using Urea

Hodges (36) of the United States Department of Agricultural Economics, stated that urea made up 12 percent of protein supplements in 1956, and 20 percent of all protein supplements in 1963, based on 44 percent protein equivalent used to average between 42-45 percent materials available. This represents 20 percent increase in the last two years but does not include an estimated 13 percent increase in use of fertilizer grade urea fed to cattle. Production of urea and biuret have increased five times in seven years to an annual production of ten million tons.

Domestic urea sales of feed grade urea for the 1956 to 1960 period have doubled and fertilizer urea sales increased by four times the beginning level. Five and six-tenths million tons of urea was fed to cattle and sheep between September 1962 and September 1963. Increases in oil-meal by-product sales also increased as did production. More cattle were fed protein, less forage and pasture, more grain was fed, and there was an increase in slaughter cattle weight as more pounds of oil-meals were fed per head. Cattle compete with poultry and hogs for available oil-meal by-products.

Some sources of feed grade urea today contain 45 percent nitrogen where formerly it contained 42 percent nitrogen. The higher level of nitrogen generally makes it a better buy.

Between the period from 1951 to 1964 urea has had a price advantage over oil meals with exception of a short period in 1956. The urea-corn advantage has been $26.65 per ton saving for the 1951 to 1964 period. Production of urea is expected to increase seven times in the next five years. Urea is expected to remain uniform in price but competition could lower its cost by 40 percent. More available urea could result as manufacturing plants increase the scale of production. There is an increase in plants producing urea and reduced freight rates are expected. Oil-meal production cost is expected to continue at a steady increase in spite of increased production due to the competition of humans for vegetable protein and cereal grain products.

On the early market only fertilizer grade urea appeared. This was followed by feed grade and increased nitrogen content. Biuret is gaining increased use in research as tests have shown and delayed ammonia release and that a mixture of 2/3 urea and 1/3 biuret could be more economical and a better feeding combination.

Takheim (89) reported a study from South Dakota Dairy Herd Improvement Association, where 10 pounds of urea added per ton of corn silage saved 3¢ per cow per day of protein, or a saving of $540 per 20 cow herd per year, in supplemental protein.

Lassiter (54) calculated a saving on 100 cow herd, fed corn silage with urea, 10 pounds per ton, could save

$1,800 per year by reducing the protein content of the grain mixture from 18 percent crude protein to 13-14 percent crude protein.

Dorr (27) reported in 1966 that dairymen reduce protein cost by $15.00 per cow while using urea treated corn silage containing 10 pounds per ton.

For most economical use of urea, Dr. Beeson of Purdue University suggests ten guide rules as reported by Takheim (89) in 1965.

1. Feed with readily available supply of energy-molasses or grain.
2. Supply enough calcium and phosphorous to meet daily requirement.
3. Supply enough trace minerals to meet daily requirement.
4. Watch levels of sulfur, the nitrogen to sulfur ratio should not be wider than 15 to 1.
5. Supply enough unidentified factors necessary to maintain favorable microorganism environment for high level protein synthesis.
6. Add three percent salt to grain mixture.
7. Fortify ration with enough vitamin A and D.
8. Be sure that urea is free flowing and mixed well into other feeds.
9. Feeding instructions should always be specific when using urea containing supplements.
10. Use other high quality ingredients with a high urea supplement in formulating urea supplements.

Effects of Adding Urea to Corn Silage

Altering the Crude Protein Content

The corn plant is very low in crude protein equivalent; the National Research Council (105) gives well matured corn silage in late dough stage 2.3 percent crude protein on fresh matter basis or 7.9 percent on dry matter basis. A sixteen year study of silage used in digestion trials at the Michigan station by Huffman *et al*. (42) between 1940-1956 shows corn silage contained 2.7 percent crude protein on fresh basis and 9.4 percent crude protein on dry matter basis. Morrison (63) 1957 edition, in a summary of 237, well-eared samples, well matured corn, lists crude protein values at 2.3 percent on fresh basis and 8.3 crude protein on dry matter basis. Four alternatives have been taken to increase protein content: (1) by crop fertilization, (2) breeding higher protein varieties, (3) by harvesting more mature corn, and (4) by supplementing the ensiled corn with protein or non-protein-nitrogen. Bender *et al*. (10) reported in 1934 they could not improve percentage of protein of the corn plant with nitrogen fertilizer up to 450 pounds nitrogen applied per acre. The yield increased which resulted in greater harvest of protein per acre but plant content remained relatively uniform. Leaf levels of nitrates were increased by fertilization. Further research has given similar results.

Hodges (36) reports higher protein corn is being developed which has increased 20 percent in crude protein content. It is said to contain a greater amount of amino acids but thus far has proved to be very low yielding. It is still uneconomical to use.

Johnson et al. (44) reported in 1966 the effects of maturity on dry matter and protein distribution in plants. In a two year study the highest total dry matter yield was from corn in late dent and glazed stage of ensiling. Ears did not constitute 60 percent of dry matter until after September 12 in 1962 and October 6 in 1964 in these Ohio trials. Leaves lost crude protein rapidly during early maturity, but the stalk lost protein rapidly only 15 days after tasseling, then slowly. Ears gained in dry matter and crude protein with maturity. The resulting late (more mature) silage contained more dry matter, higher total digestible nutrients and more crude protein.

Huber et al. (40) found that crude protein levels for soft, medium, and hard dough stage corn silage were 8.3, 7.9, and 8.1 percent with dry matter being 25.4, 30.3, and 33.3 percent. There was less crude fiber in hard dough stage corn silage and milk yield increased significantly when cows were fed higher dry matter content silage. Dry matter intake was greater with hard dough stage corn silage which resulted in higher production. The variation in crude protein listed at the beginning of this discussion might be explained in differences in the maturity of corn fodder when chopped for silage today as compared to immature corns a few years ago.

Hillman (35) in a study of 21 Michigan dairy farm silos observed that the higher dry matter content silage also contained the highest protein content. Lower protein content was most apparent in silage of less than 30 percent dry matter. It was suggested that these losses of up to 20 percent of the protein largely occurred through seepage. Seepage losses can account for loss of 0 to 10 percent of the dry matter of stored corn silage.

Since fertilization has given only limited nitrogen increases where soils were low in nitrogen, higher protein corn varieties are just becoming available, and maturity advantages can be utilized to increase silage protein content only limitedly, then the only method left to substantially increase protein content is by additional protein supplementation. In the past the cost of supplemental protein, namely those supplied in grain and oil-meal products, have been expensive and used limitedly. Since ruminants can utilize non-protein nitrogen and these compounds are more economical, full supplementation of a deficient protein diet can be economically accomplished. Karr et al. (48) reported in lamb tests when non-protein-nitrogen compounds were added at feeding time to the top of corn silage, or fed in grain, it reduced palatability. It was better to add urea at ensilage time. It was found by Schmutz (80) in a two year study of milk cows, that when urea was added at 10 to 20 pounds per ton of corn silage, the cattle were first hesitant to eat readily, but then proceeded to eat more treated silage and consumed more dry matter daily than when offered only untreated silage. Willett _et al_. (96) in a study of palatability suggested that cows may require time to fully adjust themselves to urea feeds, but once adjusted the intake is not restricted and digestibility of treated material may be higher. Urea is probably the only commonly used non-protein-nitrogen compound used to supplement corn silage at filling time. The study of its effect on nutrition as related to corn maturity and digestibility warrants further study. Bentley _et al_. (13) in 1955 ensiled corn with 17, 20, and 25 pounds urea per ton. In addition a mixture of 20 pounds urea and 2.0 pounds of dicalcium phosphate was added to another corn silage. Two untreated silos were also studied. The crude protein on dry matter basis was in order listed, 15.1, 14.6, 19.9; mixture-treatment (urea and dicalcium phosphate) 13.4; and controls 9.3 and 8.9 percent dry matter basis. Brooks _et al_. (14) in 1965 reported adding limestone and urea to fresh corn fodder at ensiling time. These corn silages, containing added limestone and urea, all contained higher crude protein values. Increases in crude protein have been reported by Gorb _et al_. (31) in 1961, who added 0.65 percent urea; by Goode (30) in 1955, and Palamaru et al. (70) in 1961, both used 0.5 percent urea. Klosterman et al. in 1961 (50), 1962 (51), and 1963 (52), reported similar results. They further suggested that urea added to corn silage could replace an increasing portion of the protein supplement when fed to cattle. Schmutz (80) in a two year study comparing untreated corn silage to urea treated silage containing various amounts of urea and diammonium phosphate, calcium carbonate and dicalcium phosphate. He reported in 1966 that urea at 10 pounds (0.5 percent level) increased the crude protein level on a dry matter basis from control level of 9.5 percent to 13.5 percent. With levels up to 20 pounds urea used per ton he concluded the increase in crude protein was equivalent to increases in proportion to the level of urea added.

Dorr et al. (27) in 1966 reported 0.5 percent urea added at filling time to corn silage on twelve dairy farms averaged 13.02 percent crude protein (sd $\pm$ 1.41).

Changing the Hydrogen Ion Concentration

A review of green chop and corn silage pH levels.--Benne et al. (11) in 1964 reported on various nutrients found in corn plants at different stages of growth. They found that corn plants on August 27 had a range from low of pH 5.1 in the upper stalks to high pH 6.8 in the silk; cobs pH 6.4; lower leaves pH 6.4 and upper leaves 5.7. Shanks and husks had a pH 5.3. Schmutz et al. (80) reported initial pH values for their different green chop corn to be 4.7, 6.0, and 6.3 before addition of various amounts of urea. Karr et al. (48) indicated that initial control silage contained pH 5.4, declined in pH rapidly and after 8 days reached pH 3.75 and still maintained this level 150 days later. Schmutz et al. (82) found their ensiled high moisture ear corn control dropped to pH 5.1 within 6 days. They reported that moisture content had a profound effect on pH. The dryer the ensiled corn the less drop there was in pH, the higher moisture silage produced lowest pH within the 60-day period studied. Schmutz et al. (80) reported that controlled silos of whole plant corn in a later study contained green chop with pH 5.1 and 5.4; by simple fermentation they declined to pH 3.6. They concluded that by the addition of a single additive the chemical reaction of the additives increased the resulting pH. Huffman et al. (42) reported a sixteen year average for corn silage was pH 4.0.

Non-protein-nitrogen delays acid production.--Schmutz et al. (80) found it took sixty days for whole plant silage with 15 pounds urea added to drop from pH 6.0 to 5.1. In a year later study Schmutz (80) found that treatment with 0.5 percent urea in whole plant corn silage resulted in pH 3.72. The reduction in acid production was not as great as that experienced by addition of 0.5 percent calcium carbonate; 1.0 percent, dicalcium phosphate, or a mixture of 0.5 percent calcium carbonate plus 0.5 percent urea in corn silage. Schmutz et al. (81) concluded in 1964 that urea complemented the buffering effect of calcium carbonate. Byers et al. (21) reported 1 percent limestone added to corn silage resulted in pH 4.2 as compared to control silage of pH 3.85. Karr et al. (48) found when using 1 percent urea or 1.2 percent biuret in corn silage with initial green chop values of pH's 7.9 and 5.4, after eight days fermentation reached pH 5.4 and 4.8 to final 150 day pH 4.25 to 4.05. He explained the cause as due to high buffering effect of urea by relatively high amounts of ammonia obtained from water extracts of these silages. He found that 28 percent of the urea was hydrolyzed after eight days in large experimental silos, indicating that these compounds alter fermentation to some extent. Bentley et al. (13) 1955 compared control silage to treated silage with 25 pounds urea, control silage to treated silage with 20 pounds of urea, and treated silage with 20 pounds urea plus 2.0 pounds of dicalcium phosphate. The results pH 4.70, 7.60 (25 pounds), 3.70, 4.05 (20 pounds), and 3.95 (mixture) respectively. Klosterman et al. (52) compared control silage to silage treated with 0.5 percent urea, and silage treated with 1.0 percent urea, the resulting silages had pH of 3.8, 4.1, and 4.4 respectively. Dorr et al. (27) reported 12 farm 0.5 percent urea treated silos reached average pH 4.46 with average dry matter 35.1 percent in late January of 1966.

These studies point out that: (1) the drop in pH is delayed by treatment of silage with urea. (2) The greatest drop in pH is in the early period (6 to 10 days) following ensiling, followed by a declining rate, which may be rapid enough to fix rapidly hydrolyzed urea-ammonia, as only very weak acid is needed to accomplish this task. (3) The greater the quantity of urea used the larger are the buffering effects on hydrogen ion concentration. (4) Dry matter content affects the way urea alters pH decline of corn silage. Schmutz (82) reported a steady degradation of urea throughout the fermentation period. Peak temperatures were reported by him in 6 to 12 days following filling. Lassiter et al. (57) reported peak temperatures were reached in their study 8 to 10 days following filling date. The lower the moisture in the fresh ear corn chop the greater was the temperature increase in ear corn silage. Using 0 degrees as filling date temperature, 11° F was the recorded rise in temperature for ear corn containing 76 percent dry matter.

Increasing Major Organic Acids Present

A review of green chop and corn silage major fermentation acids levels.--Barnett (5) in 1954 emphasized the fact that the objective in silage production was to stimulate lactic acid production to such a point as to inhibit other bacterial activities and preserve the crop. Watson et al. (92) stated that,

> the percentage of lactic acid in silage varies somewhat but in good silage samples the range is 1 to 2 percent of weight of the fresh silage. If preservation is to be effective the lactic acid level should reach the neighborhood of 1 percent and should always exceed in amount the volatile acids.

It was also noted that acetic acid should generally range from 0.5 to 0.9 percent of the fresh material. Barnett (5) also stated that lactic acid should be at a concentration of 0 to 1.5 percent of fresh material in normal silage. Karr et al. (48) reported control silage to contain 0.95 percent acetic and 5.45 percent lactic acid on dry matter basis. He stated that acetic and lactic acids were the only acids present in measurable amounts. Urea, and particularly biuret, tended to alter the fermentation by reducing lactic and increasing acetic acid with overall lower production of acid. Levels of urea and biuret treated silages were 0.87 and 0.95 percent acetic and 6.45 and 6.15 percent lactic acid on dry matter basis. Schmutz (80) reported organic acid levels for control and urea treated silos: acetic acid 1.08 percent, control; 2.28 percent urea treated. Lactic acid 8.42 percent control; and 13.06 percent urea treated on dry matter basis. These high lactic levels were from 1.0 percent urea-treated silage. Using 20 pounds urea per ton, silage increased in pH from 4.5 in green chop to pH 7.4 in fermented silage which were unusually high in moisture content. He concluded that all the additives to corn silage studied increased organic acid production. Simkins et al.

(85), using control and 0.5 percent limestone treated corn silage in lactation studies of dairy cows, found acetic acid increased by 53 percent, lactic acid by 80 percent, pH was 5.1 percent higher than control silage. Acetic acid content was 1.60 percent in control and 2.40 percent in limestone treated corn silage. Lactic acid content was 4.82 percent in control and 11.24 percent in limestone treated silage. Control silage had pH 3.73 compared to pH 3.92 for limestone treated silage. They reported no advantage to addition of $CaCo_3$ to corn silage when ensiled for lactating dairy cows. The marked increases in organic acids agrees with results of Byers et al. (21) reported earlier using one percent limestone added to corn silage that increased acetic acid levels by 104 percent and lactic acid level by 80 percent. These higher level organic acid silages when fed did not affect volatile fatty acid levels in the rumen. There was no significant difference in dry matter intake, milk yields, butter fat content or changes in body weight during the period this treated silage was fed and compared against control silage.

Effect of silage moisture on organic acid formation.--Waldo et al. (91) in two experiments where they compared corn silage to hay, grain, and a pelleted diet fed dairy cows found the following levels of organic acids. Corn silage with high moisture (22.96 percent dry matter) contained pH 5.48; 5.46 percent acetic acid; 0.48 percent lactic acid;

3.28 percent butyric acid; 1.43 percent propionic acid; or organic acid content 11.16 percent on dry matter basis. Corn silage with low moisture (45.11 percent dry matter) contained pH 4.71; 1.58 percent acetic acid; 3.37 percent lactic acid; 0.19 percent butyric acid; 0.34 percent propionic acid; or 6.13 percent total organic acids on dry matter basis. High moisture corn silage contained 26.88 percent ammoniacal-nitrogen as percent of total nitrogen present. The low moisture corn silage contained 10.68 percent ammoniacal-nitrogen as percent of total nitrogen present. Their study gave a good comparison as to the effect high or low moisture content has on organic acid formation in corn silage.

In summary, lower moisture, 45.11 percent dry matter corn silage contained 49 percent more dry matter, had pH level with which was 16 percent higher, 2.4 times less acetic acid; 6 times more lactic acid; 15 times less butyric acid, 3.2 times less propionic acid; 1.5 times more ammoniacal-nitrogen as percentage of total nitrogen present than high moisture 22.96 percent dry matter silage.

Schmutz _et al_. (82) in three trials compared 35-45 percent moisture corn silage and found butyric and propionic acid to be higher in wetter silages in seven different comparisons.

Relative Effects on Vitamins, Minerals and Trace Minerals

Vitamin A.--Simkins et al. (85) reported that corn silage treated with ten pounds limestone per ton had a 42 percent lower carotene level. The corn silage contained 9.09 and 5.26 mg carotene per pound of dry matter in control and treated silage, respectively. Klosterman et al. (53) in carotene studies using corn silage, made well eared mature corn under normal fertilization, and a second silage from corn fertilized with 200 pounds of additional nitrogen per acre. Using beef steers deficient in vitamin A, they found that growing and fattening steers were able to meet their daily vitamin A requirements from the carotene supplied by either the normal or excessively nitrogen fertilized corn silage. There were no differences in the feeding value of corn silage which received normal or extra nitrogen fertilizer. This work agrees with Owen's work (68) when he reported on metabolism of vitamin A and carotene.

Smith et al. (87) 1964, studied the influence urea had upon vitamin A in ruminant nutrition. Liver storage of supplemental vitamin A was lower than expected in sheep fed a purified diet containing urea as primary source of nitrogen for 97 days. Vitamin A liver storage remained low after feeding 12 percent ration soybean oil meal. By added 5 percent urea to 12 percent soybean oil meal the decreased vitamin A concentration, enlarged livers; however, this did not change total liver content of vitamin A present. Urea fed at 5 percent with 12 percent natural protein had no effect on vitamin A liver storage. Steer performance was the same as lambs, when steer diets were depleted of liver vitamin A and fed above diet containing urea and natural protein. They found vitamin A storage in liver to be same if steers were injected with a single dose of vitamin A intramuscularly or fed vitamin A supplement of equal amounts in the diet. They concluded that nitrogen nutrition greatly affected liver storage of vitamin A in lambs and steers, that purified urea must influence vitamin A storage but urea in combination with other protein sources had no effect on liver storage of vitamin A.

