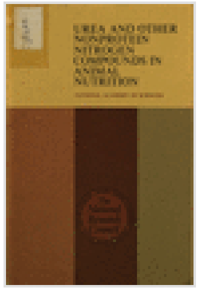

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Urea and Other Nonprotein Nitrogen Compounds in Animal Nutrition

BOARD ON AGRICULTURE AND RENEWABLE RESOURCES
Commission on Natural Resources
National Research Council

NATIONAL ACADEMY OF SCIENCES
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Contents

1 Introduction

This report reviews the utilization of supplemental nonprotein nitrogen (NPN) compounds by ruminant and nonruminant animals. It is presented as a guide for the successful feeding of these compounds in practical production programs. It also attempts to summarize research findings that established the present concepts of NPN utilization in the hope that they will suggest other means for making use of NPN. Only limited utilization of NPN compounds in nonruminant diets containing natural feedstuffs has been demonstrated to date. Whether future costs of protein and the availability of amino and alpha-keto acids make the inclusion of NPN compounds in nonruminant diets economically feasible remains to be seen. Recently, the economic savings that occurs from substitution of NPN for plant protein has stimulated renewed interest in NPN compounds and effective ways to maximize their use.

Nutritionists generally agree that protein is a major limiting nutrient for animal production around the world. This is especially true of ruminants subsisting primarily upon forages and roughages. Urea and other NPN compounds may be available in these areas and can be used to supplement the diets of ruminants under some conditions.

Ruminants have evolved to the extent that their ruminal environment fosters a dense population of microorganisms in the forepart of the digestive tract. This allows these animals to obtain energy from the lignocellulose complexes that are poorly digested by nonruminants. In addition, the ruminal microorganisms can utilize NPN compounds to synthesize protein, which can be converted by the host into meat, milk, and fiber for human usage. The nonruminant, lacking appropriate fermentation capacity, must be provided dietary protein of higher quality.

In this volume protein refers to crude protein (N × 6.25) unless otherwise specified. True protein refers to polypeptides of amino acids. Protein of natural feedstuffs contains varying proportions of true protein and naturally occurring NPN constituents.

The literature on NPN utilization is voluminous. References cited in this volume have been selected and do not cover all of the published literature on this topic. They give concepts, illustrate successful utilization of NPN compounds, and present information on controversial points. Some references will be used to delineate limitations in NPN utilization.

2 Mechanism of NPN Utilization in the Ruminant

It has been almost a century since Weiske *et al.* (1879) reported that ruminants could convert NPN to protein. During the following 60 years, this subject was intensively researched by German nutritionists. Krebs (1937) reviewed their research and summarized the status of the field at that time.

Studies on this subject in the United States began in Wisconsin. Hart *et al.* (1939) reported that either urea or ammonium carbonate was used by growing dairy heifers. They also found that dietary soluble carbohydrates increased NPN utilization. This was the forerunner of a series of experiments that had as a common goal the study of the metabolic aspects of NPN utilization by ruminants. Another landmark in NPN research was conducted by Loosli *et al.* (1949), who demonstrated that urea could serve as the sole dietary nitrogen source for lambs. Using the purified diet approach, they found that the 10 amino acids that are dietary essentials for the laboratory rat were synthesized within the rumen. Lambs fed these diets grew and remained in positive nitrogen balance during the test. Results of similar studies have yielded information on the mechanism of NPN utilization and have provided the facts for establishing the guidelines for the use of NPN in practical ruminant rations. Urea was approved in the United States as a feed ingredient in ruminant diets in 1940 by the Association of American Feed Control Officials.

AMMONIA AS A COMMON DENOMINATOR

It is important to indicate that NPN compounds are normal constituents in the biological fluids of ruminants, even when NPN is absent from the diet. Also, natural feedstuffs that are fed to ruminants contain a variable amount of NPN. Thus, the ruminant continually uses NPN as a normal dietary and metabolic constituent.

Ammonia is the common denominator in the utilization of NPN by ruminants (Hungate, 1966). If the rumen microorganisms cannot degrade the compound in question to yield free ammonia, it is useless as a nitrogen source to the microorganisms. When urea is the substrate, the following steps appear to be involved in its complete utilization:

1. Urea $\xrightarrow[\text{Urease}]{\text{Microbial}}$ $NH_3 + CO_2$

2. Carbohydrates $\xrightarrow[\text{Enzymes}]{\text{Microbial}}$ Volatile Fatty Acids (VFA) + Keto Acids

3. NH_3 + Keto Acids $\xrightarrow[\text{Enzymes}]{\text{Microbial}}$ Amino Acids

4. Amino Acids $\xrightarrow[\text{Enzymes}]{\text{Microbial}}$ Microbial Protein

5. Microbial Protein $\xrightarrow[\text{Abomasum and Small Intestines}]{\text{Animal Enzymes in the}}$ Free Amino Acids

6. Free amino acids are absorbed from the small intestine and used by the host animal.

Similar schemes would be appropriate for other NPN sources if enzymic action is needed for hydrolysis. However, different enzymes may be involved for each NPN compound. Bloomfield *et al.* (1960) reported that step number one usually proceeds at a faster rate than step number two. This is especially true if the lignocellulose complex of poor-quality forages is the primary carbohydrate source in the diet. In this case the keto acids necessary for amino acid synthesis are limiting; thus there may be a considerable loss of ammonia through the ruminal wall, resulting in poor utilization of dietary nitrogen. If the rate of urea intake is reduced in such diets to conform to the rate of cellulose hydrolysis, the efficiency of nitrogen utilization can be improved (Campling *et al.*, 1962). The kinetics of urea metabolism are presently receiving much study, and the basic experiments of Nolan and Leng (1972) are typical of these studies. Biuret metabolism in the ruminant is also an active field of study (Tiwari *et al.*, 1973a,b). It is beyond the scope of this review, however, to cover the kinetics of NPN utilization.

AMMONIA PRODUCTION IN THE RUMEN

Most ruminal bacteria prefer ammonia to amino nitrogen for the synthesis of microbial protein (Bryant, 1963; Hungate, 1966). Ammonia can arise from the degradation of dietary protein, microbial protein, and NPN compounds. In addition, urea is recycled to the rumen via saliva and through the wall of the rumen. A majority of dietary protein is hydrolyzed to peptides and amino acids by microbial enzymes. The free amino acids and peptides may be incorporated into microbial protein or deaminated with the production of ammonia and volatile fatty acids (VFA), which may be absorbed from the rumen or used by microbes as carbon skeletons for amino acid synthesis. The level of dietary protein and its solubility greatly influence ammonia production, which in turn affects the utilization of dietary NPN compounds. This important aspect has been discussed by Church (1969).

The optimum concentration of ruminal ammonia required for maximum cell yield has not been established. This is understandable, perhaps, because the concentration depends upon such factors as level of feeding, solubility of dietary protein, availability of carbohydrates and minerals to the microbes, frequency of feeding, etc. Recent *in vitro* research by Satter and Slyter (1974) indicated that the tungstic acid precipitable nitrogen was 90 percent of maximum when NH_3 concentration was 1–2 mg NH_3–N/100 ml fluid. Increasing the ammonia concentration to 8 mg increased the output some. *In vivo* results by (Slyter *et al.* (1973) indicate that nitrogen retention of steers was improved by maintaining ruminal ammonia concentrations above these values. E. L. Miller (1973) also studied this problem *in vivo* and reported that the greatest microbial flow from the rumen was achieved with rumen ammonia concentration of approximately 28 mg NH_3–N/100 ml fluid. Perhaps the increased performance with the higher ammonia concentrations *in vivo* than *in vitro* can partially be explained by the possible beneficial effects of ammonia outside the rumen, i.e., synthesis of nonessential amino acids in the liver, etc. This area is receiving considerable research interest at the present time.

As indicated earlier, NPN must first be converted to ammonia; the reaction is mediated by microbial enzymes. In the case of urea, the hydrolytic enzyme is urease, which is produced by many species of ruminal bacteria (Slyter *et al.*, 1968). Urea is rapidly hydrolyzed under most ruminal conditions. In fact, one danger of high levels of urea feeding is excess ammonia production, which may lead to ammonia toxicity. Results of studies on methods of inhibiting urease activity (Tillman and Sidhu, 1969) have not yielded useful information for practical application. Most ammonium salts, such as ammonium sulfate, ammonium phosphate, and ammonium salts of volatile fatty acids are ionized in the rumen and do not have to undergo enzyme hydrolysis.

In contrast to urea, both biuret and uric acid are hydrolyzed by induced enzymes (an enzyme that only appears after the substrate is present), biuretase and uricase, respectively. A long period (3 weeks) is needed for the induction of peak biuretase activity, while uricase (Oltjen *et al.*, 1968) is induced at a faster rate. Because of biuret's slow rate of hydrolysis and lack of toxicity, even when used at high dietary levels, some workers feel that it might become the NPN source of choice when it is fed to supplement the diets of ruminants grazing or fed low-quality roughages.

AMMONIA METABOLISM BY RUMEN MICROORGANISMS

A wide variety of NPN compounds will support growth of ruminal bacteria *in vitro* (Belasco, 1954; Henderickx, 1967). Allison (1969) reviewed the biosynthesis of amino acids by rumen bacteria. In general, amination and transamination reactions appear to be responsible for the major part of ammonia assimilation by the microflora. Glutamic dehydrogenase (Hoshino *et al.*, 1966) plays a key role in the initial fixation of ammonia to a carbon skeleton, and glutamate–oxaloacetate and glutamate–pyruvic transaminases are important in the transfer of ammonia to other carbon skeletons, which are present in rumen fluid. Other dehydrogenase and transaminase enzyme systems also play a part in ammonia assimilation by rumen bacteria (Chalupa, 1972).

Rumen microflora can use NPN for protein synthesis if the necessary carbon skeletons are present or if these can be synthesized fast enough from dietary carbohydrate or alternate carbon sources. The most important single fermentation characteristic is the amount of fermentable energy available in the diet for microbial growth (protein synthesis) above that needed for maintaining equilibrium in the rumen between the feed protein degraded and the microbial protein resynthesized.

Burroughs *et al.* (1971d,e, 1974a) proposed a system for the evaluation of feeds based on estimated urea fermentation potential (UFP). The UFP was estimated on the basis of the fermentable energy of a given feed and the amount of the feed or diet protein degraded in the rumen. This system recognizes that microbial protein synthesis is primarily dependent upon energy availability and that the conversion of rumen degraded dietary protein into microbial protein represents an energy cost.

Primary sources of carbon fragments that arise from carbohydrate fermentation are CO_2 and VFA's. However, there are specific requirements for isobutyrate, indole-3-acetate, isovalerate, 2-methylbutyrate, and phenylacetate to provide for the synthesis of the specific amino acids (Allison, 1969). There are potential sources of keto acids in rumen fluid, but the branched-chain VFA's arise mainly from the deamination of branched-chain amino acids provided by dietary protein. It is significant that the feeding of protein-free diets causes a depression in the concentration of these acids (Ørskov and Oltjen, 1967; Oltjen, 1969; Chalupa *et al.*, 1970), with isovalerate and isobutyrate being greatly influenced. However, evidence regarding a possible need to supplement high-urea diets with a combination of branched-chain VFA is not clear-cut; some researchers obtained increased responses (Hemsley and Moir, 1963; Cline *et al.*, 1966; Hume, 1970), while others have received little response (Oltjen *et al.*, 1971). Including branched-chain VFA in the diet did not change the rumen protozoa numbers, cellulolytic bacterial numbers, nor the microbial amino acid composition (Oltjen *et al.*, 1971). This aspect of NPN utilization needs further study, especially when high-forage or high-NPN-containing diets are fed.

The high levels of the enzyme activities present in the rumen would indicate that insufficient carbon skeletons could limit ammonia assimilation. This observation is in agreement with the calculations of Balch (1967) showing that the level and type of dietary carbohydrate has a large influence on the efficiency of NPN utilization.

Although many predominant species of rumen bacteria have been studied in pure culture, the microflora are complex and much remains to be learned about microbial interrelationships. This makes it difficult to generalize about requirements for certain nutrients, especially the B vitamins. It appears that specific B vitamins are needed by certain strains of ruminal bacteria; and, if they are not present at adequate concentrations in the rumen fluid, growth of some bacterial strains may cease. It is known that rumen bacteria produce as well as use certain B vitamins (Bruggemann and Giesecke, 1967). Apart from their importance in the metabolism of the host animal, B vitamins may play an important part in regulating microbial interrelationships in the rumen. It has been reported that dietary urea appears to stimulate B-vitamin synthesis in the rumen (Teeri and Colovos, 1963; Briggs *et al.*, 1964).

Hunt *et al.* (1954) found that rumen microbes, which use urea and decompose cellulose, have a requirement for sulfur that can be met by sulfate; sulfate is reduced to sulfide prior to being incorporated into the amino acids, cystine, and methionine. Bruggemann *et al.* (1962) found that there is an increase in the number of sulfate-reducing bacteria in rumen fluid when protein is replaced by urea. Several workers have reported that the N:S ratio in NPN-containing rations for sheep should be about 10:1 for the most effective utilization of these compounds. In cattle the ratio should be 12–15:1. The species difference is probably due to the requirement of the sheep for more sulfur-containing amino acids for wool production. There is no evidence to indicate that the sulfur needs of the ruminal microbes from sheep are different than those of cattle.

Minerals are required by rumen bacteria and the host animal, but substitution of NPN for dietary protein does not increase requirements for these beyond those stated in present feeding standards. Since NPN and grain are combined in a mixture to replace protein supplements in isonitrogenous diets, it must be remembered that this combination of NPN plus carbohydrate source may contain lower levels of essential minerals than does the replaced protein supplement. Therefore, the use of NPN compounds in ruminant diets makes it quite important for nutritionists to carefully consider the amounts and balance of all nutrients in diets containing NPN.

QUANTITY OF MICROBIAL PROTEIN SYNTHESIZED IN THE RUMEN

Many workers have conducted research that points to the abilities and limitations of ruminal bacteria to synthesize microbial protein. Hungate (1966) has summarized these factors. Synthesis of microbial protein from dietary nitrogen depends upon the amount and the nature of dietary constituents as well as the amount of high-energy materials, primarily ATP, that can be derived from these. Since the rumen is anaerobic and the major substrate is carbohydrate, the amount of digestible carbohydrate represents the level of energy-yielding materials. Also, oxygen is needed for oxidation of carbohydrate and energy production; thus, efficiency of protein synthesis is much lower in anaerobic than aerobic systems. Hungate (1966) suggested that a cell yield of 10–20 percent in anaerobic systems would be expected. Purser (1970), using Hungate's data, estimated a yield of 18.3 g of digestible protein for each digestible megacalorie in ruminants. Using the value proposed by Purser (1970) and the National Research Council (NRC) (1971b) requirements for protein in dairy cows, Chalupa (1972) calculated that a dairy cow entirely dependent upon the microbial protein synthesized in the rumen for milk protein synthesis would produce only 10 kg of milk per day. This value is close to the quantity of milk actually produced by cows fed protein-free urea containing purified diets (Virtanen, 1966). The cow actually produces considerably more than 10 kg of milk daily, leading Chalupa (1972) to suggest several possible explanations: (1) that the NRC requirements for digestible protein are too high, (2) that greater than anticipated yields of ATP are obtained, (3) that at least 50 percent of the dietary protein was not degraded in the rumen for the synthesis of microbial protein, and (4) that the combination of microbial protein and dietary protein, which presumably bypassed the rumen (50 percent) was just sufficient to meet the digestible protein requirement as set by the NRC.

A number of recent studies (Hogan and Weston, 1970; Lindsay and Hogan, 1972; Ørskov *et al.,* 1972; Bucholtz and Bergen, 1973; Thomas, 1973) indicate that microbial yields considerably greater than 18.3 g of digestible protein per digestible megacalorie occur in the rumen. Data from these and other reports indicate that the cell yield in the rumen may range from 10 to 30 g of digestible protein for each digestible megacalorie. It is not surprising that a range is indicated because of such factors as nutritional adequacy of the diet, availability of cofactors, specific microbial population in the rumen, turnover rate, lysis, etc. It is important to clearly define the cell yield to more precisely determine the usefulness of NPN in ruminant diets.

It has been known for many years that some dietary protein, depending upon its solubility and upon rumen conditions, does bypass ruminal degradation. If dietary protein is replaced with NPN, less protein is available for ruminal bypass. Therefore, the replacement of too much dietary protein with NPN compounds beyond certain limits could lead to protein deficiencies in high-producing animals. Ruminants fed urea-containing purified diets presented 10–30 percent less protein to the abomasum (Tucker and Fontenot, 1970) than those fed isolated soy protein containing purified diets.

It is possible that future research on protein nutrition in ruminants will reveal methods of feeding NPN that meet the nitrogen requirements of the bacteria and enable the animal to obtain all of the microbial protein possible by this means. In addition, the animal may be fed dietary protein that has been treated with formaldehyde, tannins (Peter *et al.*, 1971; Driedger and Hatfield, 1972; Nimrick *et al.,* 1972), or other materials to effectively reduce or prevent microbial degradation in the rumen. By the proper selection of the treated dietary proteins and/or "protected" amino acid supplements, the quantity and quality of protein presented to the abomasum could support optimum production in the ruminant with a minimum of preformed dietary protein.

QUALITY OF MICROBIAL PROTEIN SYNTHESIZED IN THE RUMEN

Chalupa (1972) summarized much of the available information on bacterial vs. protozoal protein found in rumen fluid and reported the following averages, respectively: crude protein content, 55 vs. 38 percent; true digestibilities, 66 vs. 88 percent; biological value, 78 vs. 77 percent; and net protein utilization, 55 vs. 67 percent. He points out that the lower than usual crude protein percentages reported were probably due to contamination of the samples with digesta found in rumen fluid.

Chalupa (1972) also constructed aminograms on bacterial and protozoal proteins using available data in the literature. Higher quantities of leucine, phenylalanine, lysine, and tyrosine were found in protozoal protein, indicating that it has superior nutritional value, which is borne out by results of feeding trials with laboratory nonruminants. Allison (1969) found that the amino acid pattern in the bacterial cell wall was different from that of cellular constituents. Using the data of Hoogenraad and Hird (1970), Chalupa (1972) plotted aminograms of cell wall and non-cell wall constituents and reported that cell walls contained lower levels of all amino acids. The cell walls of bacteria account for about 15 percent of the dry weight of these microbes; however, they are more resistant to the proteolytic enzymes. Thus, their presence reduces the digestibility of the bacterial protein. Therefore, the net utilization of bacterial protein, which contains a greater proportion of cell walls, is less than that of protozoal protein. These results indicate that it would be desirable to alter the ratio of bacteria to protozoa by increasing numbers of the latter. Abou Akkada and El-Shazly (1965) reported a significantly greater nitrogen retention by faunated sheep compared to defaunated sheep. Experimental results are not always in agreement: High-roughage diets promote a protozoal biomass nearly as great as that of bacterial, while high-grain diets reduce or completely eliminate protozoa. Yet the biological value of rumen fluid dry matter from high-grain diets was higher than that of hay-fed steers (Little *et al.*, 1965), suggesting that other factors were involved.

Smith *et al.* (1969) reported that 80 percent of the nitrogen found in ruminal bacteria was in protein and 20 percent was in nucleic acid. Nucleic acid in rumen fluid is primarily of microbial origin. When nucleic acids were added to rumen fluid *in vitro*, both RNA and DNA were degraded rapidly (Smith and McAllan, 1970). There was no degradation in the abomasum. Consequently, ruminants present a high and continuous supply of nucleic acids to the small intestine. Digestibility of nucleic acids in the small intestine is high (Smith and McAllan, 1970). Condon (1971) has studied the metabolic fate of nucleic acids reaching the small intestine of sheep. His results are as follows:

1. Lost to the host animal—63 percent
   a. Undigested and lost in feces—20 percent
   b. Excreted as purine derivatives in urine—43 percent
2. Possible value to the host animal—37 percent
   a. Contribution to ammonia pool from degradation of purine bases—11 percent
   b. Contribution to ammonia pool from degradation of pyrimidine bases—10 percent
   c. Contribution to ammonia pool from degradation of aminoisobutyrate and beta-alanine—16 percent.

Nucleic nitrogen is of limited value to the animal, thus a high production of nucleic acid in the microbial protein increases the loss of nitrogen. Replacement of dietary protein with NPN compounds increases the proportion of microbial protein and increases the nucleic acid content of protein presented to the abomasum and the small intestine.