Minerals.--Hubbert et al. (38) studied in vitro sulfur, potassium, phosphorus, magnesium, manganese, corn and sulfur, and effects on cellulose digestion where urea serves as the nitrogen source. They found high levels of copper, zinc, and cobalt inhibit cellulose digestion. They later reported that riboflavin can replace a part of potassium requirement of the rumen microflora for effective digestion of cellulose. Five of ten rumen bacterial strains used sulfate in forming organic sulfur compounds. Three strains of bacteria used sulfate if cystine was present. Cystine is a major sulfate containing amino acid. Lassiter et al. (55) reported from work with 24 Holstein dairy calves, fed 30, 50, and 70 percent of their dietary nitrogen as urea for 150 days and where corn cobs were the only roughage. As urea levels increased, corn cob intake decreased but not significantly. Daily gains and feed efficiency decreased significantly with higher level of urea. It was suggested in discussion that the diet was deficient in sulfur, since as urea content of rations increased, the sulfur content had decreased. Since, other research has shown similar results, low sulfur intake could have accounted for the above results.

Jones et al. (45) reported conclusive results which showed that sodium sulfate may improve the utilization of urea in a ration containing 3 percent urea and with an overall sulfur content 0.13 percent. Jones et al. (46) and Davis et al. (25) failed to confirm this observation. The type of ration fed and its original sulfur content are important factors in determining whether cattle fed urea need supplementary sulfur.

Trace minerals.--Burroughs et al. (17), using in vitro studies of trace mineral requirements of rumen bacteria, found minerals affect rumen bacteria which had control over efficient utilization of urea, digestion of cellulose, cellulose like containing feeds, and utilization of roughages. They used different trace mineral mixtures with extracts of clovers, rumen contents, manure, and molasses, and found improved urea utilization with mineral supplied by manure and molasses. Iron supplementalization aided cellulose digestion and urea utilization. Maximum utilization of urea occurred in absence of other ammonia producing material when energy and mineral requirements were supplied at normal dietary levels.

Metabolism of Urea-Nitrogen

Urea has been used more extensively than any other non-protein-nitrogen compound as a protein replacer with ruminants. Early in vitro studies by Owen (68) in 1941, by feeding experiments of Mills et al. (61)(62) in 1942 and 1944, McDonald (60) in 1952, by Arias et al. (3) in 1951, and Belasco (8) in 1956, as reported by DuPont (104) dealt with how carbohydrate effects utilization of urea into protein. They concluded that beneficial effects could be realized depending upon solubility of carbohydrate on the synthesis of bacterial protein produced from urea. They demonstrated the effect that type and amount of carbohydrates had on the cellulolytic activity and urea utilization. In vitro they determined the influence of carbohydrate on fatty acid production. They concluded that beyond a certain concentration of starch sugars depressed cellulose digestion which was accompanied by marked increases in concentration of butyric and valeric acid. This explains inefficiency of high molasses ration when compared to equivalent ration with cereal grains.

In Vitro Laboratory Studies

DuPont (104) points out that use of the artificial rumen techniques for *in vitro* studies performed by Pearson (72), Smith (86), Burroughs (19)(20) 1951 to 1953, Belasco (6)(8) in 1954 and 1956 provided information on: (1) Conversion of urea to bacterial protein. (2) The effect of various carbohydrate sources on utilization of urea. (3) Value of various non-protein-nitrogen sources. (4) The effects of antibiotics on cellulose decomposition. (5) The role of mineral salts on the digestion of roughage cellulose. (6) Effect of various carbohydrates on fatty acid formation. DuPont (104) reported *in vitro* studies compared urea with soybean, cottonseed and corn gluten meals indicated that urea was superior as source of nitrogen in promoting cellulose digestion by rumen microorganisms. These were the early, simulated, *in vitro* studies on metabolism of non-protein-nitrogen.

Winter *et al*. (97) in 1966 studied *in vitro* a number of non-protein-nitrogen compounds and concluded that utilization of these compounds usually involves their breakdown to release ammonia which is used by the bacteria for synthesis of their bacterial amino acid proteins. Wegner *et al*. (94) in 1940 first showed the conversion of urea to bacterial protein by rumen bacteria *in vitro*. Bentley *et al*. (13) showed that mixed cultures of rumen bacteria grown on cellulose utilized urea as its sole source of nitrogen and measured the increase in bacterial protein produced. In this ammonia and bacterial protein are determined as trichloroacetic acid (TCA) precipitable nitrogen. The level of TCA-nitrogen accounts for only 50-70 percent of urea nitrogen added to _in vitro_ fermentation. These tests suggest that a part of urea nitrogen was not converted to bacterial protein as determined by TCA precipitation. Other attempts are being made to identify all fractions present. These studies may open up further knowledge as to urea utilization.

Other non-protein-nitrogen materials tested.--Acord _et al_. (1) studied effects of ammonium salts, amino acids, amides, and amines as nitrogen sources for _in vitro_ digestion of starch (purified corn starch), by rumen microorganisms in comparison to urea. Ammonium sulfate, ammonium chloride, ammonium acetate and ammonium phosphate were equal to urea at all levels of 3, 6, and 9 milligrams urea per 20 milliliters of incubation mixture containing washed rumen microorganisms, minerals, buffers, and about 100 milligrams of purified corn starch. Aspartic acid was inferior to urea but more effective than other amino acids tested. The addition of argenine, serine, and methionine gave only moderate increases in starch digestion. Valine, glutemic acid and lysine were not effectively utilized. Acetamide, propionamide, succenamide, malonamide, guanidine acetate and aminoguanidine bicarbonate did not consistently stimulate starch digestion. High ammonia levels after 4 to 8 hours fermentation were associated with stimulated starch digestion.

In Vivo Studies Using Urea

Woodward et al. (101) were first to report in 1944 the addition of 10 pounds of urea per ton to corn silage. It was compared to a similar ration in which urea was contained in the concentrate. Both were fed to groups of cows receiving a low protein grain and hay ration. This was a 100 day single reversal trial. They reported palatability slightly impaired with moderate levels of urea. Palatability was highly affected with increased amounts of urea added, either as top dress to silage, or as supplement in the grain. Wise et al. (98) in 1944 used a liquified solution of urea which contained two pounds of crystals of 46 percent nitrogen dissolved in one gallon of water. They used 5 gallons to treat one ton of corn silage, and fed this treated silage to two groups of eleven cows. Silage served as the only roughage. Grain was fed on basis of individual cow's production. The daily intake of treated and untreated silages were 52.5 pounds and 60.0 pounds; On dry matter basis 15.5 and 16.9 pounds per cow. This was a greater difference in intake than was found by Woodward (101) in 1944. Hillman (34) in 1964, and Schmutz et al. (80) in 1966 found milk production for cows fed treated silage was similar even though cows fed treated silage consumed less silage dry matter. Woodward et al. (101) maintained that production was uniformly high regardless of method used to feed urea, either as silage or in the grain concentrate. However, others, as reported by Schmutz (80) experienced decreases in milk production when urea was added to maize silage at levels of either 0.5 or 1.0 percent urea.

Hoffman et al. (37) in 1965 reported that urea at a level of 4.0 kilograms per ton corn silage, fed in low protein to starch ratio, improved both daily milk yield and fat content. With higher level protein rations the effect was not apparent. Reid (76) in 1963 stated the results of long-time experiments with appreciable numbers of cows demonstrates that from the standpoint of milk yields and body maintenance there is no significant difference between the value of urea nitrogen as protein supplied, or other high protein sources if fed at levels up to 27 percent of the required nitrogen. Huber et al. (39) in 1964 compared the feed value of corn silage with various sources of protein supplementation. They used two trials with a total of 40 milking Holstein cows fed: (1) 15 percent dairy concentrate fed at 1 pound to 3-1/2 pounds milk; (2) a mixture of soybean and cottonseed meal; (3) a mixture of oil-meals and urea; and (4) urea added dry to top-dress silage. Silage intakes on dry matter basis were equal in (2), (3), and (4). Milk yield and production persistencies were highest in same order named, 51.3, 48.7, 43.6, and 36.0 pounds per day;

persistencies of production were 90, 85, 81, and 63 percent, respectively. Supplements (1) and (2) increased, (3) and (4) decreased, body weights. Dry matter intake decreased as level of protein supplementation decreased. High urea rations resulted in decreased milk production and yield of solids--not fats. Consumption and milk yields were highest from groups fed supplemental oilmeal (3) and urea (4) in one of two trials. This points out that feeding urea dry or grain supplementation of urea may be more limiting than when fed in a mixture with silage added at ensiling time.

Schmutz et al. (80)(81) in 1964-1966 lactation trials reported that cattle fed corn silage with (a) 1 percent diammonium phosphate consumed less silage on dry matter basis, less dry matter per 100 pounds body weight, less total dry matter, produced less 4 percent fat-corrected milk, (b) 0.75 percent urea silage gave the same depressing effect as with 1 percent diammonium phosphate. Cows produced the most when fed 0.5 percent urea silage, consumed 4 percent more per day, (c) there was slight depression in digestibility of dry matter, ash, and protein with urea treated silage.

Karr et al. (47), in extensive studies with urea reported in 1964, attempted to reduce the rate of hydrolysis by incorporating urea in dehydrated alfalfa meal pellets. By so doing they expected to increase microbial ability to utilize more of the available ammoniacal-nitrogen, increase tissue utilization and increase recovery of urea nitrogen recycled in the blood of feeder lambs. They concluded that the value of nitrogen source could be measured by three values: (1) its capacity to supply nitrogen that can be used by rumen microorganism; (2) its acceptability to the animal; (3) its toxicity level: if too rapidly hydrolyzed ammonia is absorbed directly into blood rather than synthesized into bacterial protein. Karr et al. (49) compared soybean oil-meal to urea and biuret in finishing rations for lambs, using steam-treated corn or dehydrated alfalfa meal with ground corn cobs. The steam treated corn did not increase rumen bacterial activity. The combination of dehydrated alfalfa meal, muscle implants of diethylstilbestrol with urea gave greater growth response. A low cost supplement is suggested by these trials. Karr et al. (48) measured the effects of urea and biuret. They were: (1) added to green chop corn and fed as treated silage to fattening lambs, or (2) added on top of silage at feeding time. He concluded that dry matter intake in sheep was higher when urea was added to green chop ensiled corn. The added non-protein-nitrogen increased gains by 26 percent, reduced feed requirements by 1.35 pounds per pound grain. The sources of nitrogen had no significant effect on rate of gains, so urea and biuret were equal. Biuret significantly increased nitrogen retention in two of three test trials. The addition of urea to silage as top-dress resulted in consistently lower nitrogen retention. Use of urea at ensiling time resulted in 13 percent less feed required per pound gain, improved dry matter and nitrogen digestion, nitrogen retention and percent of apparently digested nitrogen retained.

Wood (100) summarized factors effecting urea utilization in beef cattle rations that cattle feeders must bear in mind noted: (1) There is a limit to the amount of ammonia that rumen microorganisms can convert to microbial protein. (2) That urea is a source of nitrogen only. While soybean oilmeal also contains fat, carbohydrates, minerals and vitamins, and for one dollar spent no more protein can be purchased than in the form of urea. He suggested nutritional factors that may be best for use of urea: (1) High grain and silage rations provide added energy source for ammonia released, reduce blood absorption and excretion losses. (2) Provide adequate level of vitamins and minerals. (3) Best used in low level diet to increase protein needed. (4) Unidentified carriers of urea may influence its utilization and this needs more study.

Nitrogen Balance and Biological Values

The principal method used to measure the nutritive value of a protein or nitrogen source is by measurement of the nitrogen retained in the body originating from that source. Urea has been evaluated singly or in combination with other protein sources to determine its ability to promote nitrogen retention. From measurement of nitrogen retention has come the expression of nutritive value of nitrogen called the "Biological Value." It represents a measurement as how efficiently a nitrogen source can be utilized by a given animal. Reid (76) in 1953 indicated many of the factors, previously mythical, involving the efficient use of non-protein-nitrogen. Nehring (64)(65) in 1937 working with sheep presented data from nitrogen balance experiments indicating positive results from the presence of amides in the diet. Ammonium acetate gave positive balance and urea was somewhat less favorable. Harris et al. (32) conducted two separate studies in 1941 to evaluate urea as source of nitrogen for maintenance of growth in ruminants. Fecal nitrogen increased when lambs were switched from a low nitrogen ration to either a casein or urea supplemental diet. They assigned biological value of urea nitrogen to be 62 and casein nitrogen 79 at the point of nitrogen equilibrium in sheep maintenance. They used 23 wether lambs in a growth study, fed a basal ration of corn silage, limestone, salt and fortified codliver oil. This ration did not support nitrogen equilibrium, urea was added to bring protein equivalent from basal level 5.35 percent to three test levels of 8, 11, and 15 percent. The 11 percent ration was superior to 8 and 5.35 percent but not significantly different than 15 percent ration. The biological value of basal 8, 11, and 15 percent protein rations were 82, 74, 60, and 44 respectively. Chalupa et al. (23) fed low nitrogen (0.23 percent nitrogen) diet to steers to which was added urea at 0, 46, and 92 percent of nitrogen to meet nitrogen requirements, with corn gluten meal making up the balance. The differences in these rations were significant. The percentage of urinary nitrogen in form of urea, ammonia and creatine increased with increased amounts of urea in the ration. Lassiter et al. (56) also reported lower nitrogen balance for animals receiving high levels of urea. Gains in nitrogen retention were not improved when sulfur was equalized in test rations.

Waldo et al. (91) in nitrogen balance of hay silage compared with hay pellets and hay with grain reported those dairy heifers fed hay silage experienced reduced growth rate to those fed hay. There was lower intake of dry matter, the cause was not known but possibly due to changes in form of nitrogen or energy changes created during fermentation. A lower nitrogen utilization may be due to a change in nitrogen form which occurred in haylage.

Karr et al. (48) in 1965 reported increased nitrogen retention in two experiments with biuret added to a basal silage ration, while urea additions produced a lower retention. The addition of urea and biuret at ensiling time significantly improved daily nitrogen retention.

Reid et al. (77), fed rations one to five times maintenance levels to study the effects on 265 milking cows, found that requirement for total digestible nutrients increased with increased feed intake or milk production. Nitrogen-balance data indicated average rate of utilization of digestible protein to be 65 percent when digestible protein in the diet is equal to 154 percent of the quantity of protein in the milk, or 24.4 gram of digestible protein per pound of 4 percent fat-corrected-milk. The digestible protein to total digestible nutrients ratio was best at the 1:5.7 level accompanied with practical feeding knowledge.

Ruminant Metabolism of Fatty Acids

The acids in silage are of particular interest here in respect to the response of ruminants to feeds containing high levels of acetic and lactic acids, the effects of propionic and butyric acids on rumen digestion, and in what levels ruminants best utilize these major organic acids. DuPont (104) points out that nutritional economy of the ruminant is dependent upon the volatile fatty acids resulting from microbial fermentation. Carroll *et al*. (22) with *in vitro* studies showed that fatty acids produced in the rumen account for approximately 70 percent of the total energy received by the animal. Acetate was of greatest importance followed by propionate and butyrate. Precursors of these fatty acids are carbohydrates and proteins. Carbohydrates are oxided into fatty acids which are also converted into certain amino acids under rumen bacterial synthesis.

The metabolic role of volatile fatty acids is important because of the relative amounts produced and absorbed. In ruminants they represent the main products of carbohydrate digestion. Fatty acids are absorbed directly into blood, from the rumen, reticulum, omasum, and abomasum, also from the large intestine. Acetate and butyrate are used directly for energy and can also be stored much the same way as propionic acid is converted to glucose in the liver for later use by the animal.

Jarrett et al. (43) observed that propionate was removed from the blood stream more rapidly than acetate, causing simultaneous increases in glucogenic intermediates, namely lactic and pyruvic acid in the blood stream. However they received no similar response to direct acetic acid injections. Acetate injections increased ketone bodies and propionate injections decreased them. When injected together no increase was experienced. They concluded that the combination of acetate and propionate were oxided and were related to each other. The level of propionate production can affect acetate metabolism. Low propionate levels can inhibit acetate oxidation. Impaired acetate metabolism (acetoacetate) may be cause for the ruminant to develop ketosis.

Belasco (7) in study of protein feeds found they produced more butyric acid and less propionic acids and ketones. Isonitrogenous amounts of urea would produce more propionic acid as was desired. Pennington et al. (73) found that ketones were formed mainly from butyric and acetic acid; not propionic acid. Metabolism of pyruvic acid together with propionic acid suppressed formation of ketone bodies. Propionate suppressed ketones formed by lactates. Butyrate lowered intake of propionate and acetate lowered the intake of butyrate. Acetate was absorbed from rumen within first 12 hours. Volatile fatty acids are important in nutritional value to ruminants and for a special role played by propionate in its anti-ketogenic effects. Mayfield et al. (58) in a recent study of acetate metabolism in sheep livers concludes that the principal compounds burned by the ruminant to provide energy for body function are volatile fatty acids, acetic, propionic, and butyric. Acetate accounts for about 50 percent of carbon burned by ruminants, Sabine et al. (78). The body pool of acetate turns over very rapidly, Essign et al. (29), traced acetic acid by radiology and found half-life to be 1.5 minutes. Sabine et al. (78) reported in the intact sheep the rumen venous blood has about twice the acetate concentration as perepheral venous blood. This explains the remarkable ability of the ruminant to oxidize acetate rapidly. Senel et al. (83) studied the dietary effect of acetic and butyric acid on feed intake, lactation, blood glucose, and ketones. Using a Latin square 5 x 5 design they fed a basal ration of 50 percent alfalfa hay, 50 percent concentrate mixture to which was added extra amounts of (1) 2 percent acetic acid;

(2) 1 percent butyric acid; (3) a mixture of 2 percent acetic and 1 percent butyric; and (4) 4 percent acetic acid and 2 percent butyric. They found feed intake to be high to lowest in order presented (1, 2, 3, and 4) of 36.3, 35.8, 34.8, and 32.2 pounds on dry matter basis. Ketones reached high levels only with (4) and (1). Blood levels were 4.96 and 3.03 milligram per 100 milliliters. The high level acid intake (4) reduced dry matter intake and milk protein concentration. Variations in lactation performance were not significantly different. This points out that ruminants can utilize high level acetic and butyric acid levels in feeds. Huber et al. (41) in working with calves studied the effects of short chain fatty acids and concentration on calves fed normal milk diets. They evaluated fatty acid concentration in the small and large intestine and ceca. Calves were fed grain and milk plus (1) acetic acid; (2) 5 percent lactic acid; (3) 15 percent lactic acid. Acetic acid was predominant in all lower tracts of calves fed all rations except in (3) the high lactic ration. In (3) acetic and lactic were at similar levels. It was found that pH was inversely proportional to short chain fatty acid concentration. If pH level was high VFA concentration was low, the reverse was also true.

Senel et al. (84) in 1966 studied the relation of acetate and lactates to dry matter intake and volatile fatty acid metabolism. They added acids to a basal ration of 2/3 sorgum silage and 1/3 beet pulp and soybean oilmeal in a study of five dairy heifers. They used five test rations: (1) basal plus 9 percent lactates, (2) basal plus 2.8 percent acetate, (3) basal plus mixture of 5.9 percent lactate and 1.5 percent acetate, (4) basal ration. They found dry matter intake increased with high lactic and acetate supplementation in rations (1) and (2), but rations (3) and (4) were equal in intake. Body gains and efficiency were higher with rations (1) and (2) but not significantly. Rations high in acetate (2) increased molar percent of acetic acid whereas high lactates (1) decreased the proportion of acetic acid but increased propionic acid. In high lactate rations (1) blood levels of acetic, propionic and butyric were highest. Blood glucose and ketones were not apparently affected by ration (1). They concluded that lower dry matter consumption of grass and alfalfa hay silage and dry matter depression with other feed, is due to factors other than acetate and lactates present in high level in these silages. Ekern et al. (28) in study of young cattle observed that higher proportions of propionic acid and butyric to acetic acid in rumen favor best growth and body gains.