Protein requirements of ruminants, as is true of all animals, must be evaluated in terms of the amounts of amino acids absorbed from the intestinal tract in relation to those needed for productive purposes. Amino acids found in the abomasum and the small intestine have their origin in microbial protein, dietary protein that escaped rumen degradation, and endogenous secretions. The amount of degradation of dietary proteins is greatly dependent upon solubility of the protein in rumen fluid. Chalupa (1972) has considered the solubilities of various protein sources relative to rumen bypass and the factors affecting solubilities. The solubility of dietary protein is important in the utilization of ammonia released from NPN compounds, because certain proteins may be degraded to ammonia in the rumen as quickly as the NPN compound itself, while others are less soluble. The optimum condition would be to formulate diets using the less-soluble dietary proteins with the more-rapid, ammonia-releasing compounds in order not to have an excess of ruminal ammonia. It would be desirable to decrease ruminal proteolysis and/or deamination, thereby forcing the microbes to use ammonia from NPN sources. Also, it requires energy to synthesize amino acids. Wohlt *et al.* (1973) have classified many proteins according to their solubility. Heat treatment reduces solubility of oil meal proteins, thereby increasing their nutritive values for ruminants (Sherrod and Tillman, 1962, 1964).

The problems of the elucidation of the total amino acid requirements of ruminants for productive purposes and the determination of whether specific diets cause amino acid deficiencies or imbalances are complex; the main complication concerns the varying degrees of microbial alteration of dietary proteins in the reticulorumen. These problems have been studied by different methods: Some have examined plasma amino acid profiles of ruminants receiving postruminal administrations of a single amino acid or mixtures of amino acids, while others have measured the performance of ruminants as well as monitored the plasma profiles. Treatment of dietary proteins to reduce or inhibit microbial degradation in the rumen has also been tested by others.

Postruminal administration of specific amino acids and protein of high biological value offers promise of increasing the production of ruminants (Reis and Schinckel, 1964). This subject has been reviewed by L. F. Nelson (1970). In sheep, the first limiting amino acid is methionine, and an excellent response was obtained (Schelling and Hatfield, 1968) when supplemental methionine was placed in the abomasum. Nimrick *et al.* (1970a,b,c) found that, after the requirement for methionine was met, lysine and threonine became limiting. Cattle fed urea-containing purified diets had lowered plasma concentrations of valine, isoleucine, leucine, and phenylalanine, but increased levels of serine and glycine (Oltjen and Putnam, 1966; Oltjen, 1969) compared to cattle fed isolated soybean protein. Also, less dietary nitrogen was retained when the cattle were fed urea-containing diets. When combinations of valine, isoleucine, leucine, and phenylalanine were infused into the abomasum of steers, the utilization of the urea-containing diet was improved, almost equaling the performance of steers fed the soy protein-containing diet (Oltjen *et al.*, 1970). These workers also found that the infusion of glycine and serine depressed the utilization of the soy protein diet, demonstrating the importance of amino acid balance in ruminants. In general, dietary supplements of amino acids in ruminant diets have not given consistent responses, regardless of the response criteria used.

It must be emphasized that the urea-containing purified diets employed by the University of Illinois and USDA (Beltsville, Md.) researchers contained NPN as the sole nitrogen source making the protein in the abomasum solely of microbial and endogenous origin. This is a much different condition from that found with practical diets in which urea furnishes a smaller percentage of the total dietary nitrogen. However, even when practical diets containing some urea were fed to cattle, plasma levels of valine, isoleucine, leucine, and lysine were lower (Little *et al.*, 1969) than those found in cattle fed diets containing only natural protein. Because gains were also lower in urea-fed steers, the importance of amino acid supplementation at the lower gut when urea is fed at high levels is emphasized. If further research identifies the amino acids needed by ruminants under specific conditions, development of products for incorporation in the diet to bypass degradation in the rumen appears feasible. This is presently an exciting field for research, but the necessary practical guidelines must await results of future experiments.

AMMONIA METABOLISM IN THE HOST ANIMAL

Ammonia is absorbed from the reticulorumen as well as the omasum, small intestine, and cecum. The reticulorumen is considered to be the largest absorption area. The liver, as well as the mucosal cells of the reticulorumen, can use ammonia. The mucosal cells contain transaminases, and glutamine synthesis has been reported here (Hoshino *et al.*, 1966).

In an extensive study of the mucosal cellular structure, Chalupa *et al.*, 1970) found that transaminase activities of mucosal cells were lower per unit of cellular materials than in liver cells. The large mass of mucosal cells (almost 1 percent of the ruminant's body weight) would indicate that these cells also play a role in the overall metabolism of dietary nitrogen by this animal.

AMMONIA TOXICITY

Ammonia is a weak base with a pKa of 8.8 at 40° C; therefore, there is a close relationship between ruminal fluid pH and the ratio of ammonia to ammonium ions. The lipid layer of the rumen mucosa is permeable to ammonia, allowing rapid absorption. Also, the alkaline buffering capacity of rumen fluid is not great in comparison with its ability to buffer acids. Because of poor management or improper formulation, the feeding of high levels of dietary urea may result in a rapid accumulation of ammonia in rumen fluid. This is accompanied by a rise in rumen fluid pH with rapid absorption of ammonia across the rumen wall. When the rate of ammonia absorption exceeds the capacity of the liver to convert it to urea, ammonia accumulates in the blood and toxicity may result.

Lewis *et al.* (1957) reported that changes in the rumen ammonia concentration of sheep fed various diets were paralleled by changes in portal blood ammonia levels. Peripheral ammonia did not increase until the ruminal levels exceeded 60 millimoles/liter. Toxic symptoms were apparent when the concentration in the peripheral blood exceeded 0.6–0.9 millimoles/liter. Acute ammonia toxicity symptoms in the ruminant appear to be progressive as follows: The animal becomes nervous and uneasy, salivates excessively, and demonstrates muscular tremors; these symptoms are followed by incoordination, respiratory difficulty, and frequent urination and defecation; the front legs begin to stiffen, and the animal becomes prostrate; violent struggling, bellowing, and terminal tetanic spasms are found in most animals; the jugular pulse is marked, and bloating is common; death occurs within 0.5–2.5 h after the initial symptoms are observed.

It appears that rumen fluid NH_3–N levels of 80 mg/100 ml will cause toxicity and can be used as a diagnostic tool. Blood NH_3–N levels causing toxicity are difficult to determine, and it is suggested that levels of 1 mg/100 ml will cause toxicity in cattle. There are usually no characteristic lesions found on necropsy examination; however, congestion, hemorrhages, and pulmonary edema are common.

Predisposing factors to urea toxicity in cattle appear to be (1) lack of an adequate adaptation period to urea-containing diets, suggesting that it is important to start feeding urea at low levels and increase gradually over a period of several days, especially if high levels of urea are fed; (2) fasting prior to urea consumption; (3) the feeding of urea in diets composed primarily of poor-quality roughages; (4) the feeding of diets that promote a high pH in ruminal fluid; and (5) low water intake. Errors in formulation and improper mixing of urea with other diet ingredients are probably the major factors causing urea toxicity in the feeding of ruminants.

An effective treatment for urea toxicity for cattle, if applied before tetanic spasms occur, is to immediately administer 20–40 liters of cold water orally. Cold water will lower ruminal fluid temperature and thereby reduce ureolysis. It will also dilute the concentration of ammonia and reduce its rate of absorption from the rumen. Four liters of either dilute acetic acid or vinegar given with cold water is more effective than cold water alone. Acetic acid will neutralize the toxic effects of free ammonia. Also, Coombe *et al.* (1960) and Hogan (1961) have reported that ammonia is absorbed through the ruminal wall at a much faster rate at a high ruminal pH than at a low ruminal pH.

There is concern among animal production specialists regarding the possibility that ammonia toxicity in some members of the cow herd will increase the incidence of abortions in the surviving cows. It is felt that high levels of blood ammonia might be toxic to the fetus, even though the cow survives. Oklahoma researchers (Word *et al.*, 1969) induced severe toxicity symptoms in pregnant cows and then averted death by acetic acid treatment. They found no abortions in any of the cows, even though toxicity symptoms were well advanced in all cows before acetic acid was administered, indicating that the fetus is resistant to high levels of blood ammonia. Also, subsequent reproductive performance was not affected in cows subjected to this condition. There appears to be little likelihood of urea toxicity in ruminants if proper levels are fed and judicious management practices are used.

SUMMARY AND CONCLUSIONS

NPN compounds are widely used as dietary nitrogen sources for ruminants, but are of little, if any, value in nonruminant diets. For this reason, the following points apply almost entirely to ruminants:

1. Ammonia is the common denominator in the utilization of NPN compounds by ruminants. Microorganisms living in the rumen produce enzymes that hydrolyze the dietary NPN source to yield ammonia. Also, there is simultaneous enzymatic hydrolysis of complex dietary carbohydrates to produce carbon skeletons, which combine with ammonia to form amino acids. These amino acids are used for the synthesis of microbial protein, which is later digested, absorbed, and utilized by the animal for productive purposes.

Most rumen bacteria prefer ammonia nitrogen to that supplied by peptides and amino acids, suggesting that some dietary NPN in ruminant diets is desirable for maintaining a viable rumen microflora.

2. The quantity of protein synthesized in the rumen by microorganisms depends upon the nature and amounts of dietary constituents, a point that will be discussed in much detail in later sections of this report. However, if all dietary factors are present in the rumen and in optimum proportions, it appears that yields ranging from 10–30 g of digestible protein for each digestible megacalorie can be expected.

In purified-diet studies in which urea furnished all of the dietary nitrogen, thereby causing the animal to be entirely dependent upon microbial protein for supplying its requirements for growth or milk production, it was found that rates of growth and production were 65 percent of optimum. These results indicate that the rate of protein synthesis might be too slow, the quality of the microorganism protein might be poor, or both.

3. The quality of a bacterial protein is lower than that of protozoal protein; however, attempts to shift ruminal ratios of bacteria to protozoa have not always improved results. Microbial protein contains about 20 percent nucleic acid, which is not utilized efficiently by the animal for protein synthesis. Also, the high proportion of cell walls in the microbes reduces protein quality. When cattle are fed diets in which urea is the only nitrogen source and compared to control animals receiving natural protein, they have slower rates of growth and lower blood levels of isoleucine, leucine, phenylalanine, and valine, but increased blood levels of glycine and serine.

4. Major research is needed on the means of feeding sufficient NPN to maintain a viable microbial population that in turn would supply the major portion of the protein needed by the host animal. Accompanying research is needed to determine how to protect high-quality dietary protein from ruminal degradation. The development of such methods would make it possible to efficiently use NPN sources in diets of high-performance animals.

5. Prevention of urea toxicity is a management function; and, if proper management is exercised, the incidence of ammonia toxicity is extremely low. Toxicity symptoms were described, and treatment methods were suggested. Research results indicate that high levels of rumen and blood ammonia do not increase the incidence of abortions in cow herds. Furthermore, subsequent reproductive performances of cows surviving high levels of rumen and blood ammonia were not affected.

3 Use of Urea as a Protein Replacement for Ruminants

Urea is the NPN compound most widely used in ruminant diets. The use of approximately 800,000 tons in 1973 (Allen, 1974), with appropriate supplemental sources and levels of energy, represents 4.5 million tons of 50 percent protein supplement. Although the chapter is limited primarily to urea as the NPN source, the general principles of ammonia utilization presented earlier apply.

HOST NEEDS FOR PROTEIN

There are many similarities among species of the animal's need for protein or, specifically, amino acids at the tissue level. Physiological growth, function (maintenance, growth, production, or reproduction), levels of complementing and supplementing nutrients or additives, and environmental conditions (temperature, humidity, confinement) are among the factors that affect the animal's needs for amino acids. These influencing factors have been considered in the available feeding standard recommendations. Special consideration should be given for estimating the protein needs for the biological system of the ruminant. In addition to the amino acid needs of the host, there are nitrogen requirements for the microbiota if the ruminant is expected to use forage and other cellulose-containing energy sources, if the biological system is to synthesize nutrients not ordinarily added to the diets (such as B vitamins), and if the microbiota are to be used to detoxify dietary ingredients that may contain or be contaminated by chemical residues. Examples of possible rumen nitrogen deficiencies were observed in cattle and sheep fed limited protein, soybean meal-supplemented, high-concentrate diets; but they were not apparent in isonitrogenous, limited-protein, urea-supplemented, high-concentrate diets (Braman *et al.*, 1973). After the protein needs of the ruminant have been established for a particular performance or function, then the supplemental need can be determined after allowing for the protein contributions from the energy-furnishing ingredients in the diet. Because of the limitation in quantity and quality (patterns of essential amino acids) of protein synthesized by the microbiota of the ruminant, the decision to provide all of the supplemental needs is contingent upon the expected performance or function of the animal (total dietary protein requirement), protein contributions of other ingredients, and levels or concentration of other components necessary for microbial synthesis. For example, a rapidly growing, young ruminant may respond significantly more to diets supplemented in part with some preformed protein, such as soybean meal, than it would respond to diets supplemented entirely with urea. However, it has been demonstrated many times that urea is a satisfactory source of supplementary protein in diets for ruminants that have low total dietary protein requirements (maintaining a mature ruminant).

An experimental diet to test both the animal's protein needs and the efficiency of the source of nitrogen in fulfilling those needs should contain both a "negative control" (test diet known to be protein deficient) and a "positive control" (test diet with preformed protein furnishing the supplemental protein and eliciting a positive animal response relative to the negative control). The diet must be deficient in nitrogen or natural protein if supplementary urea is to be beneficial to the animal. This need, although well recognized, has not always been adhered to in some urea utilization experiments with cattle and sheep. Sound conclusions are not possible under the assumption that a diet, before urea supplementation, is protein-deficient when, in fact, it may contain adequate protein. Also, without a positive control diet, one might erroneously conclude that the negative control diet has adequate protein when, in fact, the animals consuming such a diet may be protein-deficient. This situation was demonstrated in cattle-feeding trials by Burroughs *et al.* (1972, 1973) in which two types of diets were identified that did not respond to urea supplementation. One type was protein-deficient and readily responded to natural protein supplementation, even though no response to urea was noted. The second type did not respond to either natural protein or urea supplementation and, therefore, could be accurately judged as being protein-adequate for the cattle being fed. This leads to the conclusion that, although the ruminant diet must be deficient in protein in order for urea supplementation to be beneficial, the reverse of this statement is not necessarily true; namely, urea will always be beneficial if added to a protein-deficient ruminant diet. The reason why this may not be true is that the negative control diet must not only be protein-deficient, but it must also have fermentation characteristics that permit it to be benefited by urea supplementation.

The degree of protein deficiency in a ruminant diet also exerts a strong influence upon the relative effectiveness of urea supplementation, as compared with natural protein supplementation. It has long been known that the relative effectiveness of urea is greatest when fed in a high-energy, mildly protein-deficient diet in which only a small quantity of urea or natural protein is needed in correcting the deficiency. It is less well known that when urea is fed in a similar high-energy diet that is much more protein-deficient, urea is very beneficial but may be somewhat less beneficial in satisfying the total deficiency compared with natural protein supplementation. This situation was also demonstrated and confirmed in recent cattle experiments (Burroughs *et al.*, 1972, 1973) in which urea was as effective as natural protein supplementation in the first type of diet and only about half as effective as natural protein supplementation in the second type of diet.

These same experiments demonstrated that the degree to which cattle performance was poorer (30–40 percent) from urea compared with protein supplementation of a protein-deficient diet fed to small cattle (160 kg) entering the feedlot decreased progressively until no differences existed during the last part of a 10-month feeding period. During this period, the supplemented cattle approximately tripled their body weight, increased TDN consumption, and thus conversion of urea to rumen microbial protein. Thus, this urea-supplemented diet that was deficient with limited TDN intake in smaller cattle progressively became a protein-sufficient diet for the same cattle with increased TDN intake. By contrast, the protein-supplemented cattle were not similarly influenced with respect to TDN intake.

In the feedlot, those cattle receiving urea supplements often have lowered performance during the initial weeks than those receiving protein supplements; however, subsequent performance is improved. With larger-size cattle, weighing 300 kg or more, the period of inferior performance observed is considerably shortened and may be of only 3–4 weeks' duration. Sometimes inferior performance for this short duration is attributed to the unpalatable nature of urea-containing feeds (Goodrich and Meiske, 1969). Such theories, however, were not sustained when tested by Varner and Woods (1970), who demonstrated that larger-sized cattle required no adaptation to urea provided TDN consumption was rapidly increased for a 3-week period prior to their initial consumption of urea supplements. Such cattle, when abruptly shifted in a single day from protein to urea feeds, suffered no setback and performed equally as well as cattle continuously maintained on nonurea protein feeds.

Experimental and industry observations with high-corn, grain–corn silage diets tend to support the consensus that urea is a satisfactory source of supplementary protein for beef cattle and sheep when limited to approximately 20–30 percent of the total protein requirement, provided the animal has attained full feeding conditions and the requirement does not exceed 12–13 percent of the diet. This consensus would assume that other factors necessary and favoring microbial synthesis are adequate. Because the effectiveness of urea supplementation is dependent upon other dietary factors, urea is a satisfactory source of all supplemental protein needs in high-grain fattening diets properly fortified with minerals for animals having attained full feeding conditions and having no more than 12 percent total dietary protein requirements. This would assume that feeding management is appropriate for maximizing urea supplementation.

There may be production situations where it would be economically feasible to accept a lower rate and efficiency of performance and use urea for all of the supplemental needs in diets of animals having high protein requirements.

DIETARY FACTORS AFFECTING MICROBIAL PROTEIN SYNTHESIS

An optimal level or concentration of the following factors will maximize rumen microbial synthesis: ammonia, readily available energy, carbon skeletons, minerals, vitamins, growth stimulators or inhibitors (antibiotics, hormones, anabolics), and factors that influence the chemical and physical environment (pH, temperature, diet particle size and density, presence or absence of oxygen, etc.).

NITROGEN

The influence of exogenous nitrogen compounds on net microbial synthesis has been measured and estimated in many experiments. As indicated earlier in this report, ammonia is a vital ingredient in microbial synthesis. Relevant points to be considered by formulators of ruminant diets include the sources and levels of ammonia precursors. The level of urea required to furnish supplemental ammonia to meet optimal ammonia concentrations in the rumen is dependent on (a) the amount of ammonia from degraded nitrogenous compounds contained in the other components of the diet, such as forages, grains, and other ingredients; (b) the amount of recycled endogenous urea; and (c) the levels of other necessary components (energy, minerals, etc.). The efficiency of nitrogen or ammonia utilization will be greatest whenever ammonia is the first-limiting factor necessary for synthesis. For example, urea utilization will be high in low-available nitrogen diets that contain abundant levels of available energy, carbon skeletons, minerals, vitamins, and other components that enhance microbial activity.

SOURCE OF ENERGY

Carbohydrates are normally the main source of energy and carbon skeletons for microbial synthesis. The availability of energy from different sources is the key to evaluating its dietary effects in the ruminant. The necessity of adequate available energy is essential for evaluating the utilization potential of any nutrient.

Pigden (1971) reported that the lignocellulose complex accounts for most of the energy in mature forages. He related the total dietary nitrogen levels to the total digestible energy of forages and indicated that 1 percent dietary nitrogen was sufficient for the utilization of forages having no more than 50 percent digestible energy. However, he suggested 1.5 percent dietary nitrogen for forages having higher levels of digestible energy and 2 percent dietary nitrogen for diets containing 20 percent or more starch. Pigden and Heaney (1969) reported that each roughage has a digestion "ceiling . . . where rate of utilization by rumen microflora is reduced to a point where for practical purposes the energy is no longer available to the animal." They have established digestion ceilings on many low-protein roughages and found that each roughage has its own rate of digestion, which affects and is interrelated with necessary nitrogen requirements. However, several investigators (Nelson and Waller, 1962; Donefer *et al.*, 1969; Williams *et al.*, 1969) reported that supplementary nitrogen did not improve the digestion of some roughages. Donefer *et al.* (1969) did observe that when the digestibility of straw was improved by treating it with sodium hydroxide, supplementary nitrogen was necessary to prevent depressed feed intake. These observations agree with those of Campling *et al.* (1962), who found that oat straw intake and digestibility were improved by infusing urea into the rumen of cows.

Two recent attempts have been made in both beef and dairy cattle diets to predict by mathematical formulae (Satter and Roffler, 1973;

Burroughs *et al.*, 1974a) the usefulness of urea supplementation based upon the total protein present in the diet as an index to rumen protein breakdown and in relation to the quantity of TDN in the diet as an index of fermentable energy. These attempts each predict that in high-concentrate diets with TDN values in excess of 75 percent on a dry matter basis, some ammonia will be synthesized into additional microbial protein at protein levels below 12 or 13 percent. At higher protein levels in these high-concentrate diets, the formulae predict that ammonia from protein breakdown will adequately support maximum microbial growth and that ammonia from added NPN will not further enhance microbial synthesis and therefore will not be useful. The formulae also predict that the breaking point between urea–ammonia synthesis and no synthesis into microbial protein is about 7 percent total protein in diets with less than 60 percent TDN on a dry matter basis. Intermediate breaking points are predicted for diets with intermediate TDN values. Further work is needed to clarify and confirm these values.

Starch was reported by Gallup *et al.* (1953) to be a superior source of energy for utilization of urea. This conclusion is not rejected by the widely successful use of urea as the supplementary source of nitrogen in high-grain diets. Feed molasses (most of which is sugarcane molasses) is the foundation ingredient for liquid supplements. Klett (1971) reviewed the use of urea–molasses liquid supplements in winter diets of cows maintained in the plains area of the United States. He indicated that economics of feeding the liquid supplement was often an overriding factor favoring its selection.

In experiments in which animal performance appeared to be superior on preformed protein-supplemented diets over performance on NPN-supplemented diets and when protein needs were low, the differences were likely due to the energy contributions of the preformed protein in low-protein diets containing liberal levels of lignocellulose-complex compounds. In addition to furnishing energy, dietary preformed proteins are sources of the branched-chain carbon skeletons.

Although lipids are important sources of energy in many nonruminant diets, they are not extensively used as a source of energy in ruminant diets at the present time. Small levels of supplemental lipids are included in the ruminant diets for energy and factors other than energy (dust control, lubricant for feeding equipment, and pelleting, etc.). The long-chain fatty acids *per se*, arising from hydrolysis of lipids, are not used by rumen microbes. Supplemental levels of dietary lipids (> 5 percent) often depress voluntary diet intake, performance, and/or digestibility (Bradley *et al.*, 1966; Thompson *et al.*, 1967; Johnson and McClure, 1973). A possible explanation of lipid-depressing effects on rumen digestibility of dietary ingredients is attributable to the physical property of coating particles.

SOURCES OF CARBON SKELETONS

The main sources of carbon chains for microbial synthesis are from fermented carbohydrates and preformed dietary amino acids. The degraded proteins are the main source of the branched-chain carbon skeletons.

DIETARY SULFUR AND OTHER MINERALS

Substitution of urea for natural protein sharply changes the quality and quantity of minerals available for ruminal bacteria and the host animal, but the presence of urea does not change the requirements for any mineral for either the ruminal microorganisms or the host animal. Availability of minerals may be altered by substitution; for example, added sulfur may be less available than the sulfur source found in the natural diet. Because major diet changes are often made when urea is included, other dietary ingredients will dictate which mineral elements need to be supplemented when urea replaces intact protein (Ovejero and Hogue, 1970).

Albert *et al.* (1956) identified the relative utilization of sulfur by growing lambs as elemental sulfur $<$ sulfate $<$ methionine when these were incorporated in a diet in which 92 percent of the nitrogen was supplied by urea. Garrigus (1970) comprehensively reviewed the need for sulfur in the diet of ruminants, and Moir (1970) reported a dietary requirement for sulfur by sheep as a nitrogen:sulfur ratio of 10:1. Hatfield (1972) indicated that an N:S ratio of 15:1 was superior to 10:1 for growing–fattening cattle. Presumably this reflects a species difference in relative production of keratin. Other research results indicate that either organic sulfur, supplied as the sulfur-containing amino acids, or the inorganic sources such as sulfur and sulfate (Chalupa *et al.*, 1973) can be used as sulfur sources in ruminant diets.

The trace-mineralized salt formulations sold for ruminants usually meet the needs for trace minerals required for cellulose digestion (Martinez and Church, 1970) and for animal metabolism and tissue deposition. Concentrate finishing diets for cattle and sheep may require supplementation with potassium and sulfur. Additional sulfur is needed with low-sulfur forages such as corn silage.

OTHER FACTORS

Lipids The influence of dietary lipids as a source of energy has been indicated earlier. There has been no consistent indication that dietary lipids improve urea utilization. Although many experiments suggest some depressing effect of supplementary dietary lipids on voluntary diet intake, digestibility, and/or general feedlot performance, there is little, if any, conclusive evidence to show that dietary lipids have a direct depressing effect on microbial synthesis.

Antibiotics Theoretically, high concentrations of true antibiotics would be expected to have some direct or indirect inhibitory effect on the rumen microbiota. However, at low concentrations there may be little, if any, inhibitory effect. Prescott (1953) was one of the first investigators to report that many antibiotics are nonspecific inhibitors of urease in rumen fluid. Visek *et al.* (1959) have also shown that oral antibiotics decrease the gastrointestinal urease activity of chicks and rats. Since urea utilization is influenced by the rate of urea hydrolysis, the effect of antibiotic supplementation may be due to a reduced urea hydrolysis. Brown *et al.* (1960b) fed four starter diets containing different protein levels to 42-day-old calves. After 3 weeks, calves receiving the antibiotic-fortified diets had the greatest gains, but feed consumption was not affected. Calves receiving the antibiotic made satisfactory gains on diets containing 3 percent less protein than levels required to produce similar gains without antibiotics. Cahill and McAleese (1964) reported beneficial effects for chlorotetracycline in urea-supplemented diets for growing–fattening lambs. Although a low level of antibiotic supplementation in ruminant diets is often reported to have a favorable influence, it is difficult to indicate specifically the mode of action of the beneficial effect—if there is one.

Urease Inhibitors Ruminal hydrolysis of urea usually occurs at a faster rate than subsequent microbial utilization of the liberated ammonia (Bloomfield *et al.*, 1960). Maximum urea utilization for microbial protein synthesis occurs when there is the simultaneous appearance of ammonia from urea and carbon skeletons from other dietary constituents. Some researchers have treated dietary carbohydrates to increase the rate of hydrolysis, while others have attempted to inhibit ureolytic activity in the rumen fluid. It has been shown that high levels of dietary urea inhibit urease activity (Caffrey *et al.*, 1967a; Chalupa *et al.*, 1970). However, there appears to be enough activity to completely hydrolyze urea under a variety of conditions. Clifford *et al.* (1968) found no beneficial effects on urea utilization from adding urease inhibitors such as barbituric acid, copper, or nitrates. A high level of acetohydroxamic acid decreased ruminal ammonia production and increased nitrogen retention in ruminants (Streeter *et al.*, 1969). Brent *et al.* (1971) have considered the kinetics of urease inhibition by acetohydroxamic acid. Oklahoma workers (Glimp and Tillman, 1965; Harbers *et al.*, 1965; Sidhu *et al.*, 1968, 1969) injected purified jackbean urease subcutaneously into cattle and sheep to produce circulating antibodies to urease. Ureolytic activity was reduced throughout the intestinal tract, and animal performance was improved when urea was the dietary nitrogen source. The practical application of this method is questionable. Chemical inhibitors do not presently appear to offer much promise for improving urea utilization.

FEEDING PROCEDURES TO IMPROVE UREA USE

ADAPTATION

Retention of nitrogen by ruminants fed urea sometimes appears to increase with length of the feeding period until a plateau is attained. This period of increased efficiency of utilization is sometimes referred to as an "adaptation period." Some investigators (Repp *et al.*, 1955a; Anderson *et al.*, 1959; McLaren *et al.*, 1959; Smith *et al.*, 1960; J. R. Campbell *et al.*, 1963) have noted this adaptation, while others (Miller and Morrison, 1942; Ewan *et al.*, 1958; Hirose *et al.*, 1960; Karr, 1964; Schaadt *et al.*, 1966; Caffrey *et al.*, 1967a; Oltjen *et al.*, 1969) have not. Caffrey (1965) presented experimental evidence that the response with time noted by some workers is an adjustment to the nutritional regimen rather than an adjustment to urea. Even with infusion of urea intravenously for 60 days, he noted no change in blood or ruminal ammonia.

LEVEL AND FREQUENCY OF FEEDING

Previous discussions have enumerated effects of total and/or supplemental dietary protein levels. Although frequency-of-feeding effects are sometimes confounded with changes of total diet intake, most of the data support the hypothesis that a constant or continuous intake of urea will improve its utilization over abrupt or periodic intake.

Most of the monitoring of ruminal ammonia concentrations show that, after a single administration of dietary urea, the ruminal ammonia increases rapidly, peaking in 60–90 min, and then declines, reaching initial ruminal ammonia concentrations in 4–5 h after administration. Obviously, this type of erratic ammonia environment for the microbiota can be improved by decreasing the ammonia concentrations, by feeding less urea, and by increasing the frequency of feeding.

Several investigators have reported improved performances by increasing the frequency of feeding (Campbell and Merilan, 1961; J. R. Campbell *et al.*, 1963; Simpson and Woods, 1965; Goodrich and Meiske, 1966; Deif *et al.*, 1970). Prior (1974) found essentially the same performance from lambs fed twice or 12 times daily soybean meal-supplemented diets. However, he found that feeding urea-supplemented diets twice daily produced negative nitrogen balances that were positive when urea was fed 12 times daily. Contrary to the favorable effects of more frequent feeding, other workers have observed only small or no favorable responses (Schoenemann and Kilian, 1960; Bloomfield *et al.*, 1961; Knight and Owens, 1973).

THOROUGH MIXING

Field and laboratory observations have shown that inadequate mixing can increase the incidence for acute ammonia toxicity by permitting overconsumption of urea. Diet acceptability is often reduced by insufficient mixing of the dietary ingredients. Also, the utilization of the ingested urea will be less with an inadequately mixed diet.

ADDITION AT ENSILING

One of the most effective ways to ensure thorough mixing is to add urea to chopped, whole plant, corn forage at ensiling; and this method has been advocated and adopted in a number of states. A diluting effect occurs that will reduce the possibilities of urea toxicity when the urea-enriched silage is fed. The levels of urea supplementation to silages can be made to meet specific nitrogen requirements for a particular purpose (maintenance, growing, fattening, etc.) or just to upgrade the silage for later formulation (Hatfield and Garrigus, 1967). Fortifying the fresh material to be ensiled with approximately 0.5 percent urea has given good results. Assuming that the fresh material is 35–40 percent dry matter, the addition of 0.5 percent urea on an "as is" basis at ensiling will raise the protein equivalent level of the dry matter about four percentage points. Adding higher levels of urea at ensiling time, although successful in some instances, may create problems by buffering the silage-preserving acids sufficiently to create storage problems. Both the acid-buffering property of urea and the acid production potential of the material to be ensiled should be considered in determining the level of urea to fortify materials to be ensiled. For example, silage materials having little or no grain should have a lower level of urea supplementation than high-grain or all-grain materials.

LIQUID SUPPLEMENTS

These products are primarily molasses-based materials with urea or other NPN compounds as the major nitrogen source plus an array of minerals. Their use has become increasingly widespread in recent years because of the availability and price of ingredients used and the convenience of handling and feeding liquids. Some of the advantages of the molasses-based liquid supplements include:

1. Supplies available energy for the rumen microbiota that convert NPN to microbial nitrogen.
2. Provides a suitable delivery system for many soluble micronutrients and other dietary nonnutritive additives in properly formulated supplements.
3. Reduces dust hazards and wind erosion.
4. Provides a cohesive medium for combining the supplement with other ingredients of the diet that will improve diet uniformity—particularly high-forage diets.
5. Improves acceptability of diets containing high levels of low-quality forages.
6. Fits well into certain mechanized feeding systems.

Some problems reported with liquid supplements include:

1. Needs to be kept in solution or suspension over a period of time with different environmental temperatures.
2. Requires special equipment for convenient addition and mixing into the rest of the diet.
3. Possibly leads to overconsumption and great variation in individual intakes when self-fed.
4. Causes some corrosive effect on equipment.

The choice of dry or liquid supplements is likely determined by the economics and/or convenience for a particular operation.

Although the lick wheel feeder has significant advantages for group-fed cattle, especially those on pasture, variation in consumption from lick wheel feeders is large (Webb *et al.*, 1973). Specific reviews on the use of liquid supplements are those by Loosli and McDonald (1968), Wornick (1969), and Huber (1972).

ANABOLIC AGENTS

A review of numerous experiments in the literature indicate that anabolic-like substances elicit some favorable biological response over comparable controls. The responses to these compounds were not correlated with any specific dietary nitrogen source and occurred as often in animals receiving diets supplemented with natural proteins as with NPN. It appears that the effect of these compounds occurs at the tissue level; consequently, methods of administration have some influence on concentrations that get to the tissue level and appear as a residue in excreta. There appears to be no substantial effect of these substances on the microbiota in the rumen.

SUMMARY AND CONCLUSIONS

Since dietary urea is used as a source of nitrogen for microbial protein synthesis, which in turn furnishes amino acids for the host animal, it is apparent that nutritional management must be considered that will maximize microbial protein production. Because urea is hydrolyzed so rapidly in the rumen, which in rare cases could produce urea (ammonia) toxicity, feeding procedures that consider safety must have first priority. Many of the recommended practices that reduce the chances of ammonia toxicity will, coincidentally, be practices that will improve urea utilization. For example, recommendations for continuous feeding of low levels (or avoiding periodic feeding of high levels that are hazardous) will provide a relatively continuous supply of ammonia in the rumen for microbial activity whenever other necessary ingredients are present.

The microbiota of the rumen will adapt rather quickly to their chemical environment. Whenever urea or urea-containing supplements are first introduced in the diet, a few days adaptation appear to be necessary for maximum utilization. Part of the adaptation may be due to diet acceptability and adaptation to diet components other than urea.

For maximum utilization, the level of urea supplementation should be adequate to meet the total nitrogen requirements of the animals, but frequency of feeding should be often. Urea cannot be recommended as a safe or satisfactory source of supplementary nitrogen if the management involves infrequent feedings. However, urea is an excellent source of supplementary nitrogen whenever the levels are low and the diet is fed frequently or *ad libitum*. Most ruminants will eat 10–16 meals daily when fed *ad libitum*, which distributes the urea intake over the day.

Because urea is very soluble and quickly hydrolyzed, care must be exercised to insure that it is completely and uniformly mixed with the remainder of the diet. Top-dressing other ingredients with urea or urea-containing supplements is not a recommended practice and may be hazardous.

The addition of urea to forage at ensiling time is a convenient method of urea supplementation to silage diets.

The use of liquid supplements as a method for supplementing the diet with urea has several advantages, such as furnishing available energy for the microbiota that are necessary in urea utilization, providing a delivery system for micronutrients and other dietary additives, and improving nutrition management to insure diet uniformity and reducing losses due to dust and wind erosion.

The response of ruminants to anabolic agents appears to be at the tissue level of the host with little or no direct effect on the microbiota; consequently, anabolic agents would generally improve the utilization of all nutrients, including either natural proteins or NPN compounds.

4 Use of Other NPN Products for Protein Replacement

UREA-CARBOHYDRATE COMBINATIONS

Several new products containing urea combined with carbohydrate feeds have recently been marketed for use in ruminant diets. These products differ from earlier ones in that manufacturing procedures usually include a process step or the addition of an ingredient designed to slow down the rate of ammonia release from urea while the product is in the rumen. One such product makes use of heat and pressure generated by forcing mixtures of urea and air-dry cereal grain through an extruder die. Another product relies on a different process, in which water is added to the air-dry cereal grain before the mixture is passed through the extruder die. Two other new products make use of molasses–urea mixtures to which a material is added for purposes of slowing down ammonia release.

These new products usually contain from 10 to 20 percent urea and are free-flowing with good handling and storage characteristics, allowing them to be mixed into diets with minimal segregation. Although the principal claimed benefits of these products over earlier mixtures are assumed to be improved efficiency and greater utilization of urea–nitrogen via slower-released ammonia from diets fed twice daily, this assumption may not be entirely valid based upon recent research results such as those by Knight and Owens (1973). Instead, the manufacturing procedures imposed may bring about alterations within the mixtures other than slower-released ammonia demonstrated by Helmer *et al.*

(1970a), which may be responsible for some and possibly the major part of the claimed benefits. For example, some of the extruded products take on a dark amber appearance following processing, which suggests alterations in the organic constituents of the cereal grains as a result of processing. Such alterations might increase palatability of the new product, and increased feed (energy) consumption by cattle or sheep might be the more important explanation for the claimed improved urea utilization. Insufficient research data have been reported for appraising the validity of this palatability hypothesis; however, the negative results reported by Harris *et al.* (1973) and the positive results reported by Helmer *et al.* (1970b) and Stiles *et al.* (1970) are supportive. This is a worthy area for additional research, since greater energy consumption is known to increase urea–nitrogen conversion into microbial protein as shown by Bloomfield *et al.* (1964) and used by Satter and Roffler (1973). Four recent growth trials reported by Thompson *et al.* (1972) and Schmidt *et al.* (1974), comparing an extruded urea–grain product with urea failed to demonstrate appreciable palatability differences between the two products; and cattle performance, likewise, did not differ appreciably. Despite these recent trials, greater palatability and increased energy intake resulting from the use of any new type of urea product would be a useful feature in ruminant diets, especially in the high-producing cow, young growing calf and lamb, and feedlot cattle and lambs starting on feed when energy consumption is lowest.

The future prospect of these new NPN- or urea–carbohydrate combinations being as beneficial as oilmeals in supplements incorporated into cattle and sheep diets would appear to be better when the diet contains less natural protein than about 13 percent of its dry matter content. When feedstuffs contain larger amounts of natural protein and there is a need for protein supplementation, oilmeal-type supplements would be expected to out-perform NPN supplements.

Although these new urea–carbohydrate products may have slower ammonia release rates than former mixtures, this feature should not be regarded as safety insurance against their misuse in feeding practice. Where a decision has been made to use these products, they should be fed in accordance with good feeding-management practices, as recommended by manufacturers.

AMMONIATED PRODUCTS

Ammonium salts have also been fed to ruminants as nitrogen sources. There have been attempts to ammoniate molasses and other carbohydrate sources in order to increase their nitrogen content. Millar (1942) and Stiles (1952) have developed procedures for ammoniating industrial and agricultural by-products. It was suggested that the nitrogen from these compounds could be used by ruminants (Millar 1941, 1944).

AMMONIUM SALTS

Ammonium salts of organic and inorganic acids have been tested as nitrogen sources for ruminants. Belasco (1954) and Hale (1956) demonstrated that the nitrogen of several ammonium salts, especially the organic salts, was used to a greater extent than that in urea. Belasco (1954) suggested that the organic component of these compounds was well utilized by the rumen microflora and that this stimulated rapid nitrogen uptake. Ammonium sulfate (Burroughs *et al.*, 1950; Acord *et al.*, 1966), ammonium chloride (Acord *et al.*, 1966), ammonium acetate (Repp *et al.*, 1955a,b; Kay *et al.*, 1967a,b; Varner and Woods, 1970), ammonium bicarbonate (Kirsch and Jantzon, 1934; Hart *et al.*, 1939; Hudman *et al.*, 1953), ammonium propionate (Repp *et al.*, 1955a,b; Varner and Woods, 1970), ammonium lactate (Allen and Henderson, 1972; Dutrow *et al.*, 1974), ammonium butyrate (Varner and Woods, 1970), ammonium carbonate (King and Hale, 1955), ammonium formate (Repp *et al.*, 1955a,b), monoammonium phosphate (Acord *et al.*, 1966), diammonium phosphate (Cowman and Thomas, 1962; Lassiter *et al.*, 1962; Russell *et al.*, 1962; Oltjen *et al.*, 1963; Schaadt *et al.*, 1966; Klosterman *et al.*, 1967), and ammonium polyphosphate (Colenbrander *et al.*, 1971a,b) have been studied as nitrogen sources for ruminants *in vivo* and/or *in vitro* with varying degrees of success. Some ammonium salts, as would be expected, have been shown to be relatively toxic to ruminants (Repp *et al.*, 1955a). Anhydrous ammonia has also been used directly as an additive for corn forage at ensiling, either in aqueous solution (Huber *et al.*, 1973) or as a condensed liquid (Kjelgaard and Anderson, 1974).

In an excellent review of the world literature on ammonium salts, Loosli and McDonald (1968) concluded that they are well used by ruminants. However, urea is the most widely used source of NPN, mainly because of economics.

AMMONIATED MOLASSES

Molasses is high in readily available carbohydrates; consequently, it is an attractive source of energy in solution that can be impregnated with ammonia. *In vitro* tests have suggested that only the "free" ammonia from these products is used by rumen microorganisms, while "bound" nitrogen is not significantly used (Stallcup, 1954; Davis *et al.*, 1955; Hershberger *et al.*, 1955). Indeed, *in vivo* digestion trials have shown that ammoniated molasses is of limited value as protein replacement in ruminant diets (Tillman and Swift, 1953; Tillman *et al.*, 1955, 1957a,b; King *et al.*, 1957). When fed at the rate of approximately 10 percent of the diet, ammoniated molasses has supported growth rates similar to that of urea and/or preformed protein in dairy heifers (Knodt *et al.*, 1950, 1951; Frye *et al.*, 1954; Parham *et al.*, 1955) and feedlot cattle (Tillman and Kidwell, 1951; McCall and Graham, 1953). However, other work has demonstrated that the nitrogen from ammoniated molasses was not well used as evidenced by growth trials with beef cattle and sheep (Tillman *et al.*, 1957a,b) and dairy calves (Davis *et al.*, 1955; Bartlett and Broster, 1958).