Radloff et al. (75) working with two groups of 25 milking cows fed one to 21 pounds daily of a mixture of lactates, and found increased sugar in blood and reduced ketones with lactate supplementation. Added lactates had no effect in causing ketosis or on milk production. Eight other cows fed lactose and sodium propionate showed similar results.

Resume

Corn silage is the highest yielding forage crop in terms of both dry matter and nutrients (TDN) produced per acre of land adapted to growing corn. Corn silage is outstanding nutritionally by its high starch (carbohydrate) content, but it is lacking in protein content. Utilization of corn silage is improved by protein supplementation. When properly supplemented with protein, corn silage can improve and/or maintain high levels of milk production in most dairy herds.

The addition of protein can be done by adding natural protein sources, but the most economical method is to utilize a non-protein-nitrogen source such as urea. Research has demonstrated that high digestibility of carbohydrates and retention of nitrogen result when urea is added to corn silage at ensiling time. The recommended rate is 10 pounds urea per ton of green chopped material. Further research is needed to determine the most economical and nutritional level. Levels of urea that can be added to grain concentrates have limits because of palatability and toxicity. The maturity of corn silage to which urea is added can greatly affect the fermentation process and resultant product. Harvesting at 32 to 35 percent dry matter has reduced silage seepage losses in corn nutrients. Seepage losses and undesirable fermentation can be wasteful of invested cost of adding urea nitrogen.

More mature corn silage (up to hard dough stage) provides highest level of dry matter yield per acre, and the resultant product contains more protein than immature corn silage. Cattle also consume more of the dryer corn silage and produce more milk because of greater daily dry matter intake.

Urea and other chemical compounds added to ensiled whole plant corn silage change the resulting fermentation as well as the feeding value and animal performance. Addition of urea in usual amounts to corn silage has increased crude protein equivalent of the silage about 4 percent on a dry matter basis. The addition of urea resulted in the silage containing slightly higher pH than in untreated corn silage. Fermentation as result of urea treatment prolonged both volatile fatty acid and lactic acid production. Acetic acid is the most important volatile fatty acid in energy metabolism of cattle and its level in silage is greatly increased during fermentation. Lactic acid levels in corn silage have been moderate to high as result of urea treatment. Results on the utilization of high lactic containing feeds differs among research studies. Urea treated silages contain higher levels of propionic and butyric acid. The increased level is thought to be advantageous in regard to the way the proportion is best maintained between acetate and propionate in volatile fatty acid metabolism in the ruminant.

An adequate diet of essential minerals must be maintained, only sulfur has been found seriously lacking when urea was fed with very low quality roughages. However, potassium, magnesium and carotene are under study; related research is not reported in this thesis.

Additives of limestone (0.5 percent), urea (0.5 percent) per ton of corn silage gave improved feed efficiency and rate of gains 6 to 7 percent with beef cattle. Milk production trials indicate no benefit for use of limestone in silage or in combination with urea. Milking cattle were reported to lower dry matter intake and loss in body weight during some feed trials.

The addition of urea to corn silage was found to be comparable to, or slightly less gain for young cattle compared to those fed oilmeals. In lactation studies, urea silage has provided production equal to oilmeal supplementation but not superior to other forms of protein suppliers. An economical source of nonprotein nitrogen is the main value in using urea to supplement corn silage.

EXPERIMENTAL PROCEDURE

The data reported in this research study covered corn silage grown in the 1965 crop year and made from entire field corn plant (excluding roots), harvested between August 31 and October 28, 1965 on 24 mid-Michigan dairy farms, and stored in vertical cement or tile silos for the purpose of feeding producing herds of dairy cows.

Selecting Research Silos

Increasing numbers of central Michigan dairy farmers have adopted the practice of adding urea to corn silage at filling time. A list of thirty dairy farmers located within twenty mile radius of the University was secured from Extension Agriculture agents in Ingham and Clinton counties. This group was divided into possible urea users and non-users of urea in corn silage. The experimental plan was to use ten farmer-treated silos compared to ten untreated silos in the same general farming location, with enough extras of both types to insure complete information from 20 silos.

It was more difficult to obtain untreated (control) silos as many of the farmers had decided to add urea in 1965.

A total of twenty-four silos were studied, eleven untreated (control) and thirteen treated with urea.

A total of four samples were drawn from each quarter. To make statistical analysis easier to program in the computer, two urea treated silos were dropped from the study. An uninformed, disinterested party wrote two numbers on a sheet of paper thus eliminating the data from two previously numbered silos from the study. Eleven treated silos were compared to eleven untreated control silos, by sampling each four times, one-fourth of the depth of the silage, as these preselected levels were reached and fed out by farmers. The following is a tabulation of silo samples:

Corn Silage Treatment	Number Silos	Number Sampled	Number Sampled/Silo	Total Number Analyzed
Urea treated	13	13	4	52
Untreated	11	11	4	44
Total	24	24	..	96

	Number Used In Statistical Summary	Number Used In Quarter Comparison
Urea treated	44	11
Untreated	44	11
Total	88	22

Securing Background Information

A farm information questionnaire was made out and each silo was marked with a 10 x 15 inch sign near the silo chute entrance, assigning it a number in the "Urea-Corn Silage Study of Michigan State University Dairy Department." Farmers were asked to provide information on a prepared questionnaire about silo size, make, type of chopper, length of cut, filling dates, leveling or distribution devices; kind, amount, and method of adding urea; and addition of any other materials such as water or calcium carbonate and whether seepage had occurred, and how long.

Further information was collected concerning corn variety used, beginning feeding date and possible emptying date, herd size, milking level, if available; feeding method for roughage, silage and grain and any change made in methods during the study period. Each farmer was asked to mail a pre-addressed postcard notice when he removed a pre-tagged silo door. This was to insure uniformity of quarter samples that came from the middle of each pre-selected and tagged quarter of the silo. Some selected bottom samples were taken later. Eight green chop samples were secured at filling time, and labeled for comparison to the quarter sample they represented later.

Climatic Effects on 1965 Corn Crop

Mid-central Michigan farmers had an unusual corn crop to harvest. It was low yielding, immature, and harvested later and dryer than normal. Spring planting had been delayed by lingering winter and late spring frosts. Late April and early May planted fields received adequate moisture and started growing quickly. These fields later produced the best crop in yield and maturity. Unusually low soil moisture in the preceding year made the below normal rainfall in late June and July more critical. Lack of adequate water in effect held back corn crop growth by two to six weeks. Farmers delayed harvest to allow the crop to ripen. A few of the test silos started filling early and were filled slowly. These silos juiced and seepage occurred for a long time into feeding period. Only three started filling before September 20 and ten were started to be filled after October 2. The result was two extremes: Four silos contained high moisture, immature, juicing silage. Three of these were treated. Five late filled silos represented four control and one treated silos which were extremely dry, leached, frosted corn, to which much water was added at filling time. The remaining fifteen silos contained near normal dry matter material, nine of these were treated, six were control silos. The following is a tabulation of silo filling information:

Corn Silage Treatment	Number Silos	Early May 28 to Sept.24	Middle Sept. 24 to Oct. 6	Late After Oct. 6 - 30
Urea	13	3	9	1
None	11	1	6	4
Total	24	4	15	5

Inequality of seasonal growth, filling dates, and maturity affected the silage product. Late rains and extremely muddy field conditions further delayed silo filling on test farms. Farmers who had normally stored one-third to one-fourth of their crop for silage used the entire 1965 crop for silage. Most farmers were short on corn to entirely fill their silos. Feed resources for coming fall were low, farmers fed corn silage at lower rates and supplies were stretched over a longer period. The depth of settled silage 7 to 10 days after filling was used to determine silage depth. Silage depth was divided in four equal quarters. The center silo door in each quarter was identified and tagged for sample testing.

General Silo Characteristics

Below are listed the variations in diameter and depth of urea treated and untreated corn silage. The following is a tabulation of silo diameters in feet:

Corn Silage	11 to 12 ft.	14 ft.	16 ft.	20 ft.	24 ft.	30 ft.	Totals
Urea treated	2	4	0	4	1	0	11
Untreated	6	0	1	3	0	1	11
Total	8	4	1	7	1	1	22

A tabulation of settled silage depth in feet is as follows:

Corn Silage	24 to 29 ft.	30 to 38 ft.	42 to 49 ft.	50 to 51 ft.	Totals
Urea treated	2	4	4	1	11
Untreated	1	5	3	2	11
Total	3	9	6	3	22

Untreated silos contained an average of 1.4 feet more depth of silage. Treated silos held 2,578 tons and untreated silos held 2,529 or 49 tons more in the treated silos due primarily to the larger diameter of this group.

Twenty silos were of cement stave construction, two were tile block construction. The study silos represented eight different makes.

The method of green chop distribution in silo while filling was as follows:

	By Hand	By Mechanical Distributor
Urea treated	7	4
Untreated	4	7
Total	11	11

Of the eleven farmers using mechanical distributors nine of these were Badger, one handmade similar to a Badger, and one an Even-Flow.

Fine chopped silage, a chop of 1/4" or less in length, was found in seven of the treated silos and only four of the untreated silos. Most of the farmers agreed they preferred a fine chop but were unable to maintain a fine chop cut with present equipment. Those farmers chopping dry corn material found it difficult to maintain sharp blades, even with frequent replacement and adjustment. Some stated their field choppers were set as near as they could be operated yet were medium to long in material length.

No calcium carbonate (limestone) was added to any of these silos.

Individual farmers reported eleven silos juicing or run off after filling. Seven of these were treated, two of them were early-filled silos which juiced heavily and one excessively throughout entire feeding period. Water was reported added to four silos of which two were treated, but others had water added to last few refill loads.

All except one of the silos were unloaded by external silo chutes; this one model had a center molded hole. The wall of this molded hole of high dry matter silage did not seal. Air was not excluded from the silage which was loose and fluffy. This condition favored aerobic bacterial development. This silage was covered with white mold which extended into most of the upper one-half of this silo. Dairy cattle refused to eat this feed.

Adding Urea to Green Chop Corn for Ensilage

Nine farmers added urea to eleven silos by spreading dry urea on the surface of green chop in chopper wagon before blowing combined green chop and urea treated material to the top of the silos. Most reported weighing early loads of green chop, then pouring urea into a pail and estimating or weighing out 35 to 50 pounds per load based on tonnage estimate of 3-1/2 to 5 tons of green chop corn per chopper wagon. Farmers intended to apply ten pounds of 45 percent nitrogen urea per ton of corn silage. One silo received 20 pounds per ton corn silage. Variations in the amount of corn silage contained on a chopper wagon load, resulted in some miscalculations in estimating the amount of urea added to green chop. Settled silage depth was used to calculate silo capacity in tons and then corrected back to amounts of urea used in treated silos. Of eleven treated silos one received 20 pounds per ton; five received 10 pounds per ton; one silo received 9.7 pounds per ton; one 9.6 pounds per ton; two 8.9 pounds per ton, and one 7.5 pounds per ton. The average addition of urea to these silos was 10.4 pounds per ton of settled silage. Six reported using fertilizer grade urea which contained 45 percent nitrogen. They paid an average price of $97.92 per ton. Five farmers reported using feed grade urea with 45 percent nitrogen and paying an average of $113.60 per ton. The average cost for urea used was

$0.0525 per pound. The average cost per silo for urea treatment of 0.5 percent was $105.56 for treating 234 tons of corn silage. The average cost of urea added to silos amounted to $0.45 per ton of silage.

Sampling Procedure

Green chop samples were taken at the blower as chopper wagons arrived from field. Eight green chop samples were obtained on first farm visits. Samples were taken by the hand grab method from the top surface, or unloading face, of chopper wagons. Green chop samples were collected before the addition of urea. The filling height of silage was recorded to insure a later comparison to material after fermentation, and to measure treatment effects on one-quarter of these eight silos. Four silos from which green chop samples were obtained received urea treatment and four samples did not.

Silo doors were selected to represent a central sampling position of each quarter. A reminder tag was attached to the removal handle asking the farmer to mail a "test now" card to the research worker. A tentative sample schedule was also planned, based on farmers' feeding schedules to insure obtaining a silage sample near the center of quarter-samples. Samples were collected weekly and frozen within 1 to 5 hours after sampling. Samples were carried in a car trunk, the weather was cold and there was no evidence of excessive heating of samples before freezing.

Silage was core-sampled using a 2" diameter straight tube core sampler 24" in length. In 21 silos it was driven by a 1/2" electric power drill, in three silos it was driven by a hand wood-auger brace. Eight core samples were taken and comprised one composite sample. Cores were expelled by a 28" length 3/4" water pipe plunger from sampler tube directly into 8" x 4" x 18" polyethylene airtight plastic bag. A 3-1/2 to 4 pound composite sample was mixed by hand in the plastic bag and one-half of the material placed in a separate plastic sack similarly labeled. Duplicate samples were stored in two locations either in the dairy department refrigerator at 20° C. or the bio-chemistry department cooler and later in the freezer room.

In selecting a place to remove core sample on face of silo, two samples were taken in each 1/4 pie wedge at varying distances from center but never closer than 18" from outside wall. No samples were taken in first quarter of silos until 7 to 10 days had elapsed from filling of that particular position. There was some heat in core samples in the first quarter samples when sampled followed filling by 10 to 17 days. From nine silos, five of which were treated, the silos were sampled during this early period after re-filling.

The necks of the plastic sacks were twisted and doubled back and sealed with rubber bands and had an identification tag attached giving it a sample number, material description, researcher's name, farmer's name, date, and height in silo sampled. Later a laboratory test number was assigned to each sample.

Preparation of Silage Samples for Analyzing

Individual, plastic-bagged silage samples were removed from freezer storage of 2^{o} to 3^{o} F before beginning each chemical analysis. Each composite sample became a mixture of reduced particle size by running semi-frozen wet samples through a Wiley mill using a 3/8" coarse screen. These ground and mixed wet silage samples were placed in large-mouth one-quart glass jars, and sealed with vapor proof lids to prevent moisture loss. These 3/4 pound samples were either held in a cooler at 38^{o} to 40^{o} F, and tested within a week or stored in a walk-in cooler at 20^{o} F.

Silage samples for testing were drawn in duplicate or triplicate preceding each chemical analysis, from one-quart holding jars. All tests were conducted on this fresh high moisture material. It was believed that ammoniacal-nitrogen could be more accurately measured from the wet material and that total nitrogen could be made to represent a truer measure of "as fed conditions" of this silage, if by careful handling and refrigeration, further bacterial action could be slowed down or impaired. Green chop, four quarter-samples and samples from the bottom of silos were tested for hydrogen ion concentration, moisture, ammoniacal-nitrogen, urea-nitrogen, total nitrogen, acetic, propionic, butyric, and lactic acid content. Supplementary feeds were also tested from each farm. Haylage and cornage (high moisture ear corn) samples collected from the same farmer were tested for hydrogen ion activity, moisture, ammoniacal-nitrogen, and Kjeldahl nitrogen. These samples were similarly ground and stored in refrigerator quart jars. Dry hay and farm grain mixtures were finely ground and tested for moisture and total nitrogen and then stored for future reference at room temperature in glass jars.

Statistical Tests Used in Study

1. Analysis of variance of effects of urea-treated silage and location in the silo.

Model: $Y_{ijk} = u+T_i+S_{ij}+L_k+(TL)_{ik}+(SL)_{ijk}+E_{ijk}$

Y_{ijk} = observation on a sample from the k<u>th</u> location in the j<u>th</u> silo, given treatment i

u = overall true mean

T_i = average true effect of treatment i

S_{ij} = effect of the j<u>th</u> silo given treatment i

L_k = effect of the k<u>th</u> location in the silo

$(TL)_{ijk}$ = interaction of treatment and location

$(SL)_{ijk}$ = interaction of silo and location in the silo

E_{ijk} = random error - effects of all other things influencing the magnitude of Y_{ijk}.

Sources of Variation	Degree of Freedom	Expected Mean Squares
Treatments	1	$\sigma^2 + 4\sigma_S^2 + 44k_T^2$
Location in silo	3	$\sigma^2 + \sigma_{SL}^2 + 22k_L^2$
Treatment x location interaction	3	$\sigma^2 + \sigma_{SL}^2 + 11k_{TL}^2$
Silo within treatment group	20	$\sigma^2 + 4\sigma_S^2$
Silo x location interaction	60	$\sigma^2 + \sigma_{SL}^2$

where a k^2 is a fixed component, a σ^2 is a random component.

It is obvious from the Expected Mean Squares that treatment differences must be tested by silo differences and that location differences and treatment by location interaction must be tested by the interaction of silos and locations.

Location differences were partitioned by orthogonal polynomial contrasts to study the response of certain characteristics to depth in the silo.

Also treatment by location interaction was partitioned by orthogonal polynomial contrast to study the optimal combinations of treatment and depth in the silo.

2. Correlation analysis

Simple correlations of traits studied were tested for statistical significance by standard methods.

Differences between correlations in treated and untreated silage were tested by transforming the simple regression coefficient to normally distributed values. Snedecor (88) gives r to z transformation in a chart.

3. Differences in nitrogen and acid levels between green chop corn and silage from the same material

Standard paired comparisons between green chop and silage were tested by students t-test for urea treated and untreated silage separately.

4. Simple linear regression predictions, Ostel (67)

Standard linear regression techniques were used to predict the pH of urea-treated and untreated silages, from dry matter content or from various nitrogen or acid components.

Also dry matter content was used to predict various nitrogen and acid levels and acetic acid content was used to predict ammoniacal-nitrogen and lactic acid content. Lactic acid was used to predict ammoniacal-nitrogen content.

All of these regressions fit the bivariate normal case rather than the strict assumption that an independent regression variable is measured without error. Therefore, the utility of any of the prediction equations must be demonstrated in additional data.

Dry matter percent was predicted from number of days delayed in harvest. This prediction does not suffer from the limitation of the others; i.e., in this case the independent variable is fixed.

CHEMICAL ANALYSIS

Moisture, pH, Ammoniacal-Nitrogen, Total Kjeldahl Nitrogen, Crude Protein Equivalent

Samples of green chop, corn silage as well as haylage and cornage were tested in duplicate for the above named constituents. Average of the results obtained were expressed on the natural moisture basis, corrected to a dry matter basis and are presented as such in subsequent tables.

Moisture in green chop silage, haylage and cornage was determined by drying weighed portions of suitable size in a hot air oven at 100° to 105° C for 24 hours. Moisture in air-dried ground grain and hay was determined by heating two-gram portions of the finely ground material in hot air oven at 100° to 105° C for 5.0 hours. The weight loss was considered as moisture originally present in same samples.

pH, representing the reciprocal of logarithmic expression of hydrogen ion concentration was measured by using external glass rod electrode of a Beckman pH meter inserted into wet silage mixture at room temperature and enough added distilled deionized water to wet sample and the electrode to obtain an electronic reading.