It has been reported that ammoniated molasses causes nervous symptoms in cattle and sheep (Richardson *et al.*, 1954; Rusoff *et al.*, 1954; Tillman *et al.*, 1957a,b). Feeding high levels of ammoniated molasses has caused cattle to run wildly and to injure themselves (Tillman *et al.*, 1957a) and caused endocardial hemorrhages in sheep (Tillman *et al.*, 1957b). Production of toxic 4-methylimidazole has been observed in the ammoniation of molasses and other agricultural products (Wiggins, 1956; Nishie *et al.*, 1970). It is possible that this compound is responsible for the toxic symptoms found in cattle and sheep.

AMMONIATED RICE HULLS

Recently, products of the milling industry have been ammoniated for use as ruminant feed. Several trials have been conducted to evaluate ammoniated rice hulls (ARH) as a feed source. ARH improved the digestibility of low-energy diets (Eng and Riewe, 1963; Eng, 1964), but had no effect on the nitrogen digestibility in high-energy diets when fed at levels less than 10 percent of the total diet (Furr and Carpenter, 1967). Feeding diets containing 20–40 percent ARH decreased the nitrogen digestibility in high-concentrate diets (White, 1966). Feeding less than 10 percent ARH did not affect performance of feedlot cattle fed high-concentrate diets (Furr and Carpenter, 1967; Tillman *et al.*, 1969). The combination of urea and rice hulls, fed to be isonitrogenous to the ammoniated rice hulls, promoted gains and efficiencies equal to those obtained with the ammoniated product (Tillman *et al.*, 1969). The urea-containing diets were more economical. Digestive disturbances were noted, and performance was decreased when 20 and 40 percent ARH were fed to cattle fed high-concentrate diets. ARH have been shown to be less toxic than urea when fed to sheep (Eng and Riewe, 1963).

AMMONIATED BEET PULP AND CITRUS PULP

Citrus pulp and sugar beet pulp are agricultural by-products that are commonly used as energy sources in ruminant diets. Their nutritive value can be enhanced by ammoniation (Millar, 1944). However, Ferguson and Neave (1943) concluded that ammoniation of sugar beet pulp decreased the utilization of nitrogen and seriously lowered the nutritive value of the nonnitrogenous portion of the beet pulp when fed with a low-energy diet. Later studies with diets containing some concentrate have also demonstrated the utilization of nitrogen from ammoniated beet pulp to be low, but when fed at adequate levels they could supply the supplemental nitrogen needs of dairy cows without adverse effects (Broster *et al.*, 1960). Ammoniated beet pulp and citrus pulp, containing no more than 20 percent crude protein equivalent, can be fed to beef cattle and dairy cattle in diets containing some concentrate and no more than 30–40 percent ammoniated pulp without adversely affecting intake or performance (Connell *et al.*, 1944; Davis *et al.*, 1946, 1952; McCall and Graham, 1953). The feeding of levels in excess of 40 percent of the diet generally results in a decrease in intake and performance (Kirk *et al.*, 1954, 1957), especially with younger cattle (Davis *et al.*, 1952).

BIURET

Earlier in this volume, urea was described as being soluble and quickly hydrolyzed to ammonia by urease. For efficient utilization, released ammonia must be used by rumen bacteria as it becomes available. Ammonia production greatly exceeding that utilized by bacteria is readily absorbed and, if sufficiently high, causes toxicity.

Compounds resulting from the condensation of urea consist primarily of biuret with some cyanuric acid, triuret, and others—all considerably less soluble than urea. Crude biuret contains variable levels of biuret (about 70 percent) together with some 10 percent each of urea and moisture, with the remainder being other condensation products of urea. At high dietary levels and in high-roughage diets, biuret is more readily accepted by the ruminant and is less toxic than urea (Berry *et al.*, 1956; Hatfield *et al.*, 1959). It is also more stable in ensiled mixtures

(Condon *et al.*, 1969). Biuret is not currently approved by the Food and Drug Administration for feeding to animals producing milk for human consumption because of the possibility of carry-over into milk.

The safety of biuret for ruminant animals is due to a relatively slow degradation in the rumen resulting in slow release of ammonia (Gaither *et al.*, 1955; Hatfield *et al.*, 1955; Meiske *et al.*, 1955; Repp *et al.*, 1955a; Berry *et al.*, 1956; and Clark *et al.*, 1965). Ewan *et al.* (1958) and Hatfield *et al.* (1959) reported that utilization of biuret nitrogen was similar to that of urea by sheep, but that an adaptation period was needed before rumen microorganisms were able to utilize biuret nitrogen efficiently. An adaptation period of 3 weeks for sheep (Tomlin *et al.*, 1967) and for steers (Oltjen *et al.*, 1969) has been suggested. However, Clark *et al.* (1963) observed that maximum nitrogen retention was obtained only after 6–8 weeks of feeding biuret to sheep. Variations in reports about adaptation periods to biuret appear to be related to the nitrogen status of the experimental animals and the level of protein in the basal diet to which biuret was added. The adaptation period may be shortened by the addition of readily available energy to a low-protein diet (Gilchrist *et al.*, 1968). The mechanism of adaptation seems to involve an increase in the numbers of biuretolytic microorganisms present in the rumen (Ewan *et al.*, 1958; Gilchrist *et al.*, 1968; Slyter *et al.*, 1968; Schroder and Gilchrist, 1969).

Results of utilization studies involving supplementation of low-quality roughages available for range livestock have been promising when biuret was compared to urea (Ammerman *et al.*, 1972; Fick *et al.*, 1973). Release of energy from such roughages is relatively slow, conforming to the slow release of ammonia from biuret (Tiwari *et al.*, 1973a,b). An increase in the consumption of low-quality hay by sheep fed a crude biuret supplement was observed by MacKenzie and Altona (1964). Their observations have been supported by the studies of Oltjen *et al.* (1969) and Raleigh (1969). Biuret has been used successfully as a nitrogen supplement under range conditions for cattle (Meis *et al.*, 1967) and for sheep (Tomlin *et al.*, 1967).

Lactating cows, calves, and finishing steers fed low-quality roughage supplemented with feed-grade biuret performed as well as with urea supplementation (Pickard and Lamming, 1968; Tollet *et al.*, 1969; Clanton and Brown, 1971). In feeding trials with wintering calves, Thomas *et al.* (1969) observed that feed-grade biuret promoted faster gains than urea, but not as good as with soybean meal. Feed-grade biuret proved an effective, readily acceptable, NPN supplement for long-term feeding of beef cows (Thomas *et al.*, 1971). Supplementation of low-energy diets with biuret under range-grazing conditions appears to be more suitable than supplementation with urea from the point of view of animal safety and acceptability.

When biuret was withdrawn from the diet in a "deadaptation" test, Schroder and Gilchrist (1969) found that there was an abrupt decrease in biuretolytic activity, irrespective of diet. More importantly, a practical consideration is the fact that "readaptation" must start over again once biuret is withdrawn, and readaptation does not take place at a rate faster than the original adaptation. Clemens and Johnson (1973) reported that sheep fed poor-quality hay free choice plus biuret at 4-day intervals could not maintain rumen biuretolytic activity compared to feeding biuret at 1- or 2-day intervals. The rate of adaptation to biuret by ruminal microbes is enhanced by feeding low levels of starch and moderate quantities of biuret (Clemens and Johnson, 1974). Thomas and Armitage (1972) reported essentially equal gains from steers on range and fed a biuret supplement either at a rate of 0.9 kg daily or 1.8 kg every other day.

Biuret in silage is relatively stable and usually has a higher feeding value for lambs, ewes, and cattle than urea in silage (Karr *et al.*, 1965a,b; Albert *et al.*, 1967; Owens *et al.*, 1967; Meiske *et al.*, 1968; Condon *et al.*, 1969). Urea hydrolysis produces ammonia, which neutralizes silage acids prolonging fermentation, whereas biuret remains stable during fermentation. Addition of biuret does not diminish palatability of the silage, and there is no loss of biuret nitrogen. Mild fungistatic action of pure biuret (Garrigus, 1970) may temporarily reduce losses in silage exposed to aerobic oxidation.

Biuret-supplemented finishing diets for lambs and cattle appear to be about as well used as similar finishing diets supplemented with urea (Gaither *et al.*, 1955; Hatfield *et al.*, 1955, 1959; Meiske *et al.*, 1955; Karr *et al.*, 1965b; Meis *et al.*, 1967; Thomas *et al.*, 1969; Hooper and Mudd, 1971). However, biuret supplementation of diets for full-fed ruminants on high-energy diets does not appear to be superior to urea when fed in similar diets (Oltjen *et al.*, 1974). While slow release of ammonia is probably not advantageous under feedlot conditions, it does provide safety from ammonia toxicity.

CYANURIC ACID

Some cyanuric acids and triuret results from the controlled pyrolysis of urea to produce feed-grade biuret. Cyanuric acid appears to be used by sheep at an efficiency similar to biuret (Garrigus *et al.*, 1959; Boston *et al.*, 1966). Clark *et al.* (1965) found nitrogen retention from urea, biuret, triuret, and cyanuric acid to be essentially equal by sheep fed a low-protein roughage diet.

Because urea, biuret, cyanuric acid, and triuret each has an ammonia release rate that differs from the others, there is need for more research on the value of specified mixtures of two or more of these compounds in ruminant diets.

SUMMARY AND CONCLUSIONS

Many of the ammonium salts, both organic and inorganic, have been fed to ruminants as nitrogen sources. Also, there are procedures available for ammoniating molasses and other feeds to increase their nitrogen content. Many of these have been tested in feeding and metabolism trials, and the following conclusions appear to be justified:

1. The ammonium salts of both organic and inorganic acids are well utilized by ruminants as nitrogen sources.
2. The nitrogen of ammoniated molasses is not well utilized by ruminants. Also, toxic compounds are produced in the ammoniating of molasses, and these compounds may cause nervous symptoms in sheep and cattle that result in high death losses.
3. Ammoniating beet or citrus pulp appears to increase feeding value when they are incorporated in small amounts in diets containing some grain.
4. When ammoniated rice hulls are fed at low levels in cattle diets, the nitrogen appears to be well utilized.

The major condensation products of urea are biuret and cyanuric acid. Biuret, in basic metabolism and practical feeding trials, offers much promise as a nitrogen supplement for ruminants consuming low-protein roughages supplemented intermittently. Biuret is slowly hydrolyzed and is less toxic than urea. Biuretase, which hydrolyzes biuret to ammonia, is an induced enzyme and requires variable periods of time, depending upon dietary factors, to reach a peak activity. Also, animals have to consume biuret regularly to maintain biuretase activity. The adaptation period may be reduced by the addition of readily fermentable carbohydrate, and the activity of biuretase can be maintained by frequent feedings.

Although biuret is more expensive per unit of nitrogen than urea at this time, it may find use in situations where urea feeding presents special management problems involving animal safety. Biuret is not currently cleared by the Food and Drug Administration for feeding to cows producing milk for human consumption.

5 Feeding Urea-Containing Diets to Beef Cattle, Dairy Cattle, Sheep, and Goats

BEEF CATTLE

Protein requirements for beef cattle and general guidelines for urea usage in partially satisfying these requirements are given by the National Research Council (1970). Urea has been used successfully in diets for beef cattle over the past 35 years, and review articles covering these studies include those by Krebs (1937), Reid (1953), Lewis (1961), Briggs (1967), Loosli and McDonald (1968), Tillman and Sidhu (1969), and Helmer and Bartley (1971). It has been pointed out that, although urea oftentimes is a satisfactory supplement, nevertheless, under some conditions, it is not a satisfactory source of supplementary nitrogen for beef cattle, even though conventional recommendations for urea use are followed. These recommendations include: (1) feeding no more than 1 percent of urea in the diet dry matter consumed, (2) feeding no more than 3 percent in the grain mixture consumed, or (3) feeding no more than one-third of the diet nitrogen as urea nitrogen (Rupel *et al.*, 1943). Helmer and Bartley (1971) state: "Because urea by definition is considered to be 100 percent digestible, feed formulators often consider that all nitrogen in urea is utilized by the ruminant. The nitrogen content of urea is multiplied by 6.25 to get the theoretical protein equivalent that urea nitrogen contributed directly and completely towards satisfying the digestible protein requirement of the animal. Obviously, that is not true because we have not yet learned how to utilize all of the nitrogen furnished by urea."

More recently, Burroughs *et al.* (1971d, 1974a) proposed a system to quantitate urea utilization. This proposal made use of a new evaluation of feeds based upon their estimated urea fermentation potential (UFP). The UFP was estimated on the basis of the fermentable energy of a given feed or diet as proposed by Bloomfield *et al.* (1964) and the amount of the feed or diet protein degraded in the reticulorumen when consumed by cattle. In the discussion of beef cattle feeding experiments that follows, the experiments were grouped on the basis of diets fed having energy densities capable of supporting either submaintenance to low, medium, or high levels of cattle productivity. Each productivity group is discussed, starting with the trial having the unsupplemented diet with the lowest level of protein and proceeding for the most part to the next trial with the unsupplemented diet with the next higher level of protein. This approximate arrangement also appears in Table 1 for the trials reported in which the experimental design included an unsupplemented or negative control diet. The primary purpose of negative control diets in these experiments was to ascertain protein adequacy or inadequacy without supplementation based upon animal performance. Thus, in each experiment where a greater animal response was observed with either urea or protein supplementation, it was assumed that the negative control diet was inadequate in protein and that supplementation was needed to satisfy the animal's protein requirements.

SUBMAINTENANCE AND LOW-ENERGY BEEF CATTLE DIETS

Experimental evidence indicates that supplementary urea is beneficial to submaintenance as well as low-productivity beef cattle diets with less than 60 percent TDN in their DM and composed almost exclusively of low-quality roughages with less than about 7–8 percent protein. This appears to be true whether the urea supplement contains a readily fermentable carbohydrate such as cane molasses or cereal grains. On the contrary, results of experiments with higher levels of protein demonstrate the ineffectiveness of supplemental urea in diets with less than 60 percent TDN. These will be discussed later in this chapter.

Altona *et al.* (1960) in two trials found that supplemental urea improved the performance of cattle consuming on a dry matter basis veld forage containing 3.5 percent protein and 54 percent TDN. Briggs *et al.* (1947) fed mature-weathered prairie hay containing 3.5 percent protein on a dry matter basis and observed low or negative nitrogen balances when the hay was unsupplemented. Supplementing the hay with cottonseed meal or with urea and cottonseed meal approximately doubled the nitrogen balance. Replacing all nitrogen of the cottonseed meal with

TABLE 1 Summary of Urea Benefits in Beef Cattle Diets Varying in Energy and Protein Content

Protein as a % of DM	TDN as a % of DM	Protein as a % of TDN	Was Suppl. Urea Useful?	Was Suppl. Protein Better?[a]	References
Low-Energy Diets (TDN < 60)					
3.5	54	6.5	Yes		S. Afr. J. Agric. Sci. 3(1960):69–81
3.5	54	6.5	Yes		S. Afr. J. Agric. Sci. 3(1960):69–81
3.5	54	6.5	Yes	No	J. Anim. Sci. 6(1947):445–460
3.5	54	6.5	Yes	Yes	J. Anim. Sci. 6(1947):445–460
3.5	54	6.5	Yes	No	J. Anim. Sci. 6(1947):445–460
3.5	54	6.5	Yes		Queensl. J. Agric. Sci. 16(1959):223–232
3.5	54	6.5	Yes		Farming S. Afr. 47(1971):7–9
3.5	54	6.5	Yes		Farming S. Afr. 27(1952):453–454
3.6	54	6.7	Yes	Yes	J. Agric. Sci. 58(1962):173–178
3.7	54	6.9	Yes		Queensl. J. Agric. Sci. 20(1963):213–230
3.7	54	6.9	Yes		Queensl. J. Agric. Sci. 20(1963):213–230
3.9	52	7.5	Yes		Br. J. Nutr. 16(1962):115–124
4.3	57	7.5	Yes		Queensl. J. Agric. Sci. 18(1961):409–424
4.7	54	8.7	Yes		Queensl. J. Agric. Sci. 15(1958b):181–194
4.9	57	8.6	Yes		Queensl. J. Agric. Sci. 15(1958a):161–180
5.0	54	9.3	Yes		J. S. Afr. Vet. Med. Assoc. 30(1959):457–458
5.0	54	9.3	Yes		Farming S. Afr. 33(1957):30–32
5.0	54	9.3	Yes		Farming S. Afr. 35(1959):27–29
5.0	54	9.3	Yes		Farming S. Afr. 29(1954):135–138
5.5	54	10.2	Yes	Yes	J. Anim. Sci. 22(1963):330–334
6.0	54	11.1	Yes		S. Afr. J. Agric. Sci. 3(1960):69–81
6.3	58	10.9	Yes	Yes	J. Dairy Sci. 43(1960):443–444 (A)
6.5	50	13.0	Yes	Yes	Aust. Vet. J. 24(1948):197–204
6.5	50	13.0	Yes	Yes	Aust. Vet. J. 24(1948):197–204
6.5	58	11.2	Yes	Yes	Aust. Vet. J. 24(1948):197–204
6.7	54	12.4	Yes		S. Afr. J. Agric. Sci. 3(1960):69–81
6.7	55	12.2	Yes		J. Anim. Sci. 33(1971):133–136
6.7	57	11.8	Yes	Yes	Miss. Farm Res. 7(1944):8
6.7	58	11.6	Yes		Miss. Farm Res. 6(1943):8
6.8	56	12.1	Yes		J. Anim. Sci. 8(1949):24–34
7.0	56	12.0	Yes		J. Anim. Sci. 8(1949):24–34
7.2	54	13.3	No		Okla. Agric. Exp. Stn. Misc. Publ. No. MP-43(1955):51–54
8.0	52	15.4	No	Yes	Am. Soc. Anim. Prod. Proc. (1939): 404–406
8.0	54	14.8	No		Queensl. J. Agric. Sci. 17(1960):135–146
9.5	54	17.6	No		S. Afr. J. Agric. Sci. 3(1960):69–81
9.5	54	17.6	No		S. Afr. J. Agric. Sci. 3(1960):69–81
9.5	54	17.6	No		S. Afr. J. Agric. Sci. 3(1960):69–81
Medium-Energy Diets (TDN 60–75)					
4.4	60	7.3	Yes	Yes	Can. J. Agric. Sci. 29(1949):173–184
4.5	66	6.8	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R151(1971):1–12
5.0	66	7.6	Yes	Yes	J. Agric. Sci. 59(1962):125–141

TABLE 1 (Continued)

Protein as a % of DM	TDN as a % of DM	Protein as a % of TDN	Was Suppl. Urea Useful?	Was Suppl. Protein Better?[a]	References
5.4	65	8.3	Yes		J. Anim. Sci. 35(1972):859–864
5.9	60	9.8	Yes		J. Anim. Sci. 35(1972):859–864
5.9	62	9.5	Yes		J. Anim. Sci. 35(1972):859–864
6.0	60	10.0	Yes	Yes	J. Dairy Res. 9(1938):263–272
6.0	63	9.5	Yes	Yes	J. Dairy Sci. 43(1960):890 (A)
7.4	70	10.6	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R195(1974):1–9
7.7	74	10.4	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R195(1974):1–9
8.4	69	12.2	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R195(1974):1–9
8.4	70	12.0	Yes		J. Anim. Sci. 12(1953):934 (A)
8.4	70	12.0	No	Yes	Proc. Assoc. S. Agric. Workers 52(1955):64
8.4	70	12.0	No	Yes	Proc. Assoc. S. Agric. Workers 52(1955):64
8.9	64	13.9	Yes	Yes	Aust. Vet. J. 24(1948):197–204
9.5	74	12.8	Yes		Iowa State Univ. Anim. Sci. Leafl. R195(1974):1–9
9.7	68	14.3	Yes		J. Agric. Sci. 64(1965):343–350
10.0	67	14.9	No	No	J. Dairy Sci. 49(1966):450 (A)
10.1	74	13.7	No	Yes	Iowa State Univ. Anim. Sci. Leafl. R195(1974):1–9
10.2	73	14.0	Yes	Yes	Ohio Agric. Exp. Stn. Res. Bull. 766(1955):1–20
10.3	72	14.3	Yes		J. Agric. Sci. 64(1965):343–350
10.7	74	14.4	Yes		J. Agric. Sci. 64(1965):343–350
11.4	60	19.0	No		J. Anim. Sci. 8(1949):24–34
High-Energy Diets (TDN > 75)					
–	95	–	Yes	Yes	Science 153(1966):1603–1614
–	84	–	Yes	Yes	J. Nutr. 89(1966):385–391
4.0	82	4.9	Yes	Yes	J. Nutr. 25(1943):197–202
5.0	76	6.6	Yes	Yes	J. Dairy Sci. 22(1939):785–798
5.0	77	6.5	Yes	Yes	J. Dairy Sci. 22(1939):785–798
6.2	78	7.9	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R192(1974b):1–12
6.2	78	7.9	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R173(1973):1–17
7.3	82	8.9	Yes	Yes	J. Dairy Sci. 43(1960):890 (A)
7.8	78	10.0	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R192(1974b):1–12
7.8	78	10.0	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R173(1973):1–17
8.3	82	10.1	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R153(1971):1–4
8.4	77	10.9	Yes	Yes	J. Anim. Sci. 39(1974):102–107
8.8	100	8.8	Yes		J. Anim. Sci. 28(1969):256–262
8.8	76	11.6	Yes		Queensl. J. Agric. Anim. Sci. 25(1968):19–28
8.9	77	11.6	Yes	Yes	Nebr. Agric. Exp. Stn. 63d Annu. Rep. (1950):96