NH_3-N Ammoniacal-nitrogen in the moist samples was determined by adding magnesium oxide (MgO) and distilling the NH_3 into a charge of standard sulfuric acid, according to prescribed procedure of A.O.A.C. (103).

Total Nitrogen was determined by the Kjeldahl method. Crude protein-equivalent was derived by multiplying the Kjeldahl nitrogen values by the factor 6.25. Values found in results of this study were expressed in the following method: Total nitrogen and crude protein--equivalent on dry matter basis. Also as crude protein on as-received, natural moisture corn silage basis. Analytical procedures were in accordance with those outlined by A.O.A.C. (102).

Urea-Nitrogen Determination

A colormetric procedure discussed by Brown (15) was modified for use with corn silage. Urea was extracted from portions of silage with dilute sulfuric acid solution. The extracts were neutralized with sodium hydroxide (NaOH), then zinc sulfate ($ZnSO_4$), and p-diethylamenobenzaldehyde reagents were added. A yellow colored complex developed in suitable aliquots of this extract were determined using a Model B. Beckman Spectrophotometer. Concentrations of urea were evaluated from a curve relating the absorbancy of concentration of aliquots of a standard solution of urea similarly treated. Concentrations of urea-nitrogen were expressed on dry matter basis by calculating the percent of urea in aliquot to its original volume and weight in silage samples.

Volatile Fatty Acids, Acetic, Propionic and Butyric Acid Methods

The Wiseman and Irvin gas chromatography method (99) was used to determine the acetic, propionic and butyric acid consistency of the wet material. A representative silage sample of 25 grams was drawn with spoon from one quart-jar silage reserve quarter silo samples. A 25 gm semi-frozen sample was weighed into a 50 ml glass baby food jar, to which was added 25 ml of 0.4 normal sulfuric acid solution. The loose surface material was compressed with a blunt glass rod until all organic material became wet with liquid then the sample was sealed and stored for at least 72 hours at 38° to 40° F. The liquid was filtered through a double cheese cloth strainer. Residual was squeezed to expell about 10 ml of cloudy liquid collected in 10 x 150 mm test tubes. The extracts were centrifuged for 20 minutes at 2,000 rpm. A clear 5 ml of supernatant was pipetted into 10 x 100 mm storage test tubes. These supernatants were sealed in storage tubes with saran-lined cork stoppers to reduce evaporation losses. Extracts of unknowns were stored from one to ten days at 38° to 40° F until tested. Duplicate samples were extracted and used for volatile fatty acid and lactic acid analysis. The concentrations of acetic, propionic, and butyric acid were determined with Wilkens Aerograph, Gas Chromatography Models 550 and 600, equipped with hydrogen flame detector. A $\pm$ nine fast plastic column was packed with Wilkens 10 percent F.F.A.P. on thromosorb "W" acid washed D.M.C.S. 80/100. The column temperature was maintained in Model 550 at 138° C and Model 600 at 135° C. Approximately 0.4 microliters of unknown and standards were injected into the injection part with Hamilton 701 Micro Syringe. The response of each was read against a prepared standard solution of 40 micromole/ml of acetic acid, 12-1/2 micromoles/ml of propionic acid, and 12-1/2 micromoles/ml of butyric acid. Approximately one-third of the unknown extracts were diluted, two and three times to obtain a response comparable to those received with the standard solutions.

Lactic Acid Determination

The Baker and Summerson method of 1941 (4) with modifications by Pennington and Sutherland 1956 (74), and Umbriet *et al*. (90) in 1957, was used to determine lactic acid levels.

Stored silage-extract samples prepared for volatile fatty acid analysis were first deproteinized. Deproteinizing involved pipetting 1 ml of stored chillded extract into 10 x 100 mm test tube. To this solution was added 2 ml of distilled water and mixed; to mixture was added 1 ml of 1.8 percent barium hydroxide, and mixed. To this solvent 1 ml of 2 percent zinc sulfate solution added and a white precipitate was formed. These solutions were centrifuged for 10 minutes at 2,000 rpm. To reduce the concentration of lactate, 0.5 ml of supernatant was diluted with 4.5 ml of distilled water. This brought the concentration of .01 ml of unknown extract into a range comparable to that contained in the 0.1 ml of established lactate standard. Lactate standard was prepared by disolving 0.0339 grams of calcium lactate in distilled water, placed in a 1,000 ml volumetric flask and brought up to volume with distilled water. This standard was kept refrigerated and replaced frequently when absorbency decreased. The lactate standard was made up to deliver 30 micrograms of lactic acid/ml. A series of lactic standards of 0.1, 0.2, 0.5, 0.7 and 1.0 ml were run with each test group plus blank distilled water and tested against 1.0 ml of diluted deproteinized silage extract sample. Both unknowns and standards were pipetted into 15 x 125 mm test tubes and enough distilled water was added to bring volume up to 4.5 ml. To this was added 0.5 ml of 20 $CuSO_4 \cdot 5H_2O$. Approximately 0.5 grams $Ca(OH)_2$ was added and dispersed by shaking. Mixing procedure was repeated several times during a 30 minute period. The mixture was centrifuged for 10 minutes at 2,000 rpm, chilled at 38° to 40° F in a refrigerator for 15 minutes. One milliliter of the chilled aliquot was drawn off and placed slowly on 9.0 ml of ice-cold concentrated sulfuric acid, previously placed in sealable tubes: A pyrex 20 x 150 mm screw cap culture tube, with teflon liner. These tubes were stoppered, given a quick shake and heated in boiling water for five critical minutes. The tubes were cooled quickly in an ice water bath and 4 drops of 4 percent $CuSO_4 \cdot 5H_2O$ and 7 drops of p-hydroxy-diphenyl reagent were added and shaken. Tubes were allowed to set for 1 hour and mixed by shaking frequently. The later reagent was prepared by dissolving 1.5 gm of p-hydroxydiphenyl in beaker to which was added a minimum amount of distilled water and 0.5 percent NaOH. Heating over an electric plate aided in dissolving solids. The contents were transferred to a 100 ml volumetric, brought up to volume with distilled water and kept refrigerated.

Tubes were removed from ice and heated in boiling water for 90 seconds, returned to ice for 5 minutes, then allowed to return to room temperature before placing in spectrophotometer. The absorbency was read in a Beckman "B," at wave length of 565 millimicrons with distilled water blank set at zero. Duplicated sample results were held to comparable tolerance of 8 percent or less, or disregarded. A standard curve was prepared each day from a regression line obtained from ten values. Each unknown level of lactic acid reported was the average of two duplicated tests reported in micrograms of lactic acid per milliliter on wet silage basis, then expressed on a dry matter basis for comparison.

RESULTS

Chemical Data, Comparisons, and Correlations

pH Changes in Corn Silage Preservation

The variation in pH levels of eleven urea treated and untreated control silos are presented in Table 1. Urea treated corn silage had an average pH 3.96, with a range of 3.48 to 5.74. The control corn silage had an average pH 3.80, with a range of 3.42 to 4.78. Urea treated silage had a pH level which was 0.16 higher ($P > 0.05$) but significant at ($P < 0.10$).

Eight green chop corn samples were taken out of chopper wagons, at the blower site, of four urea treated and four control silos. The balance of the wagon load was traced into a particular silo quarter for later comparison with its parent material. The purpose of this study was to measure the affects of fermentation on green chop corn. The results of these studies are reported separately and presented in Table 7.

The green chop corn placed in control silos had an average pH 4.78. The comparable control silage had an average pH 3.71. The difference was significant ($P < 0.05$).

Green chop corn placed in urea treated silos had pH 5.22. The resulting urea treated silage was pH 4.09. The difference was not significant ($P > 0.05$) but was significant ($P < 0.10$). The pH level of initial green chop was not identical. Both were slightly lower than the averages of standing field corn, but within the range of initial green chop reported by Benne (11), Schmutz (81), and Karr (48).

Green chop which went into control silage was drier 37.58 percent, but increased in moisture to 34.77 percent, due in part to addition of water to more mature corn at filling time ($P < 0.05$). Green chop which went into urea treated silos increased in dry matter slightly from 34.81 to 35.89 percent. This increase was not significant.

A study of location effects on pH that had received urea and that which had not is presented in Table 6. The overall decline in pH of both urea treated and control silage, shows that differences found in all samples tested for location effects were significantly differen ($P < 0.01$). The pH of all urea treated silages was higher than the pH of control silage at all levels. The difference between pH of urea treated silage and control was least in the top quarter (difference pH 0.09), more in the second quarter (difference pH 0.12), greater in the third quarter (difference pH 0.18), and greatest in fourth quarter (difference pH 0.21). This linear decline by quarter location was found to be highly significant ($P < 0.01$). A further study of location effects shows the greatest reduction in pH was from top quarter pH 4.21 to second quarter pH 3.74. This difference was significant ($P < 0.01$). The pH of all third quarter samples of silage was higher pH 3.82 than second or fourth quarters which were identical pH 3.74. The resultant higher pH in third quarter was statistically significant ($P < 0.05$).

Dry matter variation may be a factor affecting the fermentation process. The variation in dry matter content by quarters follows this same pattern as pH. The top quarter of dry less dense corn silage did not acidify as rapidly as lower dry matter silage which was more compacted and found at the bottom quarter of the silos. The longer a silage remained in the silo the more acidified it became but at a decreasing rate. pH decline was slower in urea treated silage. Most of the top quarter silage samples were taken just after 8 to 10 days and the difference in pH between this and other quarters may have been due to the fact that with urea treatment, like dry material corn silage, delayed the fermentation process. The release of ammonia in the process may have also slowed pH decline. The lower pH in the third quarter pH 3.82 silage than in the second quarter above it or bottom quarter pH 3.74 in both, could be because movements of liquids within the silage mass. The fourth quarter was more moist and had the highest hydrogen ion concentration.

Correlations of all characteristics including all samples (N = 88) are presented in Table 3. The pH within all samples was positively correlated with total nitrogen (r = +0.44), crude protein equivalent, and ammoniacal-nitrogen (r = +0.45) concentrations on both wet and dry matter basis ($P < 0.01$). Likewise pH was negatively correlated with lactic acid content (r = -0.38) of silages in the overall analysis ($P < 0.01$). pH was strongly correlated with the dry matter content of control corn silage (r = +0.39), but not significantly, though positively correlated (r = +0.18) with dry matter content in urea treated silage (Table 4).

In urea treated silage ammoniacal-nitrogen concentrations were strongly correlated with pH (r = +0.66) but were not correlated with dry matter content in either the control of urea treated silages. In contrast ammoniacal-nitrogen concentrations were negatively correlated with pH (r = -0.39) in control silages.

Similarly urea nitrogen concentrations were positively correlated with pH (r = +0.44) in urea treated silage but not in control silage (r = +0.07).

Dry Matter Content of Silages

The average dry matter content and standard deviations of silages are presented in Table 1. Urea treated silage contained an average of 31.34 percent dry matter with a range of 27.28 to 38.23 percent. The control silage contained an average of 33.88 percent dry matter with a range of 25.58 to 55.20 percent. Control silages averaged 2.54 percent drier than those treated with urea. The difference in dry matter levels was related directly to date of harvest or filling date. The longer length of time (days) used to fill a silo gave greatest variation in dry matter content. Dry matter content was further increased by delayed or late harvested corn silage. Poor moisture conditions had delayed corn maturity, thus farmers delayed silo filling. Rains in late September and early October caused poor field conditions extending silo filling into late October. The range in number of days used by these twenty-two farmers was from one to thirty-four days. First filling occurred on August 31 and continued for 57 days up to October 28th. Later harvested corn was more mature, some frosted and leached, consequently, higher in dry matter content. Of eleven urea treated silos, seven were started before September 25th and filled more rapidly than control silos. Six of eleven control silos were started after September 25th and filled at a slower rate. The difference in initial dry matter may have affected the fermentation reaction and value reached for all characteristics studied.

A prediction formula was established, a linear regression on all 96 silage samples from 24 silos studied. The dry matter prediction formula was $\hat{y} = 25.4 + 0.207x$, the letter x equals the number of days after August 28th; 25.40 represents mean percent of dry matter on zero date; the symbol $\hat{y}$ equals given expected dry matter of silage at feeding date.

The estimated decrease in dry matter per day delay in harvest was 0.207 $\pm$ 0.034. The mean values and the standard errors for prediction of dry matter for 0, 30, and 60 days delay in harvest are 25.4 _ 1.11, 31.6 $\pm$ 0.42, and 37.8 $\pm$ 1.11 standard error. The F = test of these values was highly significant ($P > 0.05$). Six control silos with over 30 percent dry matter contained 3.43 percent crude protein on natural moisture basis, or 10.32 percent crude protein on a dry matter basis. Four control silos of under 30 percent dry matter had 2.69 percent crude protein on a moist basis, or 9.78 percent crude protein on a dry matter basis. Six urea treated silos with average dry matter content of over 30 percent had 3.84 percent crude protein on a moist basis or 14.57 percent crude protein on a dry matter basis. Four urea treated silos averaging less than 30 percent dry matter contained 3.37 percent crude protein on a moist basis, or 14.01 percent crude protein on a dry matter basis.

The effects of fermentation changes from green chop to preserved urea treated and control corn silage in selected traced locations is presented in Table 7. The control silage was 2.87 percent lower in dry matter and urea treated silage was 3.20 percent higher in dry matter than the original values of green chopped material. The lower dry matter in control silage was statistically significant ($P < 0.05$) but the higher dry matter in urea treated silage was not significantly different than the green chop material.

Dry matter levels are presented by silo quarter comparisons in Table 6. Dry matter percentage decreased from the top 34.47 percent to fourth quarter 31.50 percent of all silos, corresponding to pH decline. Dry matter decreased greatest from top quarter 34.47 percent to second quarter 31.95 percent this difference was significant ($P < 0.01$). The third quarter had higher dry matter content 32.53 percent than second or fourth quarters, which were very similar 31.95 and 31.50 percent. The difference in dry matter content was not statistically significant between second, third, and fourth quarter ($P < 0.10$). However, the fourth quarter having the lowest dry matter content was significant at ($P < 0.05$). As shown in Table 3 overall samples of the dry matter content were negatively correlated with the percent of lactic acid, total volatile fatty acids, acetic acid, urea-nitrogen on dry matter basis, and pounds of urea added per ton of green chop. Dry matter was positively correlated with urea-nitrogen as percent of total nitrogen present and propionic acid content on dry matter basis. The fact that moisture was not excessively lost from lower quarters of silos was probably because of relatively high mean dry matter content of most of the silages in the test.

The difference in correlations between urea treated and control silage summarized in Table 5 points out that the correlation between dry matter percent and urea-nitrogen percent on dry matter basis was the only characteristic concerning dry matter whose correlation was significantly different for urea treated silage than for control silage.

Total Kjeldahl Nitrogen and Crude Protein-Equivalent of Corn Silage

The concentrations of Kjeldahl total nitrogen and crude protein (Kjeldahl N x 6.25) are both presented on wet and dry matter basis in Table 1. Eleven control silos contained average of 1.601 percent nitrogen (range 1.243 to 1.873 percent), or average crude protein of 10.11 percent (range 8.16 to 11.71 percent). Eleven urea treated silages contained an average of 2.328 percent nitrogen (range 1.794 to 3.171 percent), or crude protein-equivalent average of 14.55 percent (range 12.61 to 17.09 percent). The addition of 10.4 pounds of 45 percent nitrogen urea increased the nitrogen content in urea treated silage by 45.4 percent and the crude protein-equivalent content of urea treated silage by 43.9 percentage units on a dry matter basis. This increase in both nitrogen and crude protein content as a result of urea treatment was statistically significant ($P < 0.01$) (Table 2).

The control silages averaged 3.42 percent crude protein-equivalent on a moist basis (33.88 percent dry matter) and the urea treated silages averaged 4.56 percent protein-equivalent at 31.34 percent dry matter. The difference in nitrogen content on moist basis was significant ($P < 0.01$).

Recovery of Added Urea as Increased Nitrogen

The increase in nitrogen of urea treated silage was compared to that of control silage plus the nitrogen which would have been added with 10.4 pounds of 45 percent nitrogen urea per ton silage corrected to a dry matter basis. These recovery rates are reported in Table 9 by quarters: Top quarter 100.5 percent, second quarter 94.6 percent, third quarter 98.2 percent, fourth quarter 99.9 percent. The high recovery in first quarter may be due to higher dry matter of control silage which was also higher in nitrogen content. The average recovery of all urea treated was 98.4 percent of expected level of total nitrogen.

Nitrogen and protein levels of green chop corn are compared to traced quarters of urea treated and control silage in Table 7. Green chop corn before ensiling contained 1.787 percent nitrogen or 11.17 percent crude protein, after fermentation control silage contained 1.708 percent nitrogen and 10.67 percent crude protein on dry matter basis. The difference in nitrogen was statistically significant ($P < 0.05$). Urea treated silage contained 2.292 percent nitrogen and 14.32 percent protein, whereas initial green chop contained 1.779 percent nitrogen or 11.12 percent protein on dry matter basis. The urea treated silage difference in protein content was significant ($P < 0.05$). In the fermentation process control silage lost, and urea treated silage gained in total nitrogen and crude protein content. Perhaps the gain as benefit of adding urea should be calculated from the lower fermented control silage levels of nitrogen or protein than from the higher levels of both in green chopped corn.

Quarter differences in urea treated and control silage are presented in Table 7. Quarter sample of either control or urea treated samples show no significant differences in nitrogen or crude protein content on dry matter basis. There was little variation in nitrogen or protein levels as a result of location within a silo.

Table 3 shows that levels of nitrogen and protein were positively correlated with levels of urea used per ton silage, ammoniacal and urea nitrogen, ammoniacal and urea nitrogen as percent of total nitrogen present. Acetic, butyric and total volatile fatty acid concentrations expressed on dry matter basis were positively correlated with nitrogen content.

Table 4 and its summary Table 5 express the difference in correlation of urea treated and control silage, which points out effects of urea treatment. The effect of adding urea to corn silage increased nitrogen and protein

content. This increase was correlated to increasing amounts of urea added per ton silage, ammoniacal-nitrogen, and ammoniacal and urea nitrogen as percent of total nitrogen present in treated silage. These differences were significant ($P < 0.05$).

The addition of 20 pounds of urea per ton of silage increased crude protein content 6.47 percentage units or an increase of 69.5 percent on dry matter basis. The addition of 10 pounds of urea per ton of silage resulted in crude protein content 4.23 percent or increase of 43.9 percent. The additional 10 pounds used in heavier application increased crude protein only 2.59 percentage units. Twenty pounds of urea per ton of silage was used in only one silo. This silo was high in moisture and apparently much of the added urea disappeared in the seepage from this silo resulting in lower level of nitrogen than might be expected.

Ammoniacal and Urea-Nitrogen Content

The increase of ammoniacal-nitrogen and urea nitrogen of all urea treated and control silos are compared in Table 1 and summarized in Table 2. Urea treated silage contained 0.801 percent urea nitrogen (range 0.361 to 1.522 percent). Control corn silage contained 0.288 percent urea nitrogen (range 0.009 to 0.396 percent). The standard deviation was 0.347 for overall levels of urea-nitrogen.