TABLE 1 (Continued)

Protein as a % of DM	TDN as a % of DM	Protein as a % of TDN	Was Suppl. Urea Useful?	Was Suppl. Protein Better?[a]	References
9.2	86	10.7	Yes	Yes	Iowa State Univ. Anim. Sci. Leafl. R152(1971):1-5
9.3	81	11.5	Yes		Queensl. J. Agric. Anim. Sci. 25(1968):19-28
9.3	83	11.2	Yes	Yes	Nebr. Agric. Exp. Stn. 62d Annu. Rep. (1949):75
9.6	77	12.5	Yes	Yes	J. Anim. Sci. 39(1974):102-107
10.0	77	13.0	Yes		J. Anim. Sci. 8(1949):24-34
10.5	78	13.5	No	Yes	Iowa State Univ. Anim. Sci. Leafl. R192(1974b):1-12
10.5	78	13.5	No	Yes	Iowa State Univ. Anim. Sci. Leafl. R173(1973):1-17
10.5	86	12.2	Yes		J. Anim. Sci. 30(1970):297-302
10.7	77	13.9	Yes		J. Anim. Sci. 8(1949):24-34
11.1	77	14.4	Yes		Mich. State Univ. Cattle Rep. 645(1965): 40-47
13.0	76	17.1	No		Minn. Univ. Cattle Rep. B-40(1963):1-3
13.0	76	17.1	No		Minn. Univ. Cattle Rep. B-53(1964):7-10
13.8	80	17.2	No	Yes	Anim. Prod. 9(1967):155-165

[a] Blank spaces indicate no answers were possible due to limitations of experimental design.

urea, however, was less effective in increasing nitrogen retention. In a wintering trial, pasturing 2-year-old steers on dry prairie grass, these authors reported equal performance between a cottonseed meal supplement and one containing one-third urea and two-thirds cottonseed meal nitrogen. Briggs *et al.* (1948) found that a low-protein, dry-range grass with a supplement of 1.3 kg of pellets per animal daily with urea providing 25 percent of the nitrogen satisfactorily maintained beef bulls and pregnant cows.

There is an abundance of experimental evidence to show that the inclusion of readily fermentable feeds in a urea supplement enhances the effectiveness of the supplement. Clark (1952) fed a mature veld hay low in protein to Tollier cattle. Unsupplemented cattle lost weight, while those supplemented with molasses and urea gained weight. Similar results were obtained by Bishop and Wilke (1971) and Beames (1959). Beames (1960) found that the ratio of urea to molasses could be reduced from 1:8 to 1:2 without loss of effectiveness, if the mixture supplied adequate urea. Von La Chevallerie (1965) investigated the effectiveness of various ratios of urea to molasses in licks placed before three groups of cattle. The results indicated that the ratio of urea to molasses is of importance if the narrow ratio lowers feed intake to the extent that the intake of urea is too low. Smith (1962), in a short-term experiment with the feeding of low-quality veld hay containing 3.6 percent protein in the dry matter, observed that supplements of either urea or peanut meal increased feed intake and performance in cattle.

Beames (1963) found that cattle under 18 months of age could not survive 161 days on a low-protein veld hay containing 3.5 percent protein in the dry matter. Older cattle during the same period survived on the hay alone, but lost weight. Survival in the younger cattle and body weight losses in the older cattle were not materially improved by spraying the hay with molasses. However, the addition of urea to the molasses enabled all animals to survive with only slight loss in body weight.

Campling *et al.* (1962) fed nonlactating cows oat straw containing 3.3–4.1 percent protein in the dry matter and daily continuously infused into the rumen of each cow 150 g of urea, which was in solution with or without 500 g of sucrose. They found that urea supplementation with or without sucrose increased voluntary intake of straw 40 percent and increased organic matter digestibility 41–50 percent. Cotton strings submerged in the rumen ingesta through a fistula opening were digested nearly five times as rapidly in urea-supplemented cattle as compared with unsupplemented animals. These results were confirmed by Pieterse and Lesch (1963).

Ryley (1961) fed sorghum silage unsupplemented and supplemented with two levels of urea to heifers in late pregnancy and early lactation. The unsupplemented diet contained an estimated 4.3 percent protein and 57 percent TDN on a dry matter basis. After 24 weeks of feeding, the unsupplemented cattle lost more weight than the supplemented ones. Morris (1958a) also found that cattle performance was improved when either urea or crushed sorghum grain was added to the diet of hay containing 4.7 percent protein in its dry matter.

In another study Morris (1958b) fed Hereford heifers a sorghum silage diet containing an estimated 4.9 percent protein and 57 percent TDN on a dry matter basis. During the 28-week feeding period, animals without supplementation lost weight, while those receiving urea gained.

Barrie and Clark (1959) confirmed the earlier work of Clark (1952), which indicated that urea and molasses supplementation improved the diets of cattle consuming a low-protein veld hay. Cattle without supplementation lost weight, while those supplemented gained. Bishop (1957, 1959) pastured groups of 2- and 3-year-old oxen on mature and weathered winter veld pasture of low-protein content. Cattle without supplementation lost more weight than those with supplementation. Cattle-feeding experiments by Clark and Barrie (1954) with veld hay containing 5.5 percent protein in the dry matter and supplemented with 1.8 kg of ground cobs containing urea resulted in live-weight gains compared with no gains in unsupplemented cattle. Raleigh and Wallace (1963) mixed ground hay that contained 5.5 percent protein with different amounts of urea, cottonseed meal, or a mixture of the two, to supply 6, 9, and 12 percent protein. The complete mixtures were then pelleted and fed to similar groups of cattle. Ammonia toxicity and death losses occurred with the 12 percent protein pellet containing only urea and no cottonseed meal. However, the lower urea levels promoted better live-weight gains than obtained in control animals. Gains were highest in animals fed cottonseed meal as the only supplemental nitrogen source. Intermediate live-weight gains occurred when the supplemental nitrogen was 50:50 urea and cottonseed meal.

Altona *et al.* (1960) reported benefits from urea supplementation of veld forage containing 6 and 6.7 percent protein and 54 percent TDN on a dry matter basis. Browning *et al.* (1960) reported a daily dry matter intake of 0.91 percent of body weight in cattle receiving sorghum silage compared with 1.11 percent in animals fed 160 g of urea daily with silage estimated to contain 6.3 percent protein and 58 percent TDN on a dry matter basis.

When McClymont (1948) fed wheat straw with and without small additions of cereal grains, he found that urea supplementation in diets containing 6.5 percent protein and 50–58 percent TDN improved cattle gains. Similar benefits from urea supplementation were noted in research by Chicco *et al.* (1971) when they fed a green-chop elephant grass containing 6.7 percent protein and an estimated 55 percent TDN in the dry matter.

Cullison (1943) fed control cows a sweet sorghum silage and Johnsongrass hay. The diet was estimated to contain 6.7 percent protein and 58 percent TDN on a dry matter basis. The experimental cows were fed the same diet, except that 0.5 percent urea was added to the silage at the time of ensiling. After 78 days, the control cows had lost weight, while those receiving the treated silage had maintained their body weight. Means (1944, 1945), using similar diets, confirmed Cullison's results.

In two metabolism trials, Dinning *et al.* (1949) fed a maintenance diet containing an estimated 56 percent TDN and 6.8–7.0 percent protein in the diet dry matter and found that urea additions increased nitrogen balances.

The performance of cattle consuming the many diets presented up to this point have been improved by urea supplementation. Most, if not all, have been improved more by protein supplementation. All the diets before supplementation were estimated to contain less than about 7 percent protein and less than 60 percent TDN in the diet dry matter, supporting submaintenance or low cattle production. By contrast, a number of diets containing this low level of energy and more than 7 percent protein have been investigated, and these have not responded to urea supplementation. For example, a thick stand of native grass in Oklahoma containing 7.2 percent protein on a dry matter basis was sprayed by Pope *et al.* (1955) with a mixture of six parts cane molasses and one part urea during late summer. The cattle gained slightly less weight over a 62-day period on the sprayed, as compared with the unsprayed, grass. Work and Henke (1939) fed a diet containing an estimated 8 percent protein and 54 percent TDN in the dry matter and received no significant benefits from urea supplementation. Similar results were obtained by O'Bryan (1960).

Altona *et al.* (1960) fed eragrostis hay containing 9.5 percent protein and found that urea supplementation did not improve cattle performance. In one trial no significant differences in gains were observed with 1- and 2-year-old steers fed for 85 days on eragrostis hay with licks containing molasses with or without urea. In the same trial, steers fed eragrostis hay sprayed with a solution of urea and molasses performed little differently than when a urea–molasses lick was used. In two additional trials, cattle were fed eragrostis hay with additions of silage or urea, which was dissolved in water and sprayed on the hay. Neither supplement improved gains. In the final trial, four molasses solutions with 0–275 g of urea per 1 kg were sprayed on the eragrostis hay. No urea level produced greater gains than found in the controls. Verbeek and von La Chevallerie (1960) also supplemented eragrostis hay with either urea, peanut meal, or guano meal and found no significant differences in cattle gains.

Several experiments have been reported comparing urea with protein supplementation for submaintenance and low-energy beef cattle diets in which no supplemented control diets were fed. Cattle performance in most of these experiments (Murray and Romyn, 1939; Nelson *et al.*, 1957; Berry *et al.*, 1958; Davidson and Purchase, 1961; Nelson and Waller, 1962; Kreft, 1963; Horn and Beeson, 1969; Williams *et al.*, 1969; Varner and Woods, 1970) was satisfactory but with some small advantage toward protein supplementation.

In summary, cattle performance was improved when urea was added to submaintenance and low-productivity diets having less than 7 to 8 percent protein in their dry matter. Such diets contain principally forages such as mature and weathered pasture grasses, cereal grain straws, corncobs, corn stover, and silages. The addition of readily fermentable energy sources such as the low-protein cereal grains or molasses to these low-productivity diets further improved animal performance when urea was added.

MEDIUM-ENERGY BEEF CATTLE DIETS

Medium-energy beef cattle diets are defined here as those containing 60 to 75 percent TDN in the dry matter. Composition of the diets used in the research reports reviewed were quite varied. However, they ranged from high-roughage feeds with small additions of cereal grains to high-silage (whole plant sorghum and corn) feeds with additions of cereal grains. These medium-productivity diets, which contained less than 7 to 8 percent protein in the dry matter would be expected to be benefitted by urea supplementation, since the lower-energy diets reviewed in the preceding section with this amount of protein were benefitted by urea supplementation. This proved to be true in each of the following research reports reviewed (see Table 1).

Watson *et al.* (1949) fed calves a diet consisting of timothy hay, oat straw, barley grain, cornstarch, and cane molasses. The diet TDN and protein percentages of dry matter were 60 and 4.4, respectively. The calves receiving the basal diet failed to gain, while those receiving the basal diet supplemented with urea gained 99 kg in about 1 yr. Another group supplemented with casein gained 142 kg.

Burroughs *et al.* (1971b) fed light-weight steer calves a basal diet containing 66 percent TDN and 4.5 percent protein and composed of corncobs, corn grain, cane molasses, and beef tallow. The calves receiving the basal diet lost weight, whereas similar calves fed the basal diet supplemented with urea gained 25 kg in 50 days and calves supplemented with herring and soybean meals gained twice as much.

Coombe and Tribe (1962) fed a basal diet estimated to contain 66 percent TDN and 5 percent protein in the dry matter and composed of fresh sugarcane plus a small amount of corn grain. Cattle receiving the basal diet lost weight, contrasted with the cattle receiving the basal diet supplemented with urea. The latter gained a moderate amount, while cattle receiving protein (alfalfa hay) gained substantially.

Chicco *et al.* (1972) compared three unsupplemented diets—mature chopped Guinea grass, hay, and molasses—with three similar diets to which urea was added. The protein in the three unsupplemented diets was 5.4, 5.9, and 5.9 percent, and the TDN content varied between 60 and 65 percent by feeding a constant quantity of molasses with differing quantities of hay. Highly beneficial results from urea supplementation were observed with each diet.

Bartlett and Cotton (1938), in one of the earliest reported cattle experiments with urea, fed heifers a 6 percent protein and 60 percent TDN basal diet on a dry matter basis and obtained small live-weight gains. Animals receiving a similar diet supplemented with urea gained a moderate amount, whereas those receiving a protein supplement gained more. Brown *et al.* (1960a) fed heifers a diet composed of corncob–corn silage and containing 6 percent protein and 63 percent TDN on a dry matter basis. When the diet was supplemented with either urea or soybean meal, it was found that the urea-supplemented group gained well, but those receiving the soybean meal made 11 percent greater gains. Vetter and Burroughs (1974) fed five basal diets containing 7.4, 7.7, 8.4, 9.3, and 10.1 percent protein and from 69 to 74 percent TDN to young growing calves initially weighing about 160 kg. Each diet was supplemented with an equal amount of nitrogen from urea or protein, except the 9.3 percent protein diet, which received only the urea supplement. Protein supplementation stimulated live-weight gains with all diets tested and to a greater extent than that observed with the urea supplement. However, urea supplementation resulted in substantial stimulation in all diets except the highest protein diet (10.1 percent before supplementation).

Van Arsdell *et al.* (1953) fed fattening steers corn silage *ad libitum* and supplemented it with either soybean meal or urea. The diet on a dry matter basis was estimated to contain 70 percent TDN and 8.4 percent protein. Daily live-weight gains were 10 percent greater in the group receiving the soybean meal supplement.

Goode *et al.* (1955) fed four lots of heifer calves and four lots of mature pregnant cows (1) an unsupplemented corn silage diet estimated to contain 70 percent TDN and 8.4 percent protein on a dry matter basis, (2) unsupplemented corn silage treated with 0.5 percent urea, (3) corn silage supplemented with 0.23 kg soybean meal daily per animal, and (4) urea-treated corn silage with 0.23 kg of soybean meal added. No benefits were obtained from the urea treatment of the silage. The addition of supplementary soybean meal, however, resulted in significantly faster live-weight gains in both the cows and heifers.

Robertson and Miller (1971) supplemented a corn silage diet fed to beef calves with levels of urea nitrogen varying from 0 to 28 percent of the total supplemental nitrogen. Although no unsupplemented negative control diet was fed, the authors concluded that urea could be effectively utilized up to 20 percent of the supplemental nitrogen in this type of diet.

McClymont (1948) fed a half-concentrate, half-wheat-straw basal diet to beef calves. The diet contained an estimated 64 percent TDN and 8.9 percent protein on a dry matter basis. A second group of calves received supplementary urea to make a 14 percent protein diet, while a third group received supplementary protein to make a 14 percent diet. The respective live-weight gains on the three diets were progressively larger, starting with the first diet. Various ratios of sorghum silage to sorghum grain (60:40, 40:60, 20:80) with and without urea supplementation were fed to yearling Hereford steers by Morris and O'Bryan (1965). The estimated TDN percentages of the three diets were 68, 72, and 74, and the estimated protein percentages were 9.7, 10.3, and 10.7, respectively, on a dry matter basis. Average daily live-weight gains and improved feed conversions were appreciably improved by urea supplementation of each diet.

Pope *et al.* (1959) fed a sorghum grain and silage finishing diet containing either a soybean meal or a molasses–urea supplement to heavy yearling steers. No negative control diet without supplementation was fed. The diets, before supplementation on a dry matter basis, were estimated to contain 73 percent TDN and 9 percent protein. The cattle receiving the soybean meal supplement made daily live-weight gains 10 percent greater than those receiving the urea supplement.

Three diets were fed to heifers by Lassiter *et al.* (1958a). The diets were composed of ground corncobs and grain mixtures and contained either 3, 5, or 7 percent protein equivalent from urea. Each diet, after supplementation, was approximately isonitrogenous and isocaloric, containing on a dry matter basis an estimated 62 percent TDN and 9.2 percent protein. Daily live-weight gains were highest with the diet containing the least urea and were lowest with the diet containing the largest amount of urea. The diet containing intermediate amounts of protein and urea supported an intermediate rate of gain.

Finishing diets containing sorghum grain and silage were supplemented by either soybean meal or urea by Sellers *et al.* (1960). The diets, before supplementation, contained an estimated 10.3 percent protein and 73 percent TDN in the dry matter. The urea-supplemented cattle gained almost as much (95 percent) as the soybean-meal-supplemented cattle. In a companion experiment, in which the sorghum silage was replaced with cottonseed hulls, the urea-supplemented cattle made appreciably smaller daily live-weight gains as compared with the soybean-meal-supplemented cattle. This diet had a lower TDN (70 percent) and protein (9.4 percent) content than did the sorghum grain silage diet.

Bond and Oltjen (1973) studied growth and reproductive performance of beef females fed high urea-containing diets over long feeding periods. They reported that urea-fed cows performed satisfactorily and almost as well as cows receiving soybean meal supplementation.

Reddy *et al.* (1961) fed diets estimated to contain 65 percent TDN and 9.4 percent or more protein composed of equal parts grass hay and isonitrogenous concentrate mixtures containing 0, 9, 18, or 36 percent of their nitrogen as urea nitrogen. Although no negative control diet was fed, they concluded that urea was satisfactorily used since cattle performance was approximately similar in each case. Considering the high level of protein in relation to TDN in their diet, this conclusion may or may not be valid.

Approximately the same situation seems to have existed in an experiment reported by Kirk *et al.* (1963), in which pangola grass hay and silage were supplemented with either cottonseed meal or a mixture of cottonseed meal, urea, and citrus pulp. The performance of the cattle was similar with the two supplements; but, again, the urea-containing diet had an estimated 9.6 percent protein and 71 percent TDN content on a dry matter basis. Therefore, there was probably no need for any supplemental nitrogen in the diet. Kirk (1952) supplemented yearling steers grazing native pasture with either cottonseed meal or citrus pulp meal and urea and obtained similar live-weight gains. The protein content of the pasture was not indicated.

Bentley *et al.* (1955), in a 3-year study with steer calves, fed a corn silage and hay diet in which half the corn silage was treated with urea levels of about 1 percent at the time of ensiling. In one trial with untreated and unsupplemented corn silage, steer calves gained moderately well while consuming the basal diet containing 10.2 percent protein and an estimated 73 percent TDN on a dry matter basis. Steers fed a similar diet supplemented with urea or soybean meal gained 7 and 16 percent greater, respectively. In another trial, 190-kg steers fed the untreated silage and hay diet supplemented with soybean meal outgained by 13 percent cattle receiving a similar diet supplemented with urea. When the urea added to the silage at ensiling time and the urea added at the time of feeding were not considered, this diet contained 10.7 percent protein and an estimated 71 percent TDN on a dry matter basis. These authors in a third trial obtained rather similar gains in steers fed urea-untreated or treated silage and hay diets when each diet was supplemented with urea. The untreated silage diet, before the urea supplementation, contained 9.8 percent protein and an estimated 73 percent TDN on a dry matter basis.

Davis *et al.* (1944) found that sorghum silages containing no urea and silages with 4.5 kg of urea added per 908 kg were consumed similarly by cattle. However, the cattle refused silages containing 13.6 kg of urea per 908 kg. Woodward and Shepherd (1944) reported that adding 0.5 percent urea to corn silage slightly lowered the acceptability of the diet for mature cows. In a review of eight Ohio experiments, Klosterman *et al.* (1963) found that, in half the experiments, cattle receiving limestone-urea-treated corn silage gained at a faster rate than those receiving untreated silage; in all experiments, feed requirements per unit of gain were lessened by the treatment. Essig (1968), in a review of 26 experiments, stated that there seemed only a slight benefit from adding urea at the time of ensiling as compared with adding urea as a supplement to feeds.