Urea treated silage contained 0.563 percentage units more urea-nitrogen than did control silage. The increase in urea-nitrogen as a result of adding 10.4 pounds urea per ton of corn silage represents 236.6 percent increase in urea treated silage.

Urea treated corn silage contained 0.465 percent ammoniacal nitrogen (range 0.044 to 1.336 percent). Control corn silage contained 0.116 percent ammoniacal-nitrogen (range 0.015 to 0.265 percent). The standard deviation of overall values was 0.235 for ammoniacal-nitrogen. Urea treated silage contained 0.349 percentage units more ammoniacal-nitrogen than did control corn silages. The increase in ammoniacal-nitrogen as result of adding 10.4 pounds urea per ton corn silage represents 210.2 percent increase in urea treated corn silage. Both ammoniacal and urea-nitrogen increases were significantly higher than in the control corn silage ($P < 0.01$).

The levels of ammoniacal, urea, and total nitrogen are found in Table 7. The changes are measures of green chop compared to selected quarters of urea treated and control corn silage expressed on percent of the dry matter basis. Green chop corn contained 0.421 percent urea nitrogen which was reduced in untreated corn silage to 0.264 percent urea nitrogen. Ammoniacal-nitrogen was increased from 0.039 percent in the green chop to 0.13 percent in the fermented corn silage. The green chop placed in traced

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quarters of silos contained 0.377 percent urea nitrogen which was increased in urea treated corn silage to 0.564 percent urea nitrogen. Ammoniacal-nitrogen increased in green chop from 0.044 percent to 0.503 percent in urea treated corn silage. The increase in ammoniacal-nitrogen in urea treated silage was 3.87 times greater than that found in control corn silage. Treatment of corn with urea increased the level of urea-nitrogen by 13.4 percent in comparison to its original green chop level. Urea treated corn silage contained 2.1 times more urea nitrogen than did control corn silage. The addition of 10.4 pounds of urea per ton silage resulted in higher increase in ammoniacal-nitrogen (3.81 times) also an increase in urea-nitrogen which was somewhat lower 2.1 times as compared to selected traced quarters of control silage.

The study of differences of ammoniacal and urea-nitrogen by quarter is presented in Table 6. Control corn silage in the top quarter contained 0.256 percent urea-nitrogen and decreased slightly to 0.226 percent in bottom quarter of silage. Urea treated silage in top quarter contained 0.629 percent urea-nitrogen and increased greatly within silo to a high level 0.881 percent in the bottom quarter of silos. Because control silage lost in percent of urea nitrogen content and urea treated silage gained in urea content the overall change in content of urea-nitrogen of all tested samples was not statistically significant. The eff

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effect of adding 10.4 pounds of urea per ton of silage, had the most increase in urea nitrogen in the lower three quarters of the silo. The greatest increase of urea-nitrogen was found in bottom quarters of urea treated silage.

Control corn silage in top quarter contained 0.089 percent ammoniacal nitrogen and increased to 0.131 percent in bottom quarter of silos. Urea treated silage in top quarter of silo contained 0.436 percent ammoniacal-nitrogen and increased to 0.510 percent in the bottom quarter of silos. Ammoniacal-nitrogen content was slightly increased in lower quarters of both urea treated and control silos but not significantly. The addition of urea accounted for differences in ammoniacal-nitrogen content. Urea treatment resulted in greater increase in ammoniacal-nitrogen in top quarter and third quarter (4.9 and 4.3 times greater) than bottom and second quarter (3.9 and 3.2 times) increase of ammoniacal-nitrogen content.

Ammoniacal and Urea Expressed as Percent of Total Nitrogen Present

Ammoniacal and urea-nitrogen are each only a part of total Kjeldahl nitrogen measured in control and urea treated silages and are presented in Table 1 and summarized in Table 2.

Total Kjeldahl nitrogen in control corn silage consisted of 7.23 percent ammoniacal-nitrogen, 14.93 percent urea-nitrogen, and 77.87 percent other forms of nitrogen.

In urea treated silage 19.59 percent was ammoniacal-nitrogen, 34.42 percent urea nitrogen and 46.44 percent other forms of nitrogen as percent of total nitrogen present. The differences between control and urea treated silage were highly significant at ($P < .01$). As both the amount of ammoniacal and urea-nitrogen increased as result of adding urea, the nature of total nitrogen was altered. There was an increase of 169.7 percent in ammoniacal-nitrogen and 130.5 percent increase in urea-nitrogen accompanied by a decrease of 40.3 percent in other forms of nitrogen of the total nitrogen found in urea treated silage.

The comparison of green chop to traced quarters of urea treated and control silages are presented in Table 7. In corn silage ammoniacal-nitrogen, as percent of total nitrogen, increased from the green chop level 2.21 percent to 7.68 percent respectively. Urea nitrogen as percent of total nitrogen decreased from green chop level of 23.70 percent to 15.51 percent of the total nitrogen in control silage. Other forms of nitrogen as percent of total nitrogen increased from green chop level of 74.09 percent to 76.46 percent in control silage.

Ammoniacal-nitrogen as percent of total nitrogen increased greatly from green chop level 2.51 percent to 21.85 percent in urea treated silage.

Urea-nitrogen as percent of total nitrogen increased only slightly from green chop level of 21.03 percent to

24.79 percent in urea treated silage. Other forms of nitrogen present as percent of total nitrogen decreased from the green chop level 76.46 percent to 53.36 percent in urea treated silage. Both gain in ammoniacal-nitrogen, and loss in other forms of nitrogen present as percent of total nitrogen in urea treated silage were statistically significant ($P < 0.05$).

The study by different quarters in percent of ammoniacal, urea, and other forms of total nitrogen are presented in Table 6. The interaction of location and treatment are presented in Table 8 for these same factors. All factors are expressed on dry matter basis. Control corn silage in the top quarter contained 5.43 percent ammoniacal-nitrogen, 16.25 percent urea-nitrogen and 78.34 percent other forms nitrogen as percent of total nitrogen present. Ammoniacal-nitrogen percent increased in both second and fourth quarter, 8.01 and 8.13 percent, respectively. Urea percentage decreased gradually with lowest level in bottom quarter of silos 14.04 percent. Other forms of nitrogen present decreased slightly but was rather uniform in third and fourth quarter 77.91 percent and 77.92 percent but lower than the two other quarters. Urea treated silage increased ammoniacal-nitrogen percentage by 2.70 percent, from top quarter of 18.62 percent to bottom quarter 21.32 percent of total nitrogen present. Urea percentage increased by 10.28 percent from top quarter level 26.99 percent to bottom

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quarter of 37.23 percent ($P < 0.01$). This linear trend was significant ($P < 0.05$). Other forms of nitrogen present decreased by 12.96 percent from top level of 54.39 percent to bottom quarter 41.43 percent of total nitrogen present. Table 8 in the test for interaction shows that the changes in quarters of ammoniacal-nitrogen as percentage of total nitrogen were not significant ($P < 0.10$) but changes within quarter of urea treated and control silages were significant ($P < 0.01$).

Table 8 shows the interaction between treatment and location effect. Urea-nitrogen as percent of total nitrogen increased in urea treated silage from top quarter to bottom, where in control silage it decreased in each quarter. Linear test of interaction effects was significant ($P < 0.05$). Other forms of nitrogen as percent of total nitrogen decreased from top quarter to bottom quarter in urea treated silage but remained rather stable to slightly less from top quarter to fourth quarter in control corn silage. The linear test of interaction effects was significant ($P < 0.01$).

The correlated factors of ammoniacal and urea-nitrogen as percent of total nitrogen are presented in Table 3. As percent of ammoniacal and urea-nitrogen increased as part of total nitrogen there was a decrease in other forms of nitrogen present as might be expected. The correlation of these two factors as they are effected by urea treatment as control silage is presented in Table 4 and summarized in

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Table 5. The difference between correlations that resulted by adding 10.4 pounds of urea to corn silage as ammoniacal-nitrogen as percent of total nitrogen increased, the percent of lactic acid decreased also as urea-nitrogen as percent of total nitrogen increased; there was a decrease in ammoniacal-nitrogen as a percent of total nitrogen. The large amount of urea-nitrogen present in top quarter of urea treated and control silos and low level of ammoniacal-nitrogen in this top quarter of control silage may have been responsible for this correlation ($r = -0.52$), significant at ($P < 0.05$).

Acids of Fermentation

The concentration of acetic, propionic, butyric, total volatile fatty acids and lactic acid in both urea treated and control corn silage are presented in Table 1. Control corn silage contained an average of 0.880 percent acetic acid (range 0.212 to 1.841 percent); 0.073 percent propionic acid (range 0.0 to 0.39 percent); an average of 0.034 percent butyric acid (range 0.0 to 0.3 percent); an average of 0.987 percent volatile fatty acids (range 0.261 to 1.979 percent). The average lactic acid content of control silage was 4.22 percent with range of 0.581 to 9.850 percent on dry matter basis.

Urea treated corn silage contained an average level of 1.211 percent acetic acid (range 0.46 to 2.723 percent); 0.06 percent propionic acid (range 0.0 to 1.034 percent);

and 1.456 percent volatile fatty acids, with range from 0.46 to 3.0 percent. The average content of lactic acid was 5.445 percent with a range of 2.008 to 9.288 percent. All the above values are given as a percent of the dry matter. The differences in acid content of urea treated and control silage is presented in Table 2. The most statistically significant increase as result of urea treatment is 37.6 percent increase in acetic acid concentration. The increase in butyric acid of 250.0 percent was large but even with this increase it is still present at extremely low levels. The difference is statistically significant ($P < 0.05$). Propionic acid production was reduced (-16.4 percent) as result of urea treatment. Very low levels were present and wide variation was involved. The difference was not significant ($P > 0.10$).

Total volatile fatty acids increased 47.5 percent as a result of urea treatment. The increase was almost entirely the result of increased acetic acid. The difference between urea treated and control values for VFA was significant ($P < 0.01$).

Lactic acid content was increased 29.0 percent as result of the urea treatment. Difference in urea treated and control silage content was significant ($P < 0.05$). The increase in acetic acid followed by increase in lactic acid level of urea treated silage were the most notable differences observed in silage acid content.

The content of organic acids in green chop is compared to traced quarter locations in urea treated and control corn silages in Table 7. Green chop contained 0.174 percent acetic acid, 0.029 percent propionic acid, and no percent butyric acid for a total volatile fatty acid content of 0.203 percent. Green chop samples contained 0.66 percent lactic acid on dry matter basis. After fermentation this control silage contained 0.689 percent acetic acid, 0.89 percent propionic acid, 0.052 percent butyric acid, 0.831 percent total volatile fatty acids, and 2.929 percent lactic acid on a dry matter basis. The increase as result of fermentation in control silage was significant for acetic, total volatile fatty and lactic acids ($P < 0.05$) but not significant for propionic and butyric acid ($P > 0.10$).

The green chop which later was placed in four urea treated silos contained 0.247 percent acetic, 0.035 percent propionic, no butyric, 0.282 percent total volatile fatty and 0.978 percent lactic acid on a dry matter basis. After fermentation the traced quarters of urea treated silage contained 0.806 percent acetic, 0.091 percent propionic, 0.153 percent butyric, 1.201 percent total volatile fatty acids and 3.969 percent lactic acid on a dry matter basis. The increase as result of fermentation in urea treated corn silage was highly significant for acetic, lactic, and total volatile fatty acids ($P < 0.01$), but not significant ($P > 0.10$) for differences in propionic, or butyric acid. Urea treatment resulted in 2/3 more acetic, and total volatile fatty acids and 4/5 more lactic acid than control corn silage by fermentation.

The effects of location of the sample in the silo on organic acid content of urea treated and control corn silage is presented in Table 6. The acetic and lactic acid levels are definitely different in different quarters of the silo and are significant ($P < 0.01$). In control silos acetic acid shows a gradual increase from the top quarter level 0.51 percent to fourth quarter level of 1.130 percent. Lactic acid in control silage showed this same effect. The top quarter contained 2.511 percent compared to the fourth quarter content 5.967 percent on dry matter basis.

Urea treated silage showed a remarkably different pattern. Acetic acid was present at the top quarter with content 0.957 percent, increased the most and to its highest level in the second quarter 1.313 percent, and remained relatively high in third and fourth quarter 1.279 and 1.295 percent on a dry matter basis. The quarter differences between urea treated and control silage were statistically significant ($P < 0.01$). The urea treated top quarter contained 87 percent more, the second quarter 52 percent more, the third quarter 25 percent more, and the fourth quarter 15 percent more acetic acid than control silage from comparable quarters.

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Urea treated silage showed still a different type of location effect in lactic acid content. The level was high in the top quarter (4.311 percent) and continued to increase with highest content of 6.23 percent in fourth quarter of silos. The second quarter level of lactic acid was 5.651 percent and higher than third quarter which contained 5.583 percent on dry matter basis. The quarter difference as a result of urea treatment was significant ($P < 0.01$). The urea treated top quarter contained 75 percent more, second quarter 40 percent more, third quarter 7.0 percent more, and fourth quarter 4.5 percent more lactic acid than comparable quarters in control silage.

Although there were quarter differences in content of propionic butyric and total volatile fatty acid these variations were not statistically significant ($P > 0.10$).

Acetic acid content of silage was highly correlated ($r = 0.94$) with total volatile fatty acids and butyric acid levels ($r = 0.42$) as reported for overall correlations in Table 3. Butyric acid content was positively correlated with total volatile fatty acids, and with increases in ammoniacal-nitrogen as percent of total nitrogen. All the above correlations were significantly ($P < 0.01$). Total volatile fatty acids were correlated with levels of total nitrogen, ammoniacal-nitrogen, and ammoniacal-nitrogen as percent of total nitrogen. Total volatile fatty acids were negatively

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correlated with "other forms of nitrogen" as percent of total nitrogen ($P < 0.01$) in the overall analysis.

Correlation coefficients for both urea treated and control silages are presented in Table 4 and summarized in Table 5. As a result of urea treatment as acetic acid and volatile fatty acid content increased so did lactic acid levels on dry matter basis ($P < 0.01$ and $P < 0.05$) respectively. As lactic acid content increased in urea silage the levels of ammoniacal-nitrogen and ammoniacal-nitrogen as percent of total nitrogen decreased. The above correlations are significant ($P < 0.01$).

Sampling Study

The urea-nitrogen test was used to measure if the composite sample of silage was representative of surface of silo in which each test sample was taken. A third location was used in three separate silos. Each were sampled in excentric rings starting at center circle of 18 inches and moving out 18 inches for second ring sample. Three to four core borings were made in each ring. One silo had four ring samples, two silos had five ring samples. Silo 60 and 100 contained urea. Silo 105 was untreated control silo.

It could be concluded from sampling study that the degree of error in composite samples was very insignificant; the variations across face of silage represent care, necessary for drawing representative samples of silage, in corn silage studies.

Table 1. Mean, standard deviation, and range[a] values for tested characteristics of urea treated and control corn silage

Table 1. Mean, standard deviation, and range[a] values for tested characteristics of urea treated and control corn silage

	Urea Treated Corn Silage				Control Corn Silage			
	Average[b]	S.D.[c]	High to	Low	Average[d]	S.D.[c]	High to	Low
1. pH	3.960	0.390	5.740	3.480	3.800	0.340	4.780	3.420
2. Dry matter %	31.340	2.990	38.230	27.280	33.880	6.100	55.200	25.580
5. Crude protein % (d.m.)[e]	14.550	1.410	17.090	12.610	10.110	0.820	11.710	8.160
14. Nitrogen % (d.m.)	2.328	0.226	3.171	1.794	1.601	0.137	1.873	1.243
15. NH_3-N % (d.m.)	0.465	0.219	1.336	0.044	0.166	0.041	0.265	0.015
7. Urea-N % (d.m.)	0.801	0.274	1.522	0.361	0.238	0.075	0.396	0.009
8. Acetic acid % (d.m.)	1.211	0.552	2.723	0.460	0.880	0.399	1.841	0.212
9. Propionic acid % (d.m.)	0.060	0.064	0.265	0.000	0.073	0.079	0.390	0.000
10. Butyric acid % (d.m.)	0.119	0.208	1.034	0.000	0.034	0.065	0.300	0.000
11. Total VFA % (d.m.)	1.456	0.679	3.000	0.460	0.987	0.431	1.979	0.261
12. Lactic acid % (d.m.)	5.445	1.895	9.288	2.008	4.222	2.085	9.850	0.581
16. NH_3-N as % total N	19.590	7.220	42.130	1.780	7.230	2.390	15.150	0.550
17. Urea-N as % total N	34.420	11.085	58.020	18.760	14.930	4.970	28.000	0.560
18. Other N as % total N	46.440	10.792	69.370	23.670	77.870	6.110	91.150	62.950
13. # Urea added per ton corn silage	10.400	3.160	20.000	7.500	0.000	0.000	0.000	0.000

[a]range = limits of variations in levels studied. [b]Average 44 urea treated silage.

[c]S.D. = standard deviation. [d]Average 44 control corn silage. [e]d.m. = dry matter.

2. Summary of urea treatment effects

Table 2. Summary of urea treatment effects

	All Samples Overall		Difference Treated Minus Control	Standard Error of Means Difference	% Change[f]	Statistical Significance Probability of Type 1 Error
	Average	S.D.[e]				
1. pH	3.870	0.370	0.160	0.170	4.2	$P < 0.10^{s}$[d]
2. Dry matter %	32.610	4.940	-2.540	3.700	-7.5	$P > 0.10^{ns}$[c]
5. Crude protein %	12.330	2.510	4.440	0.624	43.9	$P < 0.01$**[b]
14. Nitrogen % (d.m.)	1.965	4.100	0.727	0.308	45.4	$P < 0.01$**
15. NH_3-N % (d.m.)	0.290	0.235	0.349	0.080	210.2	$P < 0.01$**
7. Urea-N % (d.m.)	0.519	0.347	0.563	0.089	236.6	$P < 0.01$**
8. Acetic acid % (d.m.)	1.046	0.507	0.331	0.235	37.6	$P < 0.05$*[a]
9. Propionic acid % (d.m.)	0.066	0.072	-0.013	0.141	-16.4	$P > 0.10^{ns}$
10. Butyric acid % (d.m.)	0.077	0.159	0.085	0.254	250.0	$P < 0.05$*
11. Total VFA % (d.m.)	1.221	0.613	0.469	0.270	47.5	$P < 0.01$**
12. Lactic acid % (d.m.)	4.830	2.074	1.224	0.305	29.0	$P < 0.05$*
16. NH_3-N as % total N	13.410	8.200	12.270	8.370	169.7	$P < 0.01$**
17. Urea-N as % total N	24.680	13.000	19.490	3.670	130.5	$P < 0.01$**
18. Other N as % total N	62.150	18.050	-31.420	4.210	-40.3	$P < 0.01$**
3. Crude protein % (wet basis)	4.020	0.620	1.100	0.241	32.2	$P < 0.01$**
4. Nitrogen % (wet basis)	0.640	0.140	0.180	0.241	32.7	$P < 0.01$**

[a]*Significant ($P < 0.05$). [b]**Significant ($P < 0.01$). [c]ns = not significant ($P > 0.10$). [d]s = significant ($P < 0.10$). [e]S.D. = standard deviation. [f]Urea treated mean - Control mean ÷ Control mean x 100 = % of change as result of urea treatment.

Table 3. Significant correlations of tested characteristics for all (88) silage samples studied

	1	2	3	4	5	7	8	9	10	11	12	13	14	15	16	17	18
18. Percent other N as percent of total N	n.s.	0.29	n.s.	-0.57	-0.84	-0.93	-0.39	n.s.	0.35	-0.43	-0.28	0.85	-0.83	-0.77	-0.78	-0.90	0.00
17. Percent urea-N as percent of total N	n.s.	-0.28	0.29	0.42	0.67	0.98	n.s.	n.s.	n.s.	0.27	0.32	0.73	0.66	0.45	0.46	0.00	
16. Percent NH_3-N as percent of total N	0.38	n.s.	n.s.	0.62	0.81	0.55	0.43	n.s.	0.44	0.50	n.s.	0.75	0.81	0.98	0.00		
15. Percent NH_3-N (d.m.)	0.45	n.s.	n.s.	0.66	0.85	0.57	0.41	n.s.	0.44	0.47	n.s.	0.77	0.85	0.00			
14. Percent total N (d.m.)	0.33	n.s.	0.47	0.78	0.99	0.78	0.34	n.s.	0.32	0.40	n.s.	0.89	0.00				
13. # Urea per ton silage	0.26	-0.28	0.33	0.63	0.89	0.81	0.30	n.s.	n.s.	0.32	n.s.	0.00					
12. Percent lactic acid	-0.38	-0.49	n.s.	n.s.	n.s.	0.29	0.33	n.s.	n.s.	n.s.	0.00						
11. Percent total VFA (d.m.)	n.s.	-0.32	n.s.	n.s.	0.39	0.30	0.94	n.s.	0.59	0.00							
10. Percent butyric acid (d.m.)	n.s.	n.s.	n.s.	0.32	0.33	n.s.	0.42	n.s.	0.00								
9. Percent propionic acid (d.m.)	n.s.	0.38	n.s.	n.s.	n.s.	n.s.	n.s.	0.00									
8. Percent acetic acid (d.m.)	n.s.	-0.44	n.s.	n.s.	0.33	0.28	0.00										
7. Percent urea-N (d.m.)	n.s.	-0.28	0.35	0.53	0.79	0.00											
5. Percent crude protein (d.m.)	0.34	n.s.	0.47	0.78	0.00												
4. Percent total N (wet basis)	0.44	0.43	0.99	0.00													
3. Percent crude protein (wet basis)	0.27	0.66	0.00														
2. Percent dry matter	n.s.	0.00															
1. pH	0.00																
	pH	% dry matter	% crude protein wet basis	% total N wet basis	% crude protein equivalent dry matter basis	% urea-N dry matter	% acetic acid dry matter	% propionic acid dry matter	% butyric acid dry matter	% total VFA dry matter	% lactic acid dry matter	# urea per ton silage	% total N dry matter	% NH_3-N dry matter	% NH_3-N as percent of total N	% urea-N as percent of total N	% other-N as percent of total N

Note: n.s. = not significant values under 99 percent probability; $P < 0.01$ must have critical value $\pm$ 0.27; $P < 0.05$ must have critical value $\pm$ 0.21; and d.m. = dry matter.

nd control corn silage samples

Table 4. Correlations coefficients of both urea treated and control corn silage samples

	Urea Treated Silage[a]																
	1	2	3	4	5	7	8	9	10	11	12	13	14	15	16	17	18
18. Percent other N as percent of total N	0.02	-0.35	-0.26	0.01	0.35	0.78	0.18	-0.13	0.22	0.15	0.00	0.29	0.35	0.37	0.34	0.72	
17. Percent urea-N as percent of total N	-0.44	-0.37	0.02	-0.28	-0.01	0.96	-0.04	-0.32	-0.06	-0.10	0.25	0.15	-0.01	-0.31	-0.36		0.93
16. Percent NH_3-N as percent of total N	0.59	0.09	-0.35	0.46	0.54	-0.22	0.28	0.23	0.38	0.31	-0.34	0.20	0.54	0.97	Control	0.28	0.62
15. Percent NH_3-N (d.m.)	0.66	0.03	-0.25	0.54	0.70	-0.13	0.27	0.22	0.38	0.31	-0.36	0.31	0.70		0.97	0.21	0.55
14. Percent total N (d.m.)	0.52	-0.12	0.24	0.66	0.99	0.24	0.15	0.15	0.24	0.18	-0.31	0.47		0.31	0.07	-0.21	-0.14
13. # Urea per ton silage	0.22	-0.28	-0.06	0.12	0.47	0.27	-0.01	-0.22	-0.02	-0.11	-0.09		0.00	0.00	0.00	0.00	0.00
12. Percent lactic acid (d.m.)	-0.49	-0.37	-0.27	-0.51	-0.31	0.14	0.01	-0.11	-0.38	-0.07		0.00	-0.07	0.21	0.25	0.01	0.10
11. Percent total VFA (d.m.)	0.06	-0.28	-0.34	-0.06	0.18	-0.06	0.92	0.28	0.60		0.49	0.00	0.05	0.53	0.54	0.18	0.36
10. Percent butyric acid (d.m.)	0.13	0.07	-0.09	0.26	0.24	-0.02	0.41	0.01	Urea Treated	0.42	-0.16	0.00	-0.12	0.31	0.36	0.34	0.42
9. Percent propionic acid (d.m.)	0.23	0.10	0.00	0.20	0.15	-0.27	0.22		0.30	0.25	-0.18	0.00	0.05	0.31	0.31	-0.05	0.08
8. Percent acetic acid (d.m.)	0.00	-0.42	-0.45	-0.20	0.14	-0.01		0.02	0.23	0.96	0.59	0.00	0.07	0.46	0.47	0.15	0.30
7. Percent urea-N (d.m.)	-0.31	-0.37	0.11	-0.09	0.24		0.17	-0.04	0.30	0.20	-0.02	0.00	0.09	0.33	0.32	0.95	0.90
5. Percent crude protein (d.m.)	0.52	-0.11	0.24	0.66		0.23	-0.05	-0.03	0.02	-0.05	-0.16	0.00	0.79	0.19	-0.01	0.03	0.02
4. Percent total N (wet basis)	0.51	0.67	0.99		0.51	0.18	-0.40	0.40	0.16	-0.27	-0.50	0.00	0.53	0.03	-0.10	0.02	-0.02
3. Percent crude protein (wet basis)	0.02	0.63		0.99	0.50	0.14	-0.45	0.36	0.12	-0.33	-0.52	0.00	0.50	-0.09	-0.22	-0.01	-0.09
2. Percent dry matter	0.18		0.92	0.92	0.15	0.12	-0.45	0.48	0.19	-0.30	-0.51	0.00	0.25	-0.04	-0.10	0.03	0.01
1. pH		0.40	0.39	0.34	-0.05	0.07	-0.37	0.29	0.01	-0.29	-0.46	0.00	-0.08	-0.39	-0.41	0.07	-0.10

[a]Significance of correlation coefficients is as follows: P 0.01 = ± 0.36; P 0.05 = ± 0.27; P 0.10 = ± 0.21.

[b]d.m. = dry matter. Note: For comparisons read down on column and then across.

Table 5. Differences in correlation coefficients between urea treated and control corn silage (only statistically significant are calculated)

	Urea Treated Corn Silage Minus (-) Control Corn Silage											
	1	2	3	4	5	8	11	12	13	14	15	16
17. Urea-N as % of total N	-0.51*	n.s.[a]	n.s.	n.s.	0.54*	n.s.	n.s.	n.s.	n.s.	n.s.	-0.64**	-0.52*
16. NH_3-N as % of total N	1.00**	n.s.	n.s.	0.56*	n.s.	n.s.	n.s.	-0.59**	n.s.	0.47*		
15. NH_3-N (d.m.)[b]	1.05**	n.s.	n.s.	0.51*	0.51*	n.s.	n.s.	-0.57**	n.s.	n.s.		
14. Total N (d.m.)	0.60**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.47*			
13. # Urea/ton silage	n.s.	n.s.	n.s.	n.s.	0.47*	n.s.	n.s.					
12. Lactic acid (d.m.)	n.s.	n.s.	n.s.	n.s.	n.s.	0.58**	0.56*					
11. Total VFA (d.m.)	n.s.	n.s.	-0.67**									
7. Urea-N (d.m.)	n.s.	-0.49*										
5. Crude protein (d.m.)	0.57**											
1. pH												
	pH	% dry matter	% crude protein (wet basis)	% total N (wet basis)	% crude protein equivalent dry matter basis	% acetic acid (d.m.)	% total VFA (d.m.)	% lactic acid (d.m.)	# urea per ton silage	% total N (d.m.)	% NH_3-N (d.m.)	% urea-N as percent of total N

[a]n.s. = not significantly different (P < 0.05).

[b]d.m. = dry matter.

*P 0.05 difference = ± 0.43.

**P 0.01 difference = ± 0.57.

Table 6. Mean values for 14 characteristics of urea treated and control silage at each of four locations

	pH	% Dry Matter	% Crude protein (d.m.)	% Total Nitrogen (d.m.)	% NH_3-Nitrogen (d.m.)	% Urea-Nitrogen (d.m.)	% Acetic Acid (d.m.)	% Propionic Acid (d.m.)	% Butyric Acid (d.m.)	% Total Volatile Fatty Acids (d.m.)	% Lactic Acid (d.m.)	% NH_3-N as Percent of Total N	% Urea-N as Percent of Total N	% Other N as Percent of Total N
Top Quarter														
Control silage	4.15	35.92	10.35	1.658	0.089	0.256	0.510	0.058	0.021	0.589	2.511	5.43	16.25	78.34
Urea treated	4.26	33.02	14.58	2.318	0.436	0.629	0.957	0.071	0.054	1.082	4.315	18.62	26.99	54.39
Difference	0.09	-2.90	4.23	0.660	0.347	0.373	0.447	0.013	0.033	0.493	1.804	13.19	10.74	-23.95
Average	4.21	34.47	12.46	1.974	0.255	0.442	0.733	0.065	0.038	0.836	3.413	12.03	21.62	66.36
Second Quarter														
Control silage	3.68	33.40	9.99	1.596	0.129	0.236	0.867	0.090	0.031	0.988	4.020	8.01	14.69	77.30
Urea treated	3.80	30.50	14.36	2.285	0.416	0.858	1.313	0.068	0.122	1.503	5.651	17.88	37.24	44.79
Difference	0.12	-2.90	4.37	0.689	0.287	0.622	0.446	-0.022	0.091	0.515	1.631	9.87	22.55	-32.51
Average	3.74	31.95	12.18	1.925	0.266	0.547	1.090	0.079	0.077	1.246	4.835	12.94	25.97	61.04
Third Quarter														
Control silage	3.73	33.41	9.99	1.601	0.117	0.234	1.013	0.095	0.022	1.130	5.228	7.34	14.76	77.91
Urea treated	3.91	31.64	14.88	2.333	0.503	0.837	1.279	0.045	0.225	1.504	5.583	20.54	36.21	43.34
Difference	0.18	-1.77	4.99	0.732	0.386	0.603	0.266	-0.050	0.203	0.374	0.355	13.20	21.45	-34.57
Average	3.82	32.53	12.44	1.957	0.304	0.535	1.146	0.070	0.123	1.339	5.406	13.94	25.48	60.62
Fourth Quarter														
Control silage	3.64	32.80	10.11	1.627	0.131	0.226	1.130	0.047	0.064	1.241	5.967	8.13	14.04	77.92
Urea treated	3.85	30.20	14.73	2.357	0.510	0.881	1.295	0.056	0.076	1.427	6.230	21.32	37.23	41.43
Difference	0.21	-2.60	4.62	0.730	0.379	0.655	0.165	0.009	0.012	0.186	0.263	13.19	23.19	-36.49
Average	3.74	31.50	12.42	1.976	0.314	0.553	1.213	0.052	0.070	1.335	5.677	14.73	25.63	59.67
Total All Quarters														
Control	3.80	33.88	10.11	1.601	0.116	0.238	0.880	0.073	0.034	0.987	4.220	7.23	14.93	77.87
Urea treated	3.96	31.34	14.55	2.328	0.465	0.801	1.211	0.060	0.119	1.456	5.445	19.59	34.42	46.44
Difference	0.16	-2.54	4.44	0.727	0.349	0.563	0.331	-0.013	0.085	0.469	1.225	12.36	19.49	-31.43
Average of all test silos	3.88	32.61	12.33	1.965	0.290	0.519	1.046	0.066	0.076	1.221	4.833	13.41	24.68	62.15
Overall Significance	P<.01**	P<.01**	P<.10	P<.10	P<.10	P<.10	P<.01**	P<.10	P<.10	P<.10	P<.01**	P<.10	P<.10	P<.05*
Linear	P<.01**	P<.01**	...	...	...	...	P<.01**	...	...	...	P<.01**	...	...	P<.05*
Quadratic	P<.01**	P<.10	...	...	...	...	P<.10	...	...	...	P<.10	...	...	P<.10
Cubic	P<.05*	P<.05*	...	...	...	...	P<.10	...	...	...	P<.01	...	...	P<.10

Table 7. Nitrogen and acid levels in green chop corn and comparable urea treated and control silages from known locations in the silos

	Harvested Green Chop	Control Fermented Corn Silage	t[a]	Sign	Harvested Green Chop	Urea Treated Fermented Corn Silage	t[a]	Sign.	Green Chop Overall Average
1. pH	4.780	3.710	-4.610	*[c]	5.220	4.090	-3.040	n.s.[b]	5.000
2. Percent dry matter	37.580	34.770	-5.110	*	34.810	35.890	0.306	n.s.	36.190
5. Percent crude protein (dry matter)	11.170	10.670	-1.440	n.s.[b]	11.120	14.320	5.800	*[c]	11.140
14. Percent nitrogen (dry matter)	1.787	1.708	-5.180	*	1.779	2.292	1.710	n.s.	1.783
15. Percent NH_3-N (dry matter)	0.039	0.130	1.980	n.s.	0.044	0.503	1.760	n.s.	0.041
7. Percent urea-N (dry matter)	0.421	0.264	-6.670	*	0.377	0.564	2.910	n.s.	0.399
8. Percent acetic acid (dry matter)	0.174	0.689	3.190	*	0.247	0.806	23.850	*	0.210
9. Percent propionic acid (dry matter)	0.029	0.089	1.900	n.s.	0.035	0.091	2.220	n.s.	0.032
10. Percent butyric acid (dry matter)	0.000	0.052	1.730	n.s.	0.000	0.153	1.260	n.s.	0.000
11. Percent total VFA (dry matter)	0.203	0.831	8.020	*	0.282	1.201	2.720	n.s.	0.242
12. Percent lactic acid (dry matter)	0.667	2.929	5.510	*	0.978	3.969	10.530	*	0.822
16. Percent NH_3-N as percent of total N	2.210	7.680	1.960	n.s.	2.510	21.850	17.500	*	2.360
17. Percent urea-N as percent of total N	23.700	15.510	1.770	n.s.	21.030	24.790	0.837	n.s.	22.360
18. Percent other N as percent of total N	74.090	76.460	0.139	n.s.	76.460	53.360	4.990	*	75.270

[a]t = t test, Ostel (67); [b]n.s. = not significant ($P < 0.10$); [c]* = significant above ($P < 0.05$); and critical test < 0.05 = ± 3.182.

Table 8. Interaction effects of location and treatment for urea treated and control corn silage

	Top Quarter		Second Quarter		Third Quarter		Fourth Quarter		Average of All Quarters Quarter		Treatment X Location			
	U.T.	Con.	U.T.	Con.	U.T.	Con.	U.T.	Con.	U.T.	Con.	Overall Average	Linear	Qua-dratic	Cubic
Lactic acid	4.315	2.511	5.651	4.020	5.583	5.228	6.230	5.967	5.445	4.220	P >0.10	...	...	...
NH_3-N as percent of total N	18.620	5.430	17.880	8.010	20.540	7.340	21.320	8.130	19.590	7.230	P >0.10	...	...	...
Urea-N as percent of total N	26.990	16.250	37.240	14.690	36.210	14.760	37.230	14.040	34.420	14.930	P <0.01	P <0.05	P >0.10	P >0.10
Other N as percent of total N	54.390	78.340	44.780	77.300	43.340	77.910	41.430	77.920	46.440	77.870	P <0.01	P <0.01	P >0.10	P >0.10

Table 9. Urea recovery percentage determined as increased total nitrogen*

	Top Quarter		Second Quarter		Third Quarter		Fourth Quarter		Average of All Quarters	
	U.T.	Con.	U.T.	Con.	U.T.	Con.	U.T.	Con.	U.T.	Con.
% total nitrogen Kjeldahl method (d.m. basis)	2.333	1.590	2.298	1.599	2.324	1.598	2.350	1.618	2.327	1.601
Recovered percent urea as increased nitrogen	100.5%		94.6%		98.2%		99.9%		98.4%	

*Means of all urea treated location quarters compared to control silage quarters with chemical nitrogen added, which would be supplied from 10.4 pounds 45 percent nitrogen urea per ton corn silage on dry matter basis.

[a] U.T. = urea treated silages.

[b] Con. = control silages.

Table 10. Urea nitrogen. A comparison of subsamples to composite samples of three silos

	Silo No. 60[a]	Silo No. 100[a]	Silo No. 105[b]	Summary
	urea-nitrogen level on dry matter basis			
1st center 18" circle	.243	.248	.047	
2nd 18" ring	.264	.262	.043	
3rd 18" ring	.143	.196	.034	
4th 18" ring	.213	....	.040	
Average individual silo sample same level	.216	.235	.041	.157
Composite	.213 (#64)	.241 (#103)	.054 (#109b)	.169
Difference	+.003	-.006	-.013	-.012
4 and 5 outside 18" ring	.117	.226	.082	

[a]Silo 60-100 contained 0.5 percent urea.

[b]Silo 105 contained untreated corn silage.

[c]Tested separately not included for comparison of composite sample.

DISCUSSION OF RESULTS

In this study corn silage with addition of 10.4 pounds of urea added per ton decreased less in pH than the control corn silage ($P > 0.10$). The difference in urea treated and control silage pH was insignificant in statistical test. The reaction of urea in corn silage may have been more important from biological affect. The rate of change in pH may have had a definite affect on preservation and palatability of treated silage. The slightly higher pH in urea silage indicates a less concentrated acid mixture. Schmutz _et al_. (81) reported corn silage with 0.75 percent urea with pH 3.71, 0.5 percent urea treated at pH 3.72, control silage at pH 3.65. The affect of urea to alter the decline of pH was greater in this study than that experienced by Schmutz. The silage treated with 0.52 percent urea in this study in no way approaches the buffer effect reported by Schmutz when calcium carbonate, dicalcium phosphate, diammonium phosphate or mixtures of these combined with urea were used. He reported acidity for these additives to be pH 3.73 to 4.42. Byers _et al_. (21) reported that one percent limestone resulted in treated silage with pH 4.2, and control silage pH 3.85, a difference of pH 0.35. In studies by Klosterman _et al_. (52) 1.0 percent urea treated silage had pH 4.4, 0.5 percent urea silage pH 4.1, control silage pH 3.8. The last two levels are almost identical to results found in this study which may have involved more mature corn.