Brown and Jacobson (1966) fed young heifers a diet composed of equal parts orchardgrass hay and one of three concentrate mixtures containing either 1.06 percent urea, 7.88 percent soybean meal, or 2.09 percent urea. The diets, when urea and soybean meal were not considered, contained an estimated 10 percent protein and 67 percent TDN on a dry matter basis. Live-weight gains were similar, as were digestibility data collected on the three diets.

Urea supplementation of a basal diet fed to 2-year-old steers by Dinning *et al.* (1949) resulted in no additional nitrogen retention in steers. On a dry matter basis, the diet contained an estimated 11.4 percent protein and 60 percent TDN. Briggs *et al.* (1947) fed a diet estimated to contain 12.4 percent protein and 65 percent TDN on a dry matter basis before supplementation. A urea supplement gave equal performance to a protein supplement.

In summary, all medium-energy diets reviewed in this section except two were improved by urea supplementation provided they contained less than 10 percent protein on a dry matter basis or less protein than 12.0–14.5 percent of diet TDN. The two exceptions may have been due to ensiling methods, since the urea was added in each case prior to ensiling. Medium-energy diets, when benefitted by urea, were always benefitted more by protein when they were isonitrogenous and approximately isocaloric.

HIGH-ENERGY BEEF CATTLE DIETS

High-energy beef cattle diets, for the purposes of this discussion, are those diets containing 75 percent or more TDN in their dry matter. These diets have ample energy for supporting maximum live-weight gains or milk production in beef cattle. They contain considerable concentrate feeds.

Many experiments conducted with these high-productivity diets have not included a negative control basal diet and have only compared the effects of urea supplementation versus those of protein. Such experiments are valuable in measuring differences in supplements, but they may fail to measure whether a supplement had a negative influence, a positive influence, or no influence at all. In the case of growth or lactation trials, only an experimental design containing a negative control diet without urea can be relied upon to measure whether or not benefits accrue from urea supplementation. The purified diets of Virtanen (1966) illustrate the positive benefits obtained from urea when added to a high-productivity lactation diet. In the many experiments conducted by Virtanen (1966) with diets essentially devoid of protein, but containing supplemental urea and energy equivalent to 95 percent TDN on a dry matter basis, daily milk production over a 12-month period commonly averaged about 11 kg per animal. This amount of milk would be sufficient for beef cows rearing one to two calves and could not have been produced for a long period of time had not the urea been effectively utilized. However, this level of production was only about two-thirds that obtained in dairy cows (Virtanen, 1967) when they were placed on natural diets containing protein, thus suggesting that urea was not used as completely as was protein. Ryś (1967) pointed out that: "Cows with a low or average milk production (from 2,000 to 3,000 kg of milk annually) are best fitted for the full utilization of urea. . . ."

Benefits from urea were obtained by Oltjen and Putnam (1966) with young growing Aberdeen Angus steers fed a semipurified diet essentially devoid of protein and supplemented with urea containing about 84 percent TDN on a dry matter basis. Nitrogen balance data indicated substantial benefits from the urea, but animal performance was about two-thirds of that obtained when protein was fed as the nitrogen source. Oltjen and Bond (1967) reported that two beef cows reproduced successfully when raised on a purified diet containing urea as the only source of dietary nitrogen.

In at least 26 experimental trials reported in the literature, cattle were fed high-productivity diets in which negative control diets devoid of urea were fed. Benefits from urea resulted in all experiments when the basal diet contained less than 11 or 12 percent protein on a dry matter basis or less than 13.5–14.5 percent on a TDN basis (Table 1). Two such experiments representing the earliest experiments conducted with cattle in the United States were reported by Hart *et al.* (1939). In one experiment, a basal diet containing 5–6 percent protein and an estimated 76 percent TDN in the dry matter was fed to young calves. The animals receiving the basal diet made a small live-weight gain, while the calves receiving urea gained 25 percent faster and calves receiving casein gained still more rapidly. In the second experiment, a similar basal diet composed of corn grain, cornstarch, cane molasses, and timothy hay with an estimated 5 percent protein and 77 percent TDN in its dry matter was fed. Calves fed the basal diet did not gain, while urea-supplemented calves gained moderately and the casein-supplemented calves gained more rapidly.

Loosli and McCay (1943) fed a corn grain, cornstarch, cane molasses, and timothy hay basal diet containing an estimated 4 percent protein and 82 percent TDN on a dry matter basis to young calves. Calves receiving the basal diet barely maintained live weight, while the calves receiving the basal diet plus urea gained moderately and the calves receiving the basal diet plus protein gained most rapidly.

Brown *et al.* (1956) fed a basal diet composed of corn, oats, cornstarch, cane molasses and timothy hay plus liquid milk to young calves. The dry diet contained 7.3 percent protein and 82 percent TDN. Calves receiving this basal diet gained moderately; calves receiving urea in addition to the basal diet gained 6.7 percent faster; and calves receiving linseed meal added to the basal diet gained most rapidly.

Burroughs *et al.* (1971a,c), in two trials with steers, fed corn basal finishing diets containing an estimated 8.3 and 9.2 percent protein and 82 and 86 percent TDN, respectively, on a dry matter basis. The cattle receiving the basal diets gained medium well, while the cattle fed the basal diet supplemented with urea gained faster and those supplemented with protein gained still more rapidly.

An experiment reported by Putnam *et al.* (1969) made use of a basal diet composed of ground corn, cane molasses, and soybean oil that contained an estimated 100 percent TDN and 8.8 percent protein on a dry matter basis. Urea supplementation failed to improve live-weight gains during the last 63 days of the finishing period, when 6.3 kg of corn dry matter were consumed daily per animal. Feed requirements per unit of gain, however, were improved 3 percent, even though the urea-supplemented diet contained a smaller percentage of TDN in the dry matter than that present in the unsupplemented diet.

Thurbon and Winks (1968) fed cattle two diets composed of varying amounts of corn forage and corn grain with and without urea supplementation. The diets on a dry basis were estimated to contain 8.8 and 9.3 percent protein and 76 and 81 percent TDN, respectively. Urea proved beneficial to each of the diets.

Dowe *et al.* (1950) fed a corn basal diet containing an estimated 8.9 percent protein and 77 percent TDN in the dry matter to yearling cattle. When this diet was supplemented with urea, the cattle gained 17 percent faster; and when it was supplemented with cottonseed meal, the cattle gained 27 percent more rapidly than the basal cattle.

Baker *et al.* (1949) fed unsupplemented, supplemented-with-urea, and supplemented-with-soybean-meal diets composed of corn grain and whole-plant corn silage to steer calves. The unsupplemented cattle gained the least, and the supplemented cattle gained 8 and 10 percent more rapidly. The unsupplemented diet contained an estimated 9.5 percent protein and 83 percent TDN on a dry matter basis. Dinning *et al.* (1949) fed two basal diets containing an estimated 10 percent and 10.7 percent protein and 77 percent TDN on a dry matter basis. Nitrogen balance was increased by feeding supplemental urea in each of two metabolism trials.

Clark *et al.* (1970) fed cattle a corn–orchardgrass–hay finishing diet estimated to contain 10.5 percent protein and 86 percent TDN on a dry basis. Urea supplementation in one trial was not beneficial, but in another it was. Similarly, in one trial soybean meal supplementation was superior to urea, but not in the other.

Newland and Henderson (1965) compared corn silage treated with 0.5 percent urea and 0.5 percent limestone with untreated silage fed to heifers in a diet containing corn grain and protein supplement. The untreated silage diet contained an estimated 11.1 percent protein and 77 percent TDN in the dry matter. The cattle receiving the urea-treated silage diet gained 7 percent more rapidly than those receiving the untreated silage. The degree to which limestone contributed to this response was not measured.

Greathouse *et al.* (1974) reported two trials with sorghum grain basal diets containing 8.4 and 9.6 percent protein, respectively, and estimated TDN contents of 77 percent. Urea supplementation was beneficial in both trials, but also less beneficial than soybean meal supplementation.

In six additional trials in two experiments reported by Burroughs *et al.* (1973, 1974b), basal diets containing 6.2, 7.8, and 10.5 percent protein and 78 percent TDN were compared with similar diets supplemented with equal amounts of urea or protein nitrogen. Each basal diet was benefitted by protein supplementation and by a larger amount than that observed with urea supplementation. No benefits occurred from urea supplementation when the diet before supplementation contained 10.5 percent protein on a dry matter basis, but benefits from urea were observed in all lower protein diets.

Three additional trials have been conducted with higher protein diets in which no benefits from urea supplementation occurred. Harvey *et al.* (1963) compared whole-plant corn silage with and without urea additions at the time of ensiling in steer calves fed a corn grain, alfalfa–brome hay, and linseed meal diet in addition to the silage. The diet without urea in the silage contained an estimated 13 percent protein and 76 percent TDN in the dry matter and slightly outperformed the diet containing urea added to the silage. Harvey *et al.* (1964) repeated the experiment with identical results, except that the cattle receiving the control diet with 13 percent protein outperformed by a greater extent the cattle receiving additional urea. In a third high-protein diet, Stobo *et al.* (1967) fed a high concentrate-to-hay basal diet to young calves. The diet contained 13.8 percent protein and an estimated 80 percent TDN on a dry matter basis. The calves receiving urea gained no more than calves receiving the basal diet. However, the basal diet was deficient since the young calves were substantially benefitted by protein supplementation.

Turning to the beef finishing trials with high-energy diets where urea supplements were compared with protein supplements in which no unsupplemented control diet was fed reveals that in 10 trials (Baker, 1944; Briggs *et al.*, 1947; Culbertson *et al.*, 1950; Gallup *et al.*, 1953; Klosterman *et al.*, 1964; Kolari *et al.*, 1963; Perry *et al.*, 1967; Lowrey and McCormick, 1969; Muller *et al.*, 1971; and Thompson, *et al.*, 1972) urea proved to be less effective than protein supplements. There was, however, no difference in cattle performance between urea and protein supplementation in nine other trials (Briggs *et al.*, 1947; Culbertson *et al.*, 1950; Johnson *et al.*, 1955; Harvey *et al.*, 1962, 1963, 1964; Jordon *et al.*, 1965; Oltjen *et al.*, 1974; Schmidt *et al.*, 1974) where no negative control diets were fed. However, it is observed that in eight of these nine trials, the level of protein in the diets compared was rather high, ranging from 12 to 15 percent of the dry matter fed, suggesting the probability that in many of these trials, if a negative control diet had been included, no need for supplemental protein would have been demonstrated.

Where negative control diets were fed, all but two of the tested high-productivity diets containing 75 percent or more TDN in the dry matter were benefitted by urea supplementation, provided the protein content of the dry matter did not exceed 11–12 percent or 14.5 percent TDN. No high-productivity diet containing 13 percent or more protein was improved by urea supplementation in these more critical trials. Protein supplementation of nearly all high-productivity diets was superior to urea supplementation when the unsupplemented basal diet contained less that 11–12 percent protein on a dry matter basis.

One obvious reason why a unit of supplemental urea nitrogen should be somewhat inferior to a unit of supplemental protein nitrogen is that only about 80 percent of the total urea nitrogen converted into rumen microbial nitrogen is alpha amino protein (Hungate, 1966). Therefore, any supplemental alpha amino protein that escapes rumen degradation will yield more alpha amino protein postruminally than an equivalent amount of supplemental urea nitrogen converted to microbial protoplasmic nitrogen. A second, less-obvious, but more important reason why urea nitrogen has sometimes proved inferior to supplemental protein in the past has been a failure to recognize the more important characteristics (natural protein and fermentable energy) within each cattle diet that govern urea utilization as described by Satter and Roffler (1973) and Burroughs *et al.* (1974a). When these more important considerations are recognized, then it is possible to largely overcome small differences between the value of a unit of urea nitrogen compared with a unit of supplemental protein nitrogen. This can be done by feeding a slightly larger amount of the former, as compared with an equivalent amount of the latter type of supplement or by oversupplementing with each type with respect to animal protein needs. The results of Varner and Woods (1970) illustrate many of these principles, in which a feeding system was employed in transforming a urea supplement giving inferior performance into one as beneficial as a protein supplement. They used a starter protein supplement for several weeks until TDN consumption was sufficiently high for adequate ruminal urea conversion into microbial protein in satisfying body protein requirements. At this time, they abruptly switched to the urea supplement for the major part of the total feeding period. Without the use of the starter supplement, cattle performance with urea was inferior; but with the starter supplement, subsequent urea usage was as beneficial as protein supplementation.

Less-important diet considerations, such as sulfur deficiency, rumen microbial adaptability, and poor palatability characteristics of urea, doubtlessly exert some influence upon utilization; but the amount appears to be relatively minor in the case of beef cattle.

SUMMARY AND CONCLUSIONS

This review of more than 100 beef cattle reports revealed an orderly picture of benefits from urea supplementation when the experiments were grouped in accordance with the energy and protein levels present in the unsupplemented negative control basal diets. The types of basal diets with respect to their energy content were as follows: (1) Submaintenance and low-energy beef cattle diets; (2) medium-energy beef cattle diets; and (3) high-energy beef cattle diets.

Submaintenance and low-energy diets are defined as those containing less than 60 percent TDN. Such diets are composed largely of forages such as hays and silages, range grasses and pastures, straws, corncobs, and stovers. These diets were benefitted by urea with or without readily available carbohydrate being in the supplement, provided less than 7–8 percent protein was in the diet dry matter and provided the diet before supplementation had insufficient protein to meet body needs. On the contrary, low-energy diets were not benefitted by supplemental urea if their dry matter contained more than 7–8 percent protein or their protein level exceeded about 14 percent of diet TDN.

Medium-energy diets are defined as those containing between 60 and 75 percent TDN in the dry matter, and often they contain mixtures of forages and grain. Supplemental urea regularly improved most of these diets with or without readily available carbohydrates being present in the supplement, provided less than 10 percent protein was in the diet dry matter and provided the diet before supplementation had insufficient protein to meet the cattle's body needs. On the contrary, medium-energy diets were not regularly benefitted by supplemental urea if their dry matter contained more than 10 percent protein or the protein level exceeded 12.0–14.5 percent of the TDN.

High-energy diets are defined as those containing more than 75 percent TDN in the dry matter and large amounts of concentrate feeds. Urea supplementation was helpful in such diets, provided the protein level did not exceed 11–12 percent in the dry matter or 14.5 percent TDN and provided the protein level in the diet before supplementation was sufficiently low to require supplementation in meeting body requirements. Contrarily, high-energy diets with 13 percent or more protein in the dry matter were not benefitted by urea supplementation.

Despite the many beef cattle trials reviewed where supplemental urea proved beneficial, the overall conclusion is that in the past a unit of urea nitrogen most often has not been equal or equivalent in feeding value to a unit of supplemental protein nitrogen. This conclusion is based upon 41 out of 44 observations reviewed in Table 1 in which a unit of urea nitrogen failed to yield benefits equivalent to a unit of protein nitrogen. The degree of failure varied most often from 10 to 30 percent, but in six trials it amounted to 100 percent, or total failure. Recognition and understanding of this lack of nitrogen equivalent between supplemental urea and protein become important in future beef cattle feeding standards to better appraise NPN usefulness in meeting body amino acid requirements. They also call attention to further needed research to improve urea or other NPN supplements that can contribute even more significantly in the future than in the past to alleviating short world supplies of protein feedstuffs.

DAIRY CATTLE

Reviews that have included some discussion of urea in dairy cattle feeding are those by Reid (1953), Tommé (1963), Ryś (1967), Becker (1967), Loosli and McDonald (1968), Huber *et al.* (1968a), Chalupa (1968, 1970), Conrad *et al.* (1969), Helmer and Bartley (1971), and guidelines given in the National Research Council (1971b) publication on dairy cattle.

Reid (1953) concluded that for maintenance and production of lactating cows, diets containing up to 27 percent of the total nitrogen as urea were equal to diets containing protein as supplementary nitrogen. Urea nitrogen below this concentration had no adverse effects on reproductive performance, milk composition, or general health. Reid (1953) proposed that safe concentrations of urea equaled up to 3 percent of the concentrate mixture or 1 percent of the total diet for lactating cows.

In a European review, Ryś (1967) points out that utilization of urea nitrogen occurs when basal diets are deficient in protein. If a sufficient supply of amino acids, polypeptides, and protein to meet the requirements of the rumen microflora is provided, urea utilization is depressed. Ryś (1967) concluded that cows with a production level of 2,000–3,000 kg of milk annually are best suited for the utilization of urea and that high producers fail to utilize urea efficiently; therefore, he did not recommend urea for their diets. Zein, a protein of corn, is less extensively degraded in the rumen than some other plant proteins; and Ryś (1967) feels that the success with urea feeding in the United States and USSR stems from the large amounts of corn fed in these countries.

The average annual milk production in the United States now exceeds 4,000 kg per cow, and some herds average more than 10,000 kg. There is also a well-established positive relationship between production per cow and income for labor. Therefore, urea in lactating dairy cow diets is acceptable only if high production rates are maintained for extended periods of time.

The feeding of urea is justified only if it will reduce feed costs. Under present price conditions, which often prevail in the United States, least-cost diet formulation usually includes urea in the diet unless it is specifically excluded or if protein supplementation is unnecessary.

UREA IN DIETS FOR YOUNG DAIRY CALVES

The National Research Council (1971b) recommendation for total protein in calf starters is 16 percent of the total diet dry matter, with a daily gain of 750 g per day. Brown *et al.* (1956) compared the following pelleted calf starter diets: (1) basal negative control, 7.4 percent protein;* (2) basal plus 3 percent urea; and (3) basal plus linseed meal fed to calves from 2 to 86 days of age with medium-quality timothy hay. Milk was phased out from 21 to 49 days of age. The negative control diet depressed consumption and daily gain, but calves fed the urea or linseed meal diets gained equally and significantly faster. Brown *et al.* (1960b) also compared pelleted starter diets containing 1.1, 2.2, and 3.3 percent urea added to a negative control of 7.1 percent protein. Daily gains were highest in calves fed the diet containing 2.2 percent urea. Nitrogen retention was higher in all urea-fed calves than in the controls. Results indicate that urea was being used by 5 weeks of age.

Stobo *et al.* (1967) fed calves a low-protein hay plus various starter diets for 9 weeks, starting at 3 weeks of age. The diets were: (1) basal 13.3 percent protein, (2) basal plus skim milk, and (3) basal plus 2.8 percent urea. Intakes of the starter diets and hay did not differ among the treatment groups. Daily gains averaged 0.59 kg for the skim milk diet, which was significantly greater than the 0.45 and 0.48 kg for the low-protein and urea diets, respectively.

Fish meal was compared to urea (1.6 percent) plus oats, urea (3 percent) plus oats, and mixed salts (primarily ammonium acetate) of volatile fatty acids (Kay *et al.*, 1967a). The salts were given in the drinking water. Bull calves weaned at 28 days were the experimental animals. The diets contained 19 percent crude protein, and dicalcium phosphate was included in the urea-containing diets to equalize calcium and phosphorus contents. All diets were offered when the calves were 10 days of age. Intake and gain were recorded from weaning when weights averaged 50 kg until the average was 110 kg. Daily gain and feed efficiency were reduced when urea and ammonium acetate replaced fish meal.

The possibility that the branched-chain fatty acids (BCFA), isobutyric and isovaleric (plus valeric), might be limiting growth in calves fed urea-containing diets was examined by Miron *et al.* (1968). Thirty-two Holstein calves were assigned to four starter diets of 20 percent crude protein in which comparisons were: (1) soybean meal (SBM), (2) SBM + BCFA, (3) urea (1.9 percent), and (4) urea (1.9 percent + BCFA). The diets were fed free-choice, starting when the calves were 2 weeks old and ending when they were 12 weeks old. All diets were eaten readily by the calves, and the gains of calves fed SBM alone or SBM + BCFA diets were greater than those fed the urea-containing diets. As both the

*Composition data expressed on an air-dry basis was converted to a dry matter basis throughout, assuming a 90 percent dry matter content if no dry matter data were given.

starter diet and the hay contained 20 percent protein, it is doubtful whether the urea nitrogen was needed.

Naylor and Leibholz (1970) used sorghum grain plus urea to replace meat meal in four calf diets. Levels of urea were 0, 1.33, 2.67, and 3.9 percent, which supplied urea as a percent of dietary nitrogen of 0, 20.1, 39.2, and 55.6 percent, respectively. Sodium sulfate was added to obtain a nitrogen:sulfur ratio of 11:1. The concentrate portions averaged 21.9 percent protein and were offered free-choice, while cottonseed hulls were offered separately up to a level of 17 percent of total feed intake. A milk replacer was fed for 5 weeks. Between 5 and 11 weeks of age, highest gains were made on the 2.67 percent urea diet averaging 0.81 kg per day for the 6-week trial. Total feed intake was lower in calves fed no urea than in those fed the two lowest levels of urea. Lowest nitrogen balance occurred in the diet containing 3.9 percent urea. The authors suggested that their excellent response to urea could have been due to the higher sulfur content of their diets.

D. K. Nelson (1970) compared three calf starters in which SBM, urea (2.7 percent), and SBM plus urea (1.35 percent) served as the nitrogen supplements in isonitrogenous diets, which contained about 18 percent protein. Calves were assigned to these diets from 4 through 84 days of age. A limited whole-milk feeding program was used, but no forage was fed. Daily gains were greater for calves fed the diets containing SBM alone and SBM plus urea than for those fed the diet with 2.7 percent urea. D. K. Nelson (1970) also assigned calves to three diets as follows: (1) SBM as the only supplemental nitrogen, (2) SBM plus urea (1.35 percent), and (3) SBM in a diet having only 14.3 percent protein to serve as a negative control. Diets 1 and 2 contained about 18 percent protein. Calves fed the urea and 18 percent SBM diet gained 0.60 kg/day; negative controls gained 0.52 kg/day ($P < 0.07$).

Studies on the use of urea in calf starters can be divided into those that obtained results comparable to those found on conventional protein supplements (Brown *et al.*, 1956, 1960b; Naylor and Leibholz, 1970; L. F. Nelson, 1970) and those that found a lower response when urea was fed (Kay *et al.*, 1967a; Stobo *et al.*, 1967; Miron *et al.*, 1968). Brown *et al.* (1960b) and D. K. Nelson (1970) fed no separate forage, although the pellet fed by the former contained 30 percent ground timothy hay. In these studies, milk feeding varied from 28 days (Kay *et al.*, 1967a) to 49 days (Brown *et al.*, 1956). Urea concentration in the diets is another variable that may explain some of the differences. Stobo *et al.* (1967) fed 2 percent urea, while Kay *et al.* (1967a) fed 1.6 and 3 percent and Miron *et al.* (1968) 1.9 percent urea. Although Naylor and Leibholz (1970) obtained the greatest response with 2.6 percent urea, D. K. Nelson (1970) observed the greatest response at 1.35 percent urea.

Naylor and Leibholz (1970) felt that their successful results with urea diets were due to supplemental sulfur. Use of negative controls and positive responses from urea additions by several authors (Brown *et al.*, 1956; Naylor and Leibholz, 1970; D. K. Nelson, 1970) demonstrates the use of urea by young calves.

It is suggested that calf starters fed with limited milk feeding programs can contain from 1 to 1.5 percent urea and give satisfactory results if a period of adjustment is provided while milk is being withdrawn from the diets.

UREA IN DIETS FOR HEIFERS

The minimum total protein required by growing dairy heifers is given (National Research Council, 1971b) as 10 percent of the total diet dry matter at a daily gain of 750 g per day.

Hart *et al.* (1939) at Wisconsin conclusively showed that young dairy males and females used urea for growth during two 40-week studies. Negative control diets containing 6 and 7 percent protein and supplemental diets of urea, ammonium bicarbonate, and casein at 20 percent protein in one trial and graded levels of urea from 1.4–4.3 percent in a second trial raised total diet protein levels to 19.4. Although some overfeeding of protein was apparent, growth response and carcass analysis clearly demonstrated that urea was used by these cattle, although not as efficiently as casein. The widely used guidelines of 1 percent of the total diet or 3 percent of the grain mixture appears to stem from this work. Work and Henke (1939) confirmed the Wisconsin work with a 52-week study, and Mills *et al.* (1944) showed that addition of casein or starch to a molasses–urea–timothy diet sharply increased daily gains.

Bohman *et al.* (1954) determined the value of urea and corn for replacing soybean meal in growing heifers fed cane molasses and poor-quality timothy hay. The supplements were: (1) molasses and urea; (2) corn and soybean meal; (3) molasses and soybean meal; and (4) molasses, corn, and urea. The supplements were fed once per day, and those fed the soybean meal diets gained more than those fed the urea diets. Little use was made of urea in a diet of poor forage and molasses. Substitution of corn for molasses gave only a small increase in gains.

Merrill and co-workers (1959) compared cane molasses and ear corn meal as energy supplements to early and late-cut hays fed with urea and soybean meal as sources of protein for growing dairy heifers. Sixty-six heifers were used in each of 2 years in continuous trials of 24 weeks to measure growth response. Corn silage and the dry concentrates were fed at the morning feeding, while hay with molasses poured over it was fed in the afternoon. Substitution of cane molasses for corncob meal on an equal TDN basis resulted in equal gains by dairy heifers, although the group fed molasses ate more hay. Those fed the molasses- and urea-containing diets made lower gains than those receiving molasses and soybean meal or those fed corncob meal and soybean meal.

Bates *et al.* (1960) fed two mixtures that contained urea, molasses, water, and minerals with and without ethyl alcohol as essentially the sole source of digestible protein for dairy heifers. Ground corncobs fed *ad libitum* and separately were the only roughage. During a 140-day trial, gains were low and were not affected by ethyl alcohol in the diet. Two Jersey heifers were continued on the diet for an additional 438 days, and these animals grew, reproduced, and lactated.

Employing a concentrate diet of 3 percent urea (0.129 percent S) and a low-protein hay (0.065 percent S), Jones and Haag (1946) observed an improvement in growth response of dairy heifers when the grain mixture was supplemented with 1 percent sodium sulfate. Brown *et al.* (1960a) also obtained increased gains in dairy heifers fed urea-containing supplements when sodium sulfate was used to reduce the N:S ratio from 22:1 to 16:1. As in other studies, gains by groups fed urea supplements were lower than those fed supplements containing soybean meal as the supplemental nitrogen source.

A 118-day continuous feeding trial was used by Martz *et al.* (1964) to measure the growth response of 40 Guernsey heifers that were fed corn silage *ad libitum* and the following daily supplements: (1) 1,362 g of soybean meal, (2) 681 g of soybean meal, (3) 1,362 g of urea (8 percent) mixture, and (4) 681 g of a urea (8 percent) mixture. Groups fed diets 2 and 4 received 681 g of a standard grain to equalize energy intake from supplements, and the supplements were spread twice daily on top of the silage. Dry matter consumption was not affected by the level of the supplements. However, the soybean meal supplement promoted faster gains than urea.

J. R. Campbell *et al.* (1963) fed dairy heifers soybean and urea supplements twice versus six times daily. Excellent and similar gains were made by the heifers fed the soybean meal supplement either twice or six times daily and when the urea supplement was fed six times per day. Gains of heifers fed the urea supplement twice daily were lower. Fletcher *et al.* (1968) compared the effects of feeding urea–corn silage (0.5 percent urea) and a low-protein grain mixture two versus four times per day. Forty Guernsey heifers were assigned to the four treatments in a 74-day continuous trial. Feeding urea–corn silage four times daily increased daily gains, and the animals required 14 percent less TDN to produce a unit of gain. Some of the protein needed by growing heifers can be supplied by urea, and better results seem to be obtained when complete diets or urea forages are fed *ad libitum*—in which consumption can take place several times during the day. Webb and colleagues (1963) found that dairy cows exposed to hay and silage in a loose housing system ate hay 8 times and silage 11 times during a 24-h period. Corn silage provides a high-energy forage base for the effective use of urea. Only a modest level of urea (0.5–1.0 percent in the total diet) is needed with corn silage or other forage of similar protein content to meet protein requirements.

UREA IN DIETS FOR LACTATING COWS

Experiments reported with lactating cows are frequently difficult to interpret. Adaptation and adjustment seem to be important when urea-containing diets are fed, yet short-term changeover designs have been used by many workers. Also, negative controls have not been used in many studies. These factors are further complicated by the small number of animals employed. Furthermore, some researchers have used cows that were in low production. Because of the capacity of high-producing dairy cattle to mobilize both energy and nitrogen from body reserves for milk production, nitrogen balance trials are helpful to establish the nutritional status of lactating cows fed urea-containing diets. It is also important to describe the specific conditions of the study, such as the number of times per day animals are fed and characterization of the diets with respect to their content of major and trace minerals and other factors. For these reasons, primary emphasis will be given to those studies of longer duration and to some with nitrogen balance data.

Total-diet protein-requirement levels for lactating cows is given by the National Research Council (1971b) as 14, 15, and 16 percent for milk production levels of $<$ 20 kg, 20–30 kg, and $>$ 30 kg per day, respectively. A high-producing cow (600 kg of body weight) consuming dry matter at a level of 3.5 percent of body weight with 1 percent urea in the diet would ingest 210 g of urea in a 24-h period. This compares to a 400-kg steer fed a finishing diet of primarily corn plus an NPN supplement that provides about 60 g of urea/day.

The early experiment of Archibald (1943) is one of the more conclusive studies on urea utilization by dairy cows, because the study extended over 3 years. Urea at 3 percent of the grain mixture supplied 25 percent of the diet nitrogen and was compared to soybean and cottonseed meals as supplementary nitrogen sources in both changeover and continuous-type trials. The urea-fed cows ate grain less readily, exhibited similar changes in body weight, similar lactation length, similar dry periods, and nearly identical reproductive performance to those fed protein. During two lactations, the cows fed urea continuously averaged 18.1 kg/milk/day and the control cows 18 kg/day. An adjustment interval was apparent in double reversal trials.

Distillers' grains, brewers' grains, and urea were compared as protein supplements during two lactation studies by Loosli and Warner (1958). Urea was fed at a level of 3 percent in the concentrate mixture. The first week of the 6-week experimental period was considered transitional; data were used from the last 5 weeks. The urea-containing diet appeared to be less acceptable, as the cows ate it more slowly. However, total concentrate intakes did not differ among the treatments. Milk production averaged 19.4, 19.1, 18.9, and 18.2 kg/day, respectively, during both years for the cows fed diets containing distillers' grains, brewers' grains, urea, and the low-protein control. When the concentrate mixtures contained 1.5–2.0 percent urea, plus either distillers' or brewers' grains, the diets were completely acceptable.

Using a 4 X 4 Latin square design, Colovos *et al.* (1967) compared high-quality concentrate mixtures that contained 0, 1.25, 2, and 2.5 percent urea in diets of Holstein cows that were in early lactation. All diets contained a good-quality timothy hay. Diet effects were nonsignificant on dry matter intake, dry matter digestibility, milk production, milk composition, and molar proportions of rumen acetic, propionic, and butyric acids. Milk production was relatively high (ca. 27 kg/day). However, digestible and metabolizable energy levels were lower in the two highest urea diets.

An 18-month split herd comparison was made by Holter *et al.* (1968b) on the production and reproductive performance of high-producing Holstein cows fed concentrate mixtures containing 0 or 1.5 percent urea. Soybean meal, corn gluten feed, and corn distillers' dried grains in the control mixture were replaced by hominy and urea to equate the two mixtures in energy and nitrogen. A maximum of 11.3 kg of concentrate was fed with excellent hay, hay crop silage, and corn silage plus some seasonal pasture. Although there was little difference in the acceptance of the two grain mixtures, several cows were slow in starting to eat and then ate the urea-containing concentrate more slowly. Milk production averaged 7,319 and 7,306 kg per 305-day lactation for the urea and control groups, respectively. Conception rates averaged 1.6 and 1.9 services per conception in the urea and control groups, respectively. The total diet protein level appears to have been about 15 percent during early lactation. A comparison of the difference between the actual and predicted milk production of both groups during the first and second months of the study revealed a significant interaction between treatments and milk production; the urea-fed cows produced less than predicted during the first month and more later, thereby lending further support to the idea that some adaptation or adjustment is necessary when cows are first fed a urea-containing feed. This is one of the more useful field studies on the use of urea in practical diets of dairy cows.

Complete diets containing 25 percent bagasse and 75 percent concentrates were used to compare urea with urea plus tuna fish meal (TFM) as nitrogen supplements for lactating Holstein cows (Randel, 1970). Diet 1 contained 9 percent TFM and 1.5 percent urea, diet 2 contained 4.75 percent TFM and 2.25 percent urea, and diet 3 contained 3 percent urea. The diets contained 15.9, 15.9, and 16.8 percent protein, respectively. The animals were fed the diets during a 35-day preliminary period, a 7-day adjustment period, and a 105-day comparison period. Feed consumption and milk production were both depressed nearly 4 kg/day by increasing levels of urea. Ramage and Woolf (1970) fed three complete rations containing 70 percent grain and 30 percent hay with urea at levels of 0, 0.8, and 1.6 percent to Holstein cows in a 29-week continuous trial. Time for adaptation was provided by including a low level of urea in diets fed during a 6-week postpartum preliminary period. The feeds were offered *ad libitum* during the first 6 weeks, and thereafter the amount of feed was reduced weekly at the rate of 0.18 kg/day. Milk production was 22.8, 26.5, and 25.1 kg/day with the corresponding additions of urea.

UREA AND CORN SILAGE

Because corn silage is high in digestible energy but relatively low in protein, NPN addition is indicated. Brigl and Windheuser (1931) added urea to corn at ensiling. They felt that this procedure might avoid toxicity and show whether silage bacteria would use urea nitrogen to form microbial protein. Their results indicated that about 60 percent of the urea remained unaltered and that the remaining appeared to be present as ammonium salts.

Interest in adding urea to corn at ensiling in the United States developed during World War II (Wise *et al.*, 1944; Woodward and Shepherd, 1944). Both added 0.5 percent urea to the green forage at ensiling. Woodward and Shepherd (1944) fed the urea–corn silage with low-protein concentrate and hay to cows for 100 days in a single reversal experiment. Another group of cows received the same level of urea in the concentrate mixture. Method of feeding urea had no effect on production. Wise *et al.* (1944) reported that urea–corn silage was slightly less acceptable than untreated corn silage as the sole forage for lactating cows.

Davis *et al.* (1944) and Cullison (1943) added urea to sorghum silage at levels of 0.5, 1.5, and 2.5 percent of the green forage weight at ensiling. Free ammonia was observed in the silage that contained 2.5 percent urea, and cattle refused to eat the silage until the free ammonia had disappeared; however, there was no problem of acceptance when the silage contained 0.5 percent urea.

Most studies since 1944 have dealt with urea additions to corn at ensiling. Conrad and Hibbs (1961) fed cows that received only urea–corn silage (0.7 percent urea) and found that they only used 8.1 percent of their nitrogen intake for milk secretion and retention in body tissue, compared to 22 percent for a group that received alfalfa hay and grain. The poor use of urea nitrogen may have been caused by a low energy intake, and the addition of grain to provide additional readily fermentable carbohydrate in the urea-containing diets might have given improvement.

Huber *et al.* (1967) reported three experiments conducted with 91 lactating cows fed diets containing corn silage in which urea provided from 0 to 48 percent of total nitrogen. A 3-week preliminary period was followed by 12-week periods. In experiment 1, equal levels of nitrogen were provided by: (1) a grain mixture that provided 16 percent protein, (2) soybean or cottonseed meal, (3) equal nitrogen from the oilseed meals and urea, and (4) urea. Milk production was depressed from about 18.5 to 13.5 kg/day by treatment 4, but it was felt that the effect of urea was confounded by the diet energy levels.

In experiment 2, three levels of energy and three levels of urea were fed; urea provided 21 and 38 percent of the dietary nitrogen, and the nitrogen supplements were mixed with the corn silage immediately before feeding. Total milk production ranging from 16 to 24 kg/day and persistency were increased by additional energy and depressed by increasing urea level. In the third experiment, concentrate mixtures (18 percent protein) containing 0, 1.1, and 2.2 percent urea that provided 0, 10, and 20 percent dietary nitrogen, respectively, were compared. One kg of concentrate was fed per 3 kg of milk. A 7-day nitrogen balance trial was conducted near the end of the experiment. The 2.2 percent urea level depressed milk production from 22.9 to 21 kg/day, but did not reduce silage or concentrate intake, although the concentrate portion was eaten slowly. As in the two previous studies, the cows were unable to use a high level of urea when fed corn silage as the only forage. Urinary nitrogen increased, and total milk nitrogen decreased as a percentage of intake with increasing levels of urea. It is significant that in all three experiments, milk yields were lower whenever urea provided more than 20 percent of the total dietary nitrogen.

Holter *et al.* (1968a) found no depressing effect on feed intake and milk production (average 26 kg/day) when high-quality multi-ingredient concentrate mixtures containing up to 2.5 percent urea were fed with corn-silage forage. Their highest level of urea provided about 300 g of urea per day. Nitrogen balance was positive for all treatments but lower in the urea-fed cows. Urea increased rumen ammonia nitrogen from about 15 mg to 30 mg/100 ml fluid during the first hour after feeding. The concentrate mixture contained seven major energy sources, which were believed to contribute to the high feed intake and excellent performance. In contrast, the mixtures used by Huber *et al.* (1967) contained only three energy sources. Also, the calcium and phosphorus levels in the concentrate mixes used by Holter *et al.* (1968a) were higher than those fed by Huber *et al.* (1967). These factors may offer an explanation for the differences in results. Dietary levels of other mineral elements should also be equated when urea and grain replace an oilseed meal in dairy cow diets.

Polan *et al.* (1968) used a 70-day continuous trial to compare corn silages containing 0, 0.5, and 0.75 percent* urea as the only forage for lactating cows fed concentrates at the rate of 1 kg/3 kg of milk. The diets were isocaloric and isonitrogenous. Level of urea did not influence silage intake, total feed intake, or milk production of about 19 kg/day. In a second trial, whole-plant corn forage that had been ensiled with 0, 0.6, or 0.85 percent urea was fed *ad libitum* as the only forage to lactating cows in a 63-day trial. Again, no significant differences in intake or milk production were noted. Blood urea nitrogen levels tended to increase as the level of dietary urea increased. Nitrogen balance data revealed that the cows fed the 0.85 percent urea–corn silage were in negative nitrogen balance because of low protein digestibility and high urinary nitrogen losses. These results emphasize the need for production trials of long duration, negative controls, and balance data for evaluating the effect of urea on milk production.

Corn ensiled with no additive contains a variable amount of NPN that may account for 40–50 percent of the total nitrogen (Johnson *et al.*, 1967; Huber *et al.*, 1973). Corn silage with 0.5 percent urea added at ensiling contained 30–40 percent of the total nitrogen as ammonia and urea (Johnson *et al.*, 1967; Huber *et al.*, 1968a; Huber and Thomas, 1971).

Corn forage that contained 30, 36, and 44 percent dry matter was

*Urea additions are expressed on a wet forage basis.

ensiled with and without 0.5 percent urea (Huber *et al.*, 1968b). Concentrate mixtures containing 16.2 and 22 percent protein (dry basis) were fed with the control silages; the urea-containing silages were fed with a 16.2 percent protein concentrate. The silages were fed *ad libitum*, and the concentrate portion was fed at the rate of 1:2.5 kg of milk for 80 days. Increasing silage dry matter depressed milk yields from 23.8 to 23 kg/day. Urea additions apparently increased consumption of silage dry matter, and milk persistency appeared to be better in cows fed urea-treated silages. Feed intake was lower for cows fed the urea-treated 44 percent dry matter silage. Since milk production of the cows fed untreated silage plus 16.2 percent protein concentrate was similar to those receiving the 22 percent protein concentrate or urea-treated silage, it was impossible to determine the extent of urea nitrogen use because the negative control cows may have been depleting body protein reserves during the trial.

Urea (0.5 percent) was also added to whole-plant corn that contained 32 or 48 percent dry matter (Van Horn *et al.*, 1969a) at ensiling. The silages were fed *ad libitum* to 18 Holstein cows, which received 2.3 kg of hay/day plus a concentrate mixture (1 percent urea) for 63 days. Milk production was lower in cows fed the high dry matter silage, even though feed consumption was not affected. These results and others support the idea that urea should not be added to whole-plant corn containing more than 40 percent dry matter. The well-matured corn plant may contain more than 50 percent grain. Consequently, corn silage made from whole corn is in reality a mixture of forage and grain. This fact led Boman *et al.* (1969) to study restricted concentrate supplementation with corn silage *ad libitum*. Diets compared were: (1) corn silage plus a 20 percent protein grain supplement that was fed at a ratio of 1:3, (2) corn silage plus 45 percent cottonseed meal (CSM) fed at a ratio of 1:9, and (3) urea–corn silage (0.5 percent) plus CSM and shelled corn at 1:9. Twenty-four cows were fed the silages (36 percent dry matter) *ad libitum* during the 16-week continuous trial. Differences among treatment groups were small in total dry matter intake, but there was a trend in favor of groups fed restricted concentrate and more urea with respect to milk production (ca. 19 kg/day) and weight gain. Consumption of the urea–corn silage was depressed during the first month of the study. This subject deserves much more attention in high-producing cows early in lactation.

To obtain further information on levels of NPN that can be used by lactating cows, Van Horn *et al.* (1969b) employed a 4 × 3 factorial design with four levels of urea (0, 82, 160, and 232 g/day) and three levels of protein for lactating cows. Four urea levels were obtained by using a combination of corn silage, with and without 0.5 percent urea, and a concentrate with and without 1.5 percent urea. A 5-day nitrogen balance and digestion trial was conducted at the end of the 70-day trial with one-half the 24 cows. Treatments did not affect milk production, milk composition, or body weight change. The average milk production during the 70-day trial was 19.5 kg/day at the start, but declined to 12.5 kg/day during the nitrogen balance trial. All cows were in positive nitrogen balance during the metabolism phase. However, negative nitrogen balance may have occurred during the early feeding period, and the animals adjusted production accordingly. Some nitrogen reserve exists, and part of this reserve can apparently be mobilized for milk production without depressing production.

Protein levels of 9, 13.7, and 20.4 percent were used in concentrate mixtures based upon shelled corn and soybean meal (Huber and Thomas, 1971). Whole-plant corn was ensiled with (1) no additive, (2) 0.5 percent urea, (3) 0.75 percent urea, and (4) 0.75 percent urea plus 0.17 percent $CaSO_4$ and fed as the only forage. The diet combinations and some of the results of the 70-day continuous trial are shown in Table 2. The five highest-producing cows from each group were used in a 7-day nitrogen balance trial following the feeding period. Increased production on diets B and C compared to the negative control again shows that cows use urea in corn silage for milk synthesis when protein is limiting. High yields were obtained on diet E, which contained corn silage (0.5 percent urea) plus a 13.7 percent crude protein concentrate, and diet F, which contained control silage plus 20.4 percent crude protein concentrate. These results indicate that under some conditions cows are able to use urea when it is included in corn silage as well as they use nitrogen from diets without added urea.

Knott *et al.* (1972) used two intensive, continuous experiments to examine the ability of lactating cows to use urea nitrogen. Cows were adjusted to urea previous to the study. In the first experiment, urea was not well used in either 12, 17, or 22 percent protein concentrate mixes (1.5 percent urea) fed with corn silage as the only forage. Nitrogen balance confirmed the milk production indication that urea was not being used to an appreciable extent. In the second experiment, higher production throughout, negative controls, and nitrogen partition evidence confirmed that cows were using urea nitrogen when some of it was carried in the corn silage and some in the concentrate. Urea in corn silage stimulated both dry matter consumption and milk production over that of the negative controls. The reason(s) for difference in responses between these two experiments is not apparent, but higher production in the second experiment demanded more protein.

TABLE 2 Milk Yields, Milk Composition, Body Weight Change, Apparent Digestibility and Nitrogen Balance Data of Cows Fed Various Concentrate Protein Levels and Urea–Corn Silages (10 Cows per Group) (Data from Huber and Thomas, 1971)

	Diet					
	A	B	C	D	E	F
Silage fed*	1	2	3	4	2	1
Concentrate protein (%)	9.0	9.0	9.0	9.0	13.7	20.4
Milk yields						
Treatment period (kg/day)	19.2	23.7	23.8	23.9	26.4	25.7
Covariance adjusted (kg/day)	18.8[a]	22.7[b]	23.7[b]	23.7[b]	25.8[c]	25.3[c]
Milk fat (%)	3.30	3.31	3.60	3.07	2.91	3.54
Milk protein (%)	3.16	3.23	3.36	3.40	3.29	3.40
Weight change (kg/day)	–0.49[a]	–0.10[a,b]	–0.08[a,b]	+0.10[b,c]	+0.20[b,c]	+0.55
Digestibility (%)						
Protein	47.8[a]	59.8[b]	66.2[b,c]	60.9[b,c]	66.3[b,c]	68.2[c]
Dry matter	56.4[a]	59.0[a,b]	64.9[b,c]	63.1[a,b,c]	65.0[b,c]	69.2[c]
N balance (g/day)	–32	–29	+23	–3	+35	+9
Sum of N excreted (% of digested)	143[a]	118[a,b]	90[b]	102[b]	89[b]	98[b]

*Silage 1, control corn silage; 2, with 0.5 percent urea; 3, with 0.75 percent urea; 4, with 0.7 percent urea plus 0.17 percent $CaSO_4$.
[a,b,c]Treatment comparisons not sharing a common letter are significantly different.

Van Horn *et al.* (1967) assigned three groups of eight cows each to the following diets: (1) control corn silage plus concentrate (19.3 percent protein, no urea), (2) urea–corn silage (0.5 percent) plus concentrate (13.2 percent protein, no urea), and (3) urea–corn silage (0.5 percent) plus concentrate (14 percent protein, 1 percent urea). The corn silages were offered at levels up to 27.2 kg/day, and hay was fed at 2.3 kg/day. Concentrates were fed to meet energy needs of the cows. The diets were essentially isonitrogenous. The forages were fed twice and concentrate three times per day for 80 days. Feed consumption, milk production of ca. 25 kg/day, and body weight changes did not differ among treatments. It appears that when urea is included in both the silage and grain more dietary urea can be used without decreasing the acceptability of the concentrate portion of the diet. The feeding of 2.3 kg of legume hay daily and frequent feedings of the concentrate mixture may have contributed to the successful use of urea in this study.

Continuing a dual approach, Van Horn *et al.* (1969a) used a 28-day adaptation period to precede an 84-day experimental period in feeding cows. A urea-containing silage (0.5 percent) and a concentrate mixture (1 percent urea) or a control diet (no urea) were fed. All cows received 2.3 kg of legume hay/day, as in the previous trial. Milk production was depressed by urea, even though total feed intakes were similar. However, the cows ate more of the urea-containing silage while reducing their concentrate consumption. Differences between this and the previous study were in the dry matter level of the silage. In this trial, it was about 44 percent vs. 32 percent in the previous trial. These results further support the recommendation to avoid adding urea to corn forage of more than 40 percent dry matter.

OTHER ADDITIVES COMPARED TO UREA FOR CORN AT ENSILING

Schmutz *et al.* (1969) compared corn silages that contained (1) no additive, (2) 0.57 percent urea, (3) 1 percent diammonium phosphate (DAP), (4) 0.5 percent $CaCO_3$, (5) 0.5 percent urea plus 0.5 percent $CaCO_3$, and (6) 0.5 percent $CaCO_3$ plus 1 percent DAP as the forage source for Holstein cows in a 90-day continuous trial. Grain was fed to equalize energy and protein intakes when silage consumption was 35 kg/day. In a second experiment, corn silage was fed with (1) no additive, (2) 0.5 percent urea, (3) 0.75 percent urea, (4) 0.5 percent $CaCO_3$, (5) urea plus 0.5 percent $CaCO_3$, and (6) 0.75 percent urea and 0.5 percent $CaCO_3$. In the first experiment, cows fed corn silage containing DAP ate less silage and produced less milk (ranging from ca. 14–18 kg/day) than cows on other treatments. In the second experiment, cows fed the 0.75 percent urea silage ate less silage. So either DAP at 1 percent or urea at 0.75 percent may depress silage intake.

In some geographic regions, the least costly form of NPN is anhydrous ammonia. Michigan workers (Huber and Santana, 1972; Huber *et al.*, 1973) have pioneered in using ammonia solutions as NPN additives to whole-plant corn at ensiling for dairy cattle. Huber and Santana (1972) compared urea (0.5 percent) and aqueous ammonia (0.28 percent ammonia and 3 percent water) as additives for 35 percent dry matter whole-plant corn at ensiling to a control silage as the sole forage. Four groups of seven lactating cows each were fed the silages, and concentrate mixtures were formulated to provide both negative and positive controls. Feed intakes and milk yields of cows fed the urea silage, ammonia silage, and positive control diets were all similar and significantly greater than for the cows fed the negative control diet. Milk production averaged 25.7 kg/day for the cows on the experimental diets compared to 19.4 kg/day for the negative controls. More water-insoluble nitrogen and higher lactic acid was present in ammonia-treated silage than in urea-treated silage.

In further extensive work, Huber *et al.* (1973) compared no additive, urea at 0.5 and 0.75 percent, urea plus minerals at 2 percent and several ammonia solutions at 2–4 percent as NPN fortification for corn silage of low dry matter (ca. 30 percent) and high dry matter (42–52 percent). Addition of ammonia solutions to 52 percent dry matter silages or at 4 percent depressed silage lactic acid content and milk production from 23 to 18.4 kg/day. Similarly, urea added to 42 percent dry matter forage depressed milk production as shown previously. Highest milk production of about 27 kg/day was obtained with the ammonia silages. The ammoniated silages again contained more lactic acid and water-insoluble nitrogen than the urea-treated silage, but the latter contained more water-insoluble nitrogen than the control. In view of the price advantage of ammonia over urea, this form of NPN warrants more research.

UREA ADDITIONS TO BARLEY AT ENSILING

Barley silages that had been ensiled with 0 and 0.55 percent urea were fed with three concentrates (no urea) that contained protein levels of 10.2, 14.1, and 18.8 percent to 30 lactating Holstein cows in a continuous trial (Polan *et al.*, 1970). Nitrogen balances were determined midway through the trial. The concentrate portion of the diet was designed to provide 85, 100, and 115 percent of the nitrogen requirements of cows fed the control (no urea) silage and 100, 115, and 130 percent of the nitrogen requirements of cows fed the urea–barley silage. Neither urea addition nor concentrate protein level affected the *ad libitum* consumption of silage dry matter. Differences in milk production, analyzed as a percentage of pretreatment production, were significantly less for cows fed urea–barley silage than the two higher protein concentrates. However, urea met some of the nitrogen requirements; and dry matter, protein, and acid detergent fiber digestibilities were increased by urea. Nitrogen balance data revealed that urea in the silage increased nitrogen retention, but only when fed with low-protein concentrates. These results lend emphasis to the concept that only if nitrogen is limiting can urea be used for a productive function.

UREA IN LIQUID SUPPLEMENTS FOR DAIRY CATTLE

In earlier reviews Loosli and McDonald (1968), Wornick (1969), Coppock (1969), and Huber (1972) discussed the use of liquid supplements in cattle feeding. However, there are few studies that relate specifically to dairy cattle.

Owen *et al*. (1943) replaced the nitrogen of blood meal with a urea-plus starch mixture in diets for lactating Ayrshire cows. The mixture supplied 25 percent of the total dietary nitrogen requirements. The total mixed ration was fed twice daily, and no palatability problems were encountered. Though production was low (ca. 13 kg/day), nitrogen balance data indicated use of urea. Balch and Campling (1961) compared: (1) a low basal control, (2) urea in molasses plus ethyl alcohol plus phosphoric acid, (3) urea plus molasses plus phosphoric acid, and (4) groundnut meal as nitrogen supplements. They concluded that if the basal diet was low in protein and contained large amounts of starch, the nitrogen of urea could be used almost completely by dairy cows. Dietary alcohol did not increase the utilization of urea, which agrees with other results.

A liquid supplement containing both urea and ammonium polyphosphate was compared to soybean meal in complete feeds that contained 70 percent corn silage and 30 percent concentrate (wet basis) by Van Horn *et al*. (1969a). Liquid-supplement-fed cows produced less milk and lost more weight. In further work Van Horn and Mudd (1971) compared soybean meal to two levels of ammonium polyphosphate plus urea dissolved in cane molasses. The liquid supplement was fed at two levels to supplement a basal diet composed of pelleted concentrate (1 percent urea) and corn silage *ad libitum*. The liquids were spread over the corn silage, and it was found that the higher level depressed milk production from 22.2 kg/day to 20.8 kg compared to 22.5 kg for soybean meal (SBM). Liquid supplements increased plasma urea nitrogen and reduced gains compared to soybean meal.

In a second trial, complete diets containing 77 percent corn silage and 23 percent concentrate were compared (wet basis). The concentrate portion included urea, soybean meal, or liquid supplement as the primary nitrogen additive. The latter provided about 135 g of urea equivalent per cow per day. Cows fed the diet containing soybean meal ate more total feed and produced about 1.5 kg/day milk more than cows fed either added NPN. Cows fed soybean meal also had significantly lower plasma urea nitrogen levels and greater body weight gain than those fed NPN. Huber (1972) compared liquid and dry NPN supplements added to corn forage at ensiling, or the same supplements added at feeding, and found no detectable milk production differences due to NPN form or place of addition. These results and others indicate that NPN supplements fed in liquid form are comparable to similar supplements in dry form for milk production.

SULFUR AND OTHER MINERAL SUPPLEMENTATION OF UREA DIETS

Jones and Haag (1946) observed a growth response in dairy heifers fed low sulfur hay plus grain with 3 percent urea and 1 percent sodium sulfate. Lassiter *et al.* (1958a,b) and Brown *et al.* (1960a) also observed a growth response to a sulfur supplement with dairy heifers fed high levels of urea and low-sulfur forage. Other studies with sulfur have given inconsistent results. This probably indicates that the level of sulfur in the basal diets was sufficient for the production levels achieved.

Davis *et al.* (1954) obtained no increase in milk production when sodium sulfate (0.25 percent) was added to a urea-containing concentrate (2.3 percent urea). However, the animals' diets were relatively high in sulfur, giving a nitrogen to sulfur (N:S) ratio of about 5–8:1.

Based upon published values (National Research Council, 1971a) for nitrogen and sulfur, it can be shown that the use of a 0.5 percent urea–corn silage and 1 percent urea in simple concentrate mixes of practical diets for dairy cattle can result in N:S ratios of 18–20:1. The National Research Council (1971b) requirement for sulfur by lactating cows is given at 0.2 percent of the total diet, which implies an N:S ratio of 12:1 for medium-producing cows (15 percent protein in the total diet dry matter). Moreover, Allaway (1969) pointed out that a downward trend in sulfur concentration in crops is likely because of increasing use of fertilizers that contain low levels of sulfur and emphasis on environmental quality.

Jacobson *et al.* (1967) obtained a milk production response from a sodium sulfate addition that increased the sulfur level to 0.18 percent in a complete diet, but no response was obtained by Huber and Thomas (1971) with a calcium sulfate addition that narrowed the N:S ratio from 20:1 to 14:1. A similar change in the N:S ratio with sodium sulfate did not produce a change in milk production or feed intake in the studies of Grieve *et al.* (1973). Recent balance trials with lactating cows (Bouchard and Conrad, 1973) indicate that the requirement for sulfur lies between 0.12 and 0.18 percent of the total diet dry matter and that sulfur is highly available from sodium sulfate, calcium sulfate, and magnesium sulfate.

The broad area of mineral supplementation of urea diets needs much more research. For example, the addition of cobalt to urea-containing diets increased gains in steers (Bentley *et al.*, 1954) and seemed to increase diet acceptance by dairy cows (Bowstead and Fredeen, 1948). It would be helpful if authors would more completely define the mineral composition of the diets fed. For example, calcium in the corn plant is accumulated rather early in its development, and about 85 percent is found in the stalk and leaf (Chandler, 1960). As the plant matures, starch fills the kernel and the calcium content as a percentage of the whole plant declines. Morrison (1956) lists 0.35 and the National Research Council (1971a) lists 0.30 as the percentage of calcium in the dry matter of corn silage. Recent emphasis on harvesting a more mature plant for silage has resulted in corn silage (Johnson and McClure, 1968) that may have a calcium content of less than 0.20 percent. Consequently, it appears that some workers may have fed diets that were borderline in calcium and diets that contained less calcium than phosphorus. Listing the mineral contents of diets used will make future interpretation of such experiments more useful.

NEW PRODUCTS CONTAINING UREA

Ohio workers (Conrad and Hibbs, 1968; Conrad *et al.*, 1969) reported that a pelleted mixture containing 66 percent dehydrated alfalfa, 31.6 percent urea, 2 percent dicalcium phosphate, and 0.4 percent sodium metabisulfite or sodium propionate makes a very effective substitute for soybean meal in diets of lactating cows. When cornmeal and oats were combined with about 9 percent of this pellet, a concentrate mixture containing 19 percent protein and 2.84 percent urea was obtained. During a 305-day lactation test, cows fed the urea–dehy pellet concentrate averaged 6,965 kg of milk compared to 7,013 kg by cows fed a soybean meal containing concentrate (Conrad *et al.*, 1969). Also, cows fed the urea–dehy pellet received up to 40 percent of the dietary nitrogen from urea. Forage was primarily corn silage plus about 2 kg alfalfa hay/day, so some of the urea was needed. In a field trial, it was shown that an abrupt change from a soybean-meal-supplemented concentrate to one containing the urea–dehy pellet had no effect on grain consumption or milk production.

In an attempt to reduce the acceptance problem with urea-containing diets and to slow the release of ammonia from urea in the rumen, Kansas researchers (Helmer *et al.*, 1970a) developed a product in which a cereal grain and urea were combined and processed through a cooker–extruder under conditions of moisture, temperature, and pressure that cause the starch to gelatinize. This extruded urea–grain product (EUGP) supplement made from ground corn and urea was compared to urea plus corn and soybean meal as protein supplement for lactating cows (Helmer *et al.*, 1970b). Eighteen lactating dairy cows were assigned to a 3 × 3 Latin square design for 6-week periods, with the first 2 weeks being considered the changeover interval. The concentrate mixtures were fed twice daily to appetite. The preexperimental concentrate contained 1 percent urea. The experimental mixture and EUGP contained 2.8 percent urea. The cows fed EUGP and soybean supplement ate considerably more grain, produced more milk, and gained more weight than the cows fed the urea supplement. The urea–grain mixture was apparently unpalatable. Previous *in vitro* work had shown that EUGP slowed urea hydrolysis (Helmer *et al.*, 1970a) in the rumen and increased synthesis of microbial protein. Owen and Appleman (1970) also reported a significant increase in milk production when a gelatinized milo–urea mixture or a urea–dehydrated alfalfa combination was compared to a control diet containing 2.5 percent urea. These preliminary results indicate that a urea–dehy combination and an extruded urea–grain product may partially resolve one problem of feeding urea; namely, poor acceptance of the urea diet and perhaps a more constant release of ammonia in the rumen. More work is needed on these products, especially with high-producing cows in early lactation before general recommendations can be made.

RELATIONSHIP OF RUMEN AMMONIA LEVEL TO NPN USE

Roffler and Satter (1973) recently described studies that show the relationship of rumen ammonia levels to microbial growth *in vitro* and to the total protein level in the diet. Using continuous culture fermentors, Satter and Slyter (1974) found that maximum microbial protein production occurred at a rumen ammonia level of 5 mg ammonia–nitrogen/100 ml rumen fluid. From analyses of 1,038 rumen ingesta samples from 207 dairy cows fed a large range of diet protein levels (but not containing urea), it was discovered that rumen ammonia nitrogen levels exceeded 5 mg ammonia nitrogen/100 ml rumen fluid whenever diet total protein levels exceeded 13 percent of diet dry matter (Roffler and Satter, 1973); therefore, it was concluded that diets containing more than 13 percent natural protein could not be benefitted by NPN additions. If these results are applied to all lactating cow diets, then no urea or NPN should be added, because National Research Council (1971b, Table 3) requirement levels are greater than 13 percent for all lactating cows.

Recent studies by Sparrow *et al*. (1973) and Gardner and Park (1973) with nonurea diets do not suggest that National Research Council protein levels are too high for high-producing cows. Although a negative control group was not used by Conrad *et al*. (1969), a urea–dehy-containing diet with a calculated protein level of 15.9 percent resulted in average lactation yields of 7,542 kg in nine Holsteins, which was almost identical to a diet of SBM. One cow produced 9,343 kg of milk in 305 days. The calculated total protein in the diet without the urea–dehy supplement was 11 percent. It appears that these cows must have used a substantial amount of the urea from the urea–dehy supplement.

A recent study by Ramage and Woolf (1973), which extends their previous work (1970) referred to above, supports the Ohio work that indicates that high-producing cows fed near National Research Council requirements may use urea nitrogen. Urea levels from 0 to 1.6 percent of the total diet were used in complete feeds of chopped hay and concentrate. The primary protein source used in the basal diet was corn gluten, chosen because of its relative insolubility. Only high-producing cows (> 32 kg/day for a week during peak production) were used. The standard basal complete diet of 70 percent concentrate, 30 percent chopped hay, and 0.8 percent urea was fed the first 6 weeks postpartum; then experimental diets were fed for the subsequent 29 weeks. Over 1 percent urea in the complete feeds did not adversely affect intake or production.

The protein solubility of feeds in the basal diet can have a marked effect on rumen ammonia levels and the usefulness of added urea to the diet. Wohlt and others (1973) showed that the protein solubility of energy feeds ranged from 4 to 42 percent and the protein solubility of protein supplements from 3 to 93 percent. Moreover, the solubility of processed feedstuffs varies greatly from batch to batch (Sniffen, 1973). The amount of natural protein