Green chop samples in this study had an average pH 5.0. Most investigators agree freshly copped corn is acid. Schmutz et al. (81) reported green chop had averaged ph 4.5 and 5.1 in 1964; pH 5.4 in 1965. The one low pH 4.5 is not characteristic of others, or similar samples found in his study. This sample later had 1.0 percent urea added to it and increased to pH 7.4. This lower level pH in initial green chop and high pH rise during fermentation did not agree with other work reported. Karr et al. (48) reported green chop with pH 5.4. Benne et al. (11) in 1964 found a range of pH in corn plant parts August 27, 1964 to be pH 5.1 to 6.8. This would indicate that green chop may be more uniform in pH than resulting silage. Green chop corn is slightly acid at harvest, and declines rapidly in the fermentation process within six to ten days following filling as reported by some workers. This decline in pH can occur as material is lying in loaded chopper wagons, waiting to be unloaded at the silo. The slightly acid green chop is important to fixation of ammonia released from added urea, as well as other nitrogen and natural proteins.

pH decline was less affected by the presence of added urea in higher dry matter corn silage found in the top quarter of the silos. Urea treated silage shoed greatest dif

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difference in pH in the lower dry matter silage found in the bottom quarter when compared to control silage. This higher pH in drier silage was accompanied by large increases in ammoniacal nitrogen in the top half of the silos. Urea may have been more completely hydrolyzed to ammonia in the drier silage silage material.

Both urea treated and control silage in this study had higher dry matter content than that reported by others. Schmutz et al. (80) worked with silage containing 24 to 28 percent dry matter. Higher levels of nitrogen and crude protein equivalent are found in the dry matter of higher dry matter silage. This more mature, higher dry matter effect on increased protein equivalent levels agrees with Hillman et al. (35), Johnson et al. (44), and Huber et al. (40). The control silages in this study averaged 10.11 percent crude protein-equivalent on dry matter basis. Schmutz et al. (81) control corn contained 9.6 percent crude protein-equivalent. The difference may be due in part to the lower dry matter and nitrate content of silage. Schmutz reported a direct relationship between dry matter and crude protein-equivalent expressed as a percent of dry matter.

Only one silage sample had an unusually high pH. This sample (No. 101) from the top quarter silage was treated with 1.0 percent urea and had a pH 5.74 with 27.84 percent dry matter which was one of the lowest dry matter samples in the study. This silo had stood for a nine month period after filling (September 1, 1965 opened May 27, 1966). Spoilage near the surface may have caused a rise in pH in top quarter sample. Spoilage action in this quarter was indicated by a higher than normal propionic and butyric acid content. The second quarter (sample No. 102) of the same silo had pH 3.5 which was next to the lowest encountered in urea treated corn silage. Increased pH can not be explained as effect of using up to 1.0 percent urea per ton of silage. This study was not designed to determine effects of various amounts of urea in silage. Only one silo contained 20 pounds per ton. Nine silos were treated with 10 pounds $\pm$ 0.4 pounds urea. Only one reported using 7.5 pounds urea per ton. The nitrogen content of this silage was equal to others which were reported to contain heavier additions. An error in estimating the amount of urea added might account for the discrepancy.

pH differed the most in the third and fourth quarters, or bottom one-half of silos, and less in the top one-half as result of urea treatment. Both lactic acid and ammoniacal-nitrogen also increased most in the upper half of silos. It is doubtful if one could define urea as a buffer reagent in corn silage. The biological definition is: "A buffered solution resists the changes in (H+) which would otherwise result from addition of an acid or base to solution." Buffering capacity represents an important means of mair

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maintaining constant pH in the region near neutrality. A buffer defined in Webster's dictionary is any devise, person or thing used to lessen or absorb the shock or impact. In this sense the ammonia, a weak base, may have been an absorber of released hydrogen ion in the preservation process. As pH declined in the lower levels of silos the percentage of total nitrogen and crude protein equivalent increased slightly. This accumulation could be the result of movement of soluble and gaseous nitrogen compounds within the silage mass.

When farmers had not added urea in a day's harvest, the nitrogen content in silage samples was always lower. Untreated areas were located in three urea silo quarters, new quarter samples were taken seven days later. Whether or not the nitrogen level equaled untreated silage depended upon its location in relation the the adjoining treated urea mass. Juices and ammonia moved into the untreated area and increased its nitrogen content above control silage levels.

Green chop corn lost dry matter content while in the fermentation process. As urea hydrolyzed a portion of the ammoniacal-nitrogen apparently was carried upward as heat or moisture vapors raised and condensed in the upper surface of silage causing high ammoniacal-nitrogen levels in top quarter. The decline in ammoniacal-nitrogen levels in second and third quarters could denote upward movement under heat. Urea hydrolysis to ammonia was reported by Schmutz et al.

(81) to be 8 to 12 days in length and by Lassiter et al. (55) to be 6 to 10 days in length. Because of increased pressure, liquids may be moved downward, squeezed out, and drained off at the silo base. In such case urea could move downward to filter out in lower silage quarters and expelled juices.

pH was strongly correlated with the dry matter content of control corn silages (r = 0.39) but was not significantly though positively (r = 0.18) correlated with dry matter in urea treated silages. This apparent contradiction can be explained largely by the narrow range in dry matter content of treated silages (s.d. = 3.0) compared to the relatively wide range in dry matter found in the control silages (s.d. = 6.1). Likewise the relatively small amount of ammoniacal-nitrogen found in control silage probably exerted little influence toward increasing pH and in fact was negatively correlated (r - -0.39) with pH in control silage. In urea treated silage ammonia levels were strongly correlated with pH (r = +0.66) but were not correlated with dry matter content in either the control or urea treated silages. Since dry matter content was negatively correlated with lactic acid (r = -0.37) and acetic acid (r = -0.42) in urea treated silage and ammoniacal-nitrogen levels were also negatively correlated with lactic acid production (r = -0.36) it appears that the relationship of ammoniacal-nitrogen to pH is largely incidental to conditions affecting lactic acid production. The highest pH was in fact found in the top quarter of silos where silage was drier, less packing probably occurred and less fermentation time elapsed between filling and sampling.

Nitrogen recovery did not show a decline in top or fourth quarter of urea treated silos. Nitrogen recovery in this study ranged from 94.6 percent in the second quarter to 100.5 percent in the first quarter. This is a higher recovery than reported by Schmutz et al. (81) who calculated the recovery rates as crude protein equivalent to be 85 to 95 percent on as fed basis. Bentley et al. (13) reported urea recovery values ranging from 94 to 112 percent respectively, over a three year period for silages containing 17 to 25 pounds of urea per ton. The method used to determine relative concentrations can definitely alter values for recovery. If pre-treatment green chop was by chance lower in nitrogen than that analyzed as control silage the recovery might not have been so great. With a large number of samples involved as in the case of this study it appears that nitrogen only changes form, but remains present in the silage acid media. Urea nitrogen was most evident higher in bottom quarters of silo.

In the sampling check study, urea nitrogen was higher in the center and less concentrated toward the outside edge of urea treated silos. In control silage, where

urea-nitrogen was twice the level in green chop as recorded in silage, the highest concentration was at the outer walls with less in central area of silo. However, samples were few in number and these indications need further confirmation.

Urea increased the total nitrogen and crude protein equivalent content of treated silage significantly ($P < 0.01$) by 4.45 percentage units on dry matter basis. Schmutz _et al_. (81) reported 4.0 percent increase. The actual input of urea per ton of silage was higher 10.4 pounds; Schmutz used 10.0 pounds per ton of silage. Higher dry matter silage (35.3 percent) compared to 24.4 percent dry matter used by Schmutz could have reduced seepage losses. The parent material may have also been higher in nitrogen. The increased nitrogen observed agrees with work reported by Bentley _et al_. (13), Wise _et al_. (98), Gorb _et al_. (31), Goode _et al_. (30), Palamaru _et al_. (70), and Klosterman _et al_. (52)(53).

The nature of nitrogen present in silage is altered during the fermentation process. Control silage contained a low level of nitrogen 1.6 percent dry matter basis, of which 78 percent was contained in compounds other than urea and ammonia, 15 percent urea-nitrogen, and 7 percent ammoniacal-nitrogen. Urea treated silage contained 2.3 percent nitrogen on dry matter basis, of which 46 percent was in other forms of nitrogen, 34 percent urea nitrogen, 20 percent ammoniacal-nitrogen. Urea treatment resulted mostly in

an increase of ammonia and secondly in urea nitrogen. Protein and other sources of nitrogen involve ammonia release in digestion. The silage fermentation could conceivably break down cellulose as well as synthesize protein from urea for ruminant utilization. Urea treated silage could because of the altered form release ammonia over a wider period of time in the rumen. The mixing of urea in silage further reduces the risk involved when used with grain concentrates. Cattle are less likely to consume a toxic amount of urea from corn silage than from a grain mixture.

The major organic acids of fermentation were increased by addition of urea to corn silage, control silage ranged from 0.21 to 1.84 percent acetic acid and 0.58 to 9.85 percent lactic acid. Urea treated silage contained 0.46 to 2.72 percent acetic acid and 2.0 to 9.29 percent lactic acid in this study. These values are in agreement with those reported by others. Karr et al. (48) reported control silage contained 0.95 percent acetic and 5.45 percent lactic acid on dry matter basis. Urea and biuret treated silage contained 0.87 and 0.95 acetic and 6.45 to 6.15 percent lactic acid, respectively.

The levels of major organic acids observed in this study were lower than those reported by Schmutz et al. (81) who found levels of 1.08 to 2.28 percent acetic acid and 8.42 to 13.06 percent lactic acid on dry matter basis as a result of adding 0.5 percent urea. This may have been due to the fermentation of lower dry matter silage.

Acetic and lactic acids represented the greatest increase as a result of urea addition. Both of these acids were at highest levels in the lower quarter of silos. The greatest alteration in acetic acid content occurred in top quarters of urea treated silos, where control silage contained 0.510 percent compared to 0.957 percent in treated silage; second quarter 0.867 to 1.313 percent acetic acid. Lactic acid followed a similar pattern with top quarter control 2.51 to treated 4.32 percent and 4.02 to 5.65 percent in the second quarter on dry matter basis.

If Barnett (5) was correct in stating that best fermentation causes stimulation of lactic acid production in silage which can inhibit other undesirable bacteria action, then the addition of urea achieves this objective and provides more readily available acetic acid as well as lactic acids to control bacteria action and improve the energy value of silage.

If lactic acid is metabolized into pyruvic acid, which can be altered into acetic or propionic acid, as utilized into energy in the citric acid cycle, then it too is advantageous to meeting the glucose requirements of ruminants.

Watson et al. (92) suggested that acetic acid utilization in the ruminant is controlled by maintaining appropriate levels of propionic acid. Urea treatment also effects level of propionic and butyric acid. This study found butyric acid in control and urea treated silages, respectively, to be 0 to 0.3 percent and 0 to 1.034 percent, and propionic acid to be 0 to 0.39 percent and 0 to 0.265 percent on a dry matter basis. Schmutz et al. (81) suggested both butyric and propionic acids would be increased by addition of urea, but did not present substantiating data. Both butyric and propionic acid were present at very low concentrations. Butyric acid levels did not affect total volatile fatty acid levels nearly as much as did the large predominant increase in acetic acid. Schmutz et al. (81) and Klosterman et al. (50) reported that calcium carbonate had greater effect on building acetic and lactic acid levels than did addition of urea.

Lactic acid and acetic acid were negatively correlated with dry matter content in both control and urea treated silage.

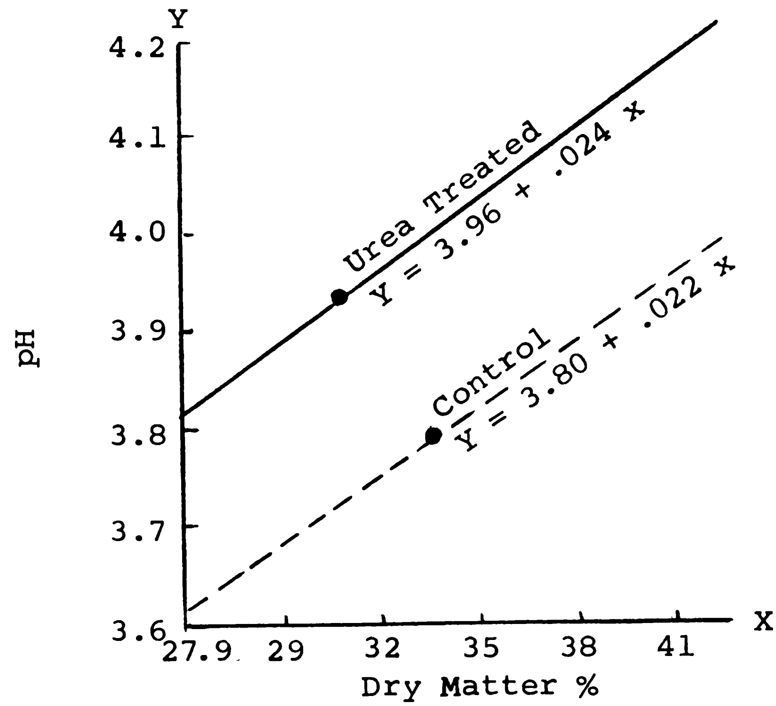

Fig. 1. Relationship of dry matter to pH.

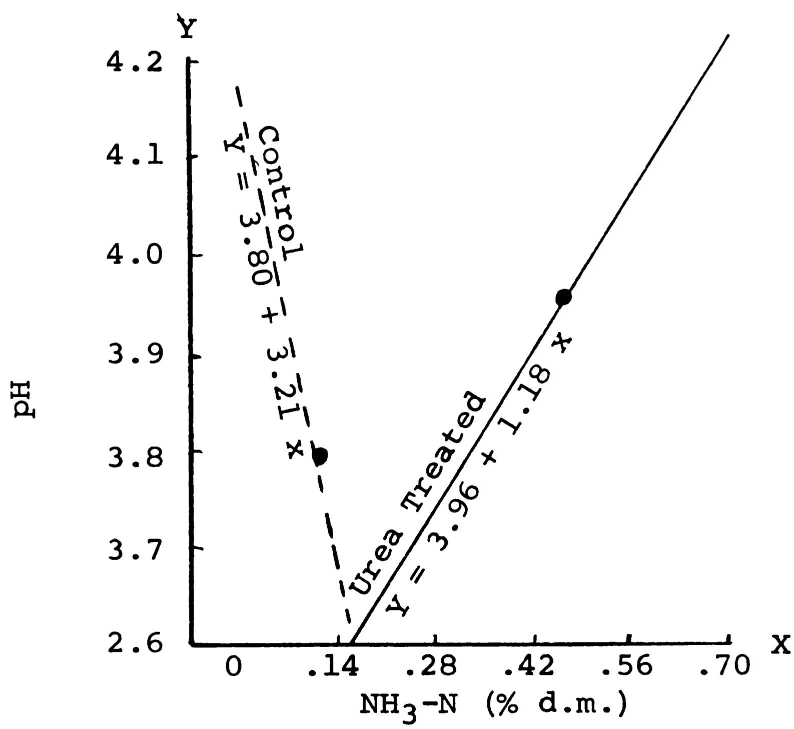

Fig. 2. Relationship of ammoniacal-nitrogen to pH.

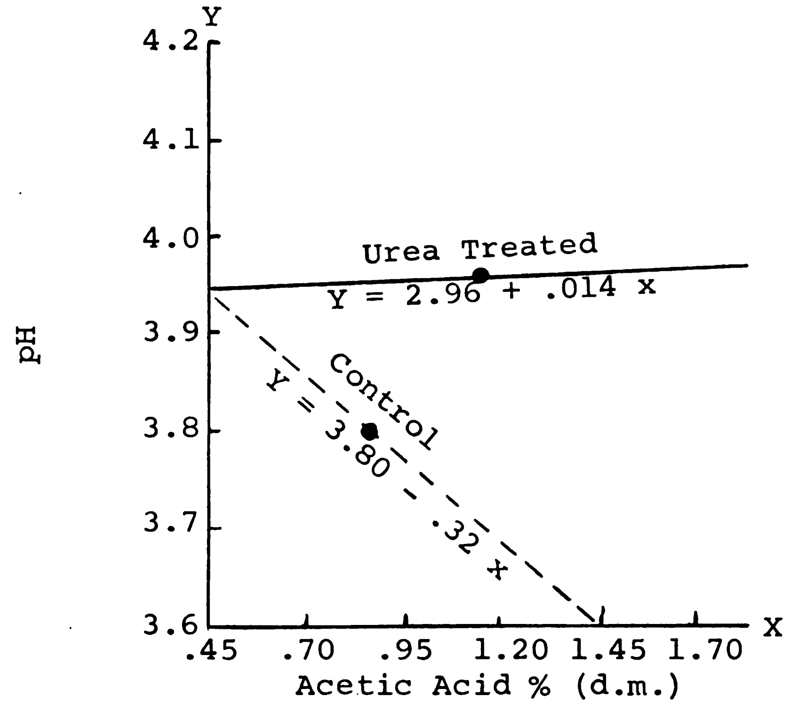

Fig. 3. Relationship of acetic acid to pH.

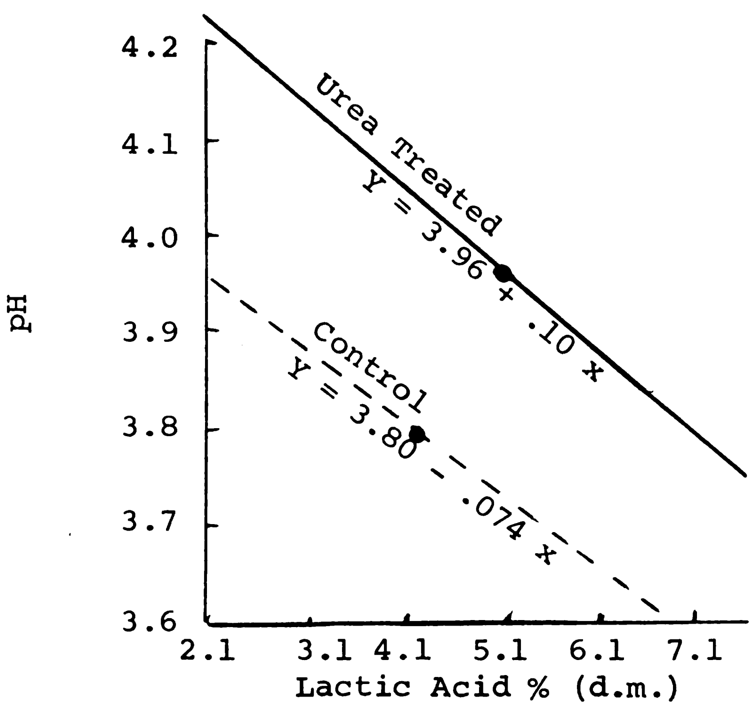

Fig. 4. Relationship of lactic acid to pH.

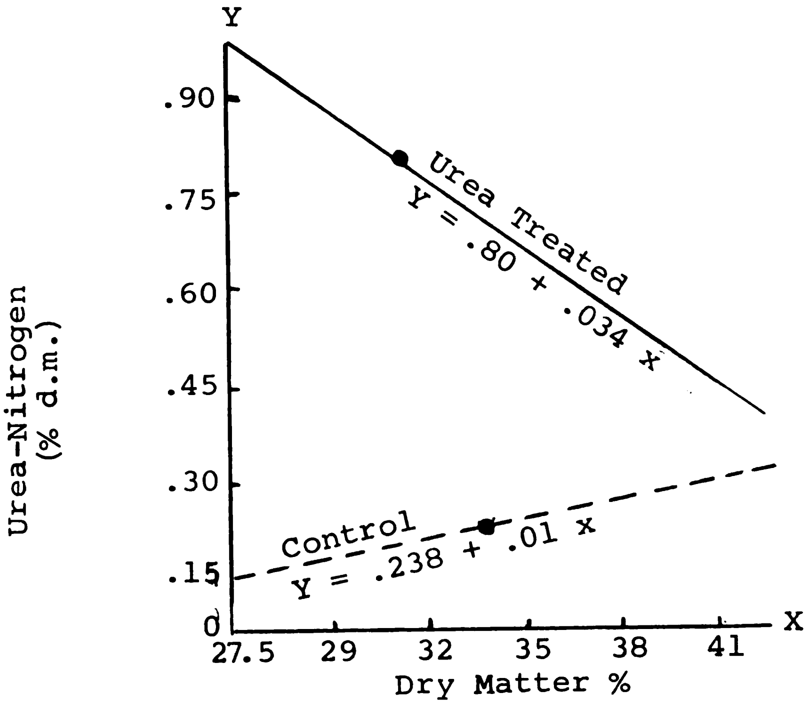

Fig. 5. Relationship of dry matter to urea-nitrogen.

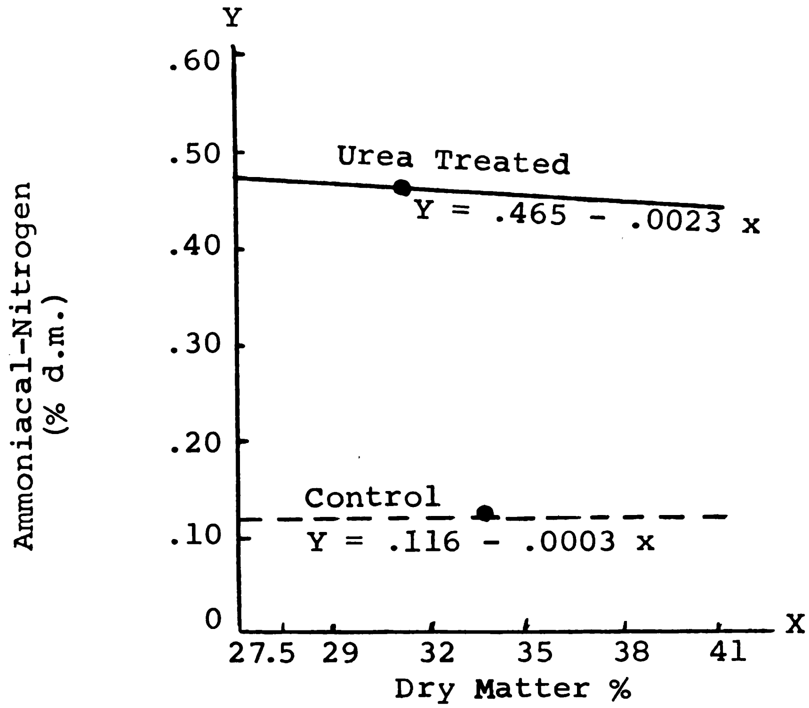

Figure 6. Relationship of dry matter to ammoniacal nitrogen.

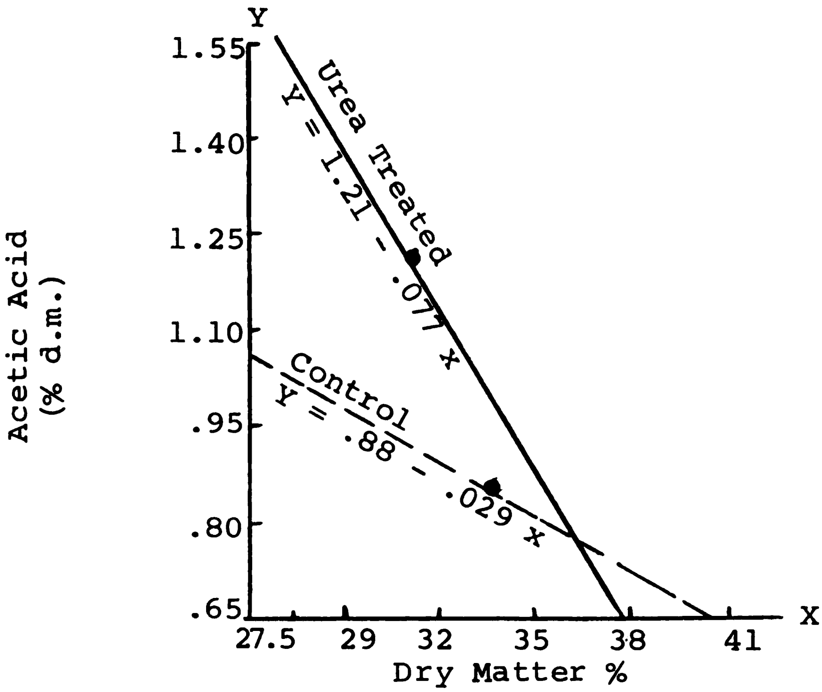

Fig. 7. Relationship of dry matter to acetic acid.

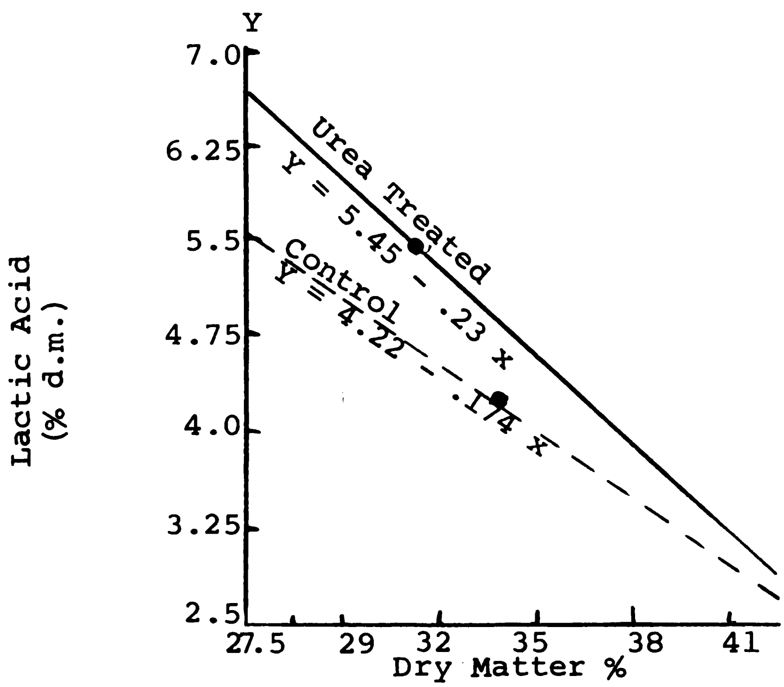

Fig. 8. Relationship of dry matter to lactic acid.

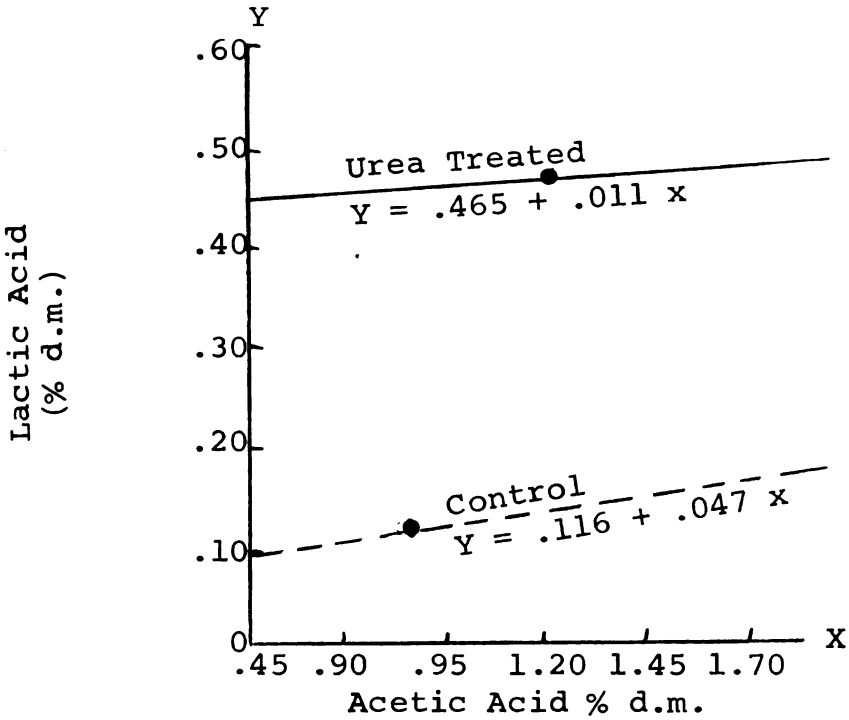

Fig. 9. Relationship of acetic acid to ammoniacal-nitrogen.

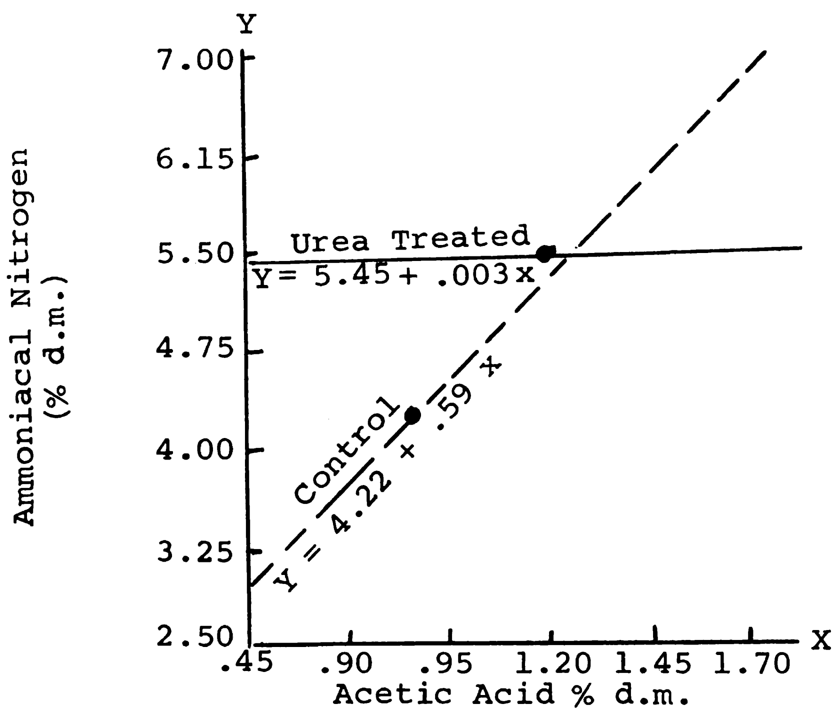

Fig. 10. Relationship of acetic acid to lactic acid.

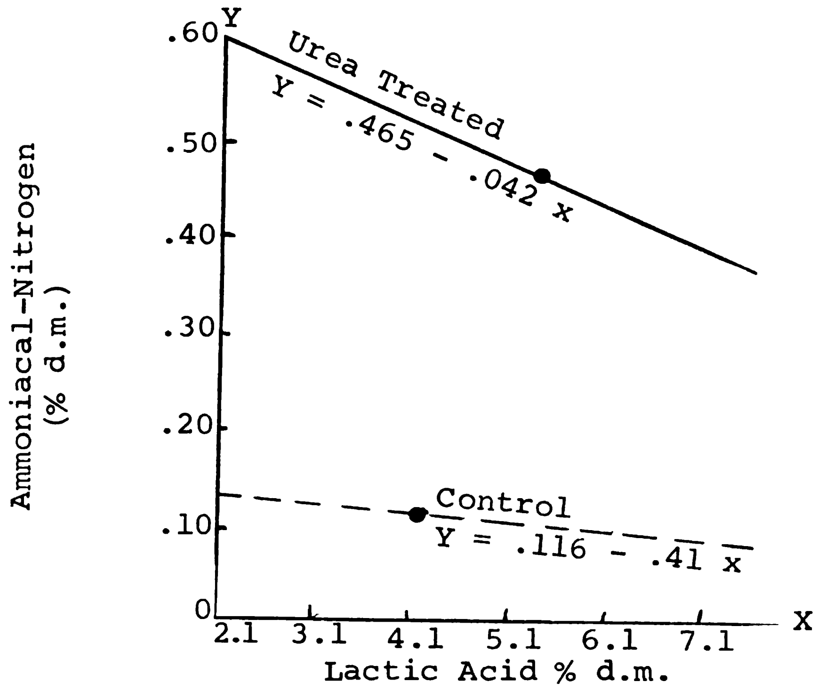

Figure 11. Relationship of lactic acid to ammoniacal-nitrogen.

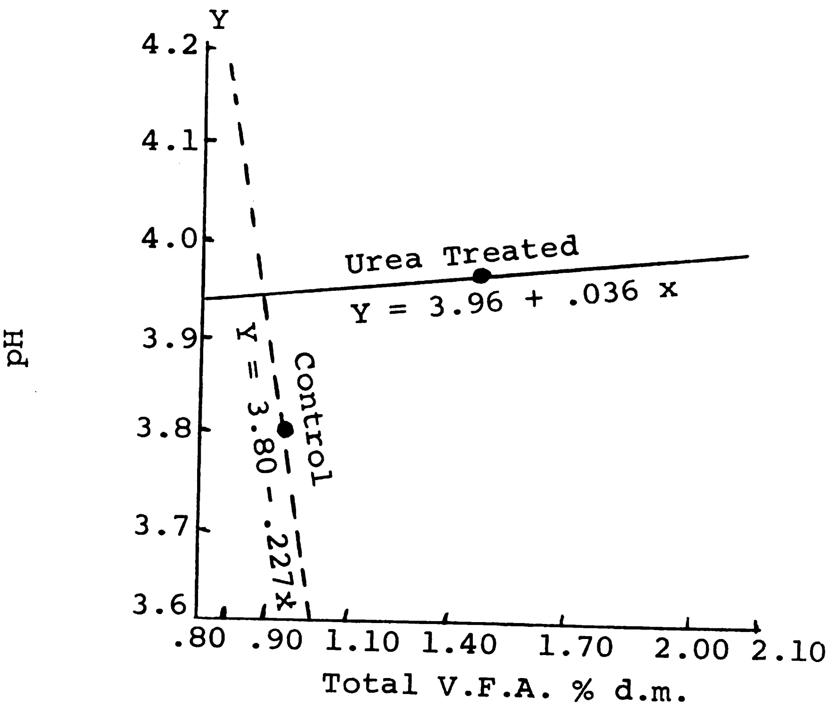

Figure 12. Relationship of volatile fatty acids to pH.

SUMMARY AND CONCLUSION

The affect of adding ten pounds of 45 percent nitrogen urea per ton to whole plant corn at silo filling time was studied on twenty-two central Michigan farms. Eleven vertical farmer-filled urea treated silos were compared to eleven vertical silos of untreated corn silages from the 1965 harvested crop. Samples were taken from each one-fourth of the depth of settled silage and frozen for later analysis. A comparison of analysis of urea treated and control silage was made to determine the effects of urea addition. A separate fermentation study was made by testing green chop corn and comparing it to similar silage from a traced location of four urea treated and four control fermented silages. Changes in silage and green chop materials were measured in pH, dry matter, total Kjeldahl nitrogen, calculated crude protein-equivalent, ammonia, and urea-nitrogen. Major acids of silage studied included lactic, acetic, propionic and butyric acid in both urea treated and control silages.

Urea treated corn silage declined less in pH and stabilized at a level only slightly higher (pH 3.96) than control silage, pH 3.80 ($P < 0.10$). Green chop had pH 5.1.

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The difference in pH between control and treated silage tended to be greater from top to bottom of silos. The changes in pH by quarters were closely correlated to differences observed in dry matter content between quarters of silos. The top quarter of both urea and control silages were drier. Lower dry matter silage obtained the lowest pH. The pH in silage was positively correlated with total nitrogen in urea treated but not in control silage. Lower pH silage also contained higher ammoniacal-nitrogen, lactic and acetic acid levels. Higher dry matter silage was found to be directly related to delay in harvest date. Drier corn silage had higher total nitrogen, protein-equivalent and lactic acid content. Most of the urea treated silage was harvested earlier and more rapidly and ranged from 27 to 38 percent dry matter, control silage ranged from 25 to 55 percent dry matter. There was more variation in dry matter content in control silage (s.d. = 6.1) than in urea treated silage (s.d. = 3.0).

The average recovery of nitrogen added as urea was 98.4 percent. A greater percent of added urea was recovered in upper quarter of silos (100.5 percent) than in second or third quarter. The second highest percentage of urea was recovered from the fourth quarter (99.9 percent), recovery in 30 percent dry matter silage. Urea recovery was a measure of percent total nitrogen compared to control nitrogen level, plus added nitrogen from urea treatment by quarters.

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The addition of 0.52 percent urea resulted in corn silage with protein-equivalent of 14.55 percent or 43.9 percentage increase in protein-equivalent on a dry matter basis. The increase represents 45.4 percent increase in protein-equivalent.

Green chop whole plant corn was found to be naturally high in urea nitrogen .399 percent and relatively low .041 percent in ammoniacal-nitrogen on a dry matter basis at harvest. Fermented control silage decreased 37.3 percent in urea-nitrogen, and fermented urea treated corn silage gained 49.6 percent in urea nitrogen when compared to their original green chop level. Both urea treated and control silage increased in ammoniacal nitrogen content. The increase in urea treated silage of ammoniacal-nitrogen was five times greater than in control silage. Urea nitrogen increased in urea treated silos more in the lower half of silo and less in the upper half. The greatest effect of urea treatment was found in increased ammoniacal-nitrogen content of top quarter with less increase in third quarter compared to control silage. The least increase occurred in fourth and second quarters of treated silages. In urea treated silage 14 percent was found to be ammoniacal-nitrogen, 9 percent was urea-nitrogen, which resulted in 23 percent decrease in other forms of nitrogen present as percentage of total nitrogen. Urea treatment resulted in a major increase in lactic and acetic acid content in the treated silage. Propionic acid remained relatively unchanged. Lactic acid increased most in the drier top and second quarters of treated compared to control silage but the lactic concentration was highest in the low dry matter silage. Acetic acid also increased 37.4 percent mostly in drier silage and accounted for major part of increase (47.5 percent) in total volatile fatty acids. Butyric acid increased 24.7 percent and propionic acid decreased 17.2 percent as a result of urea addition compared to control silage. Both were very minor in silage as well as in degree of alteration as result of treatment.

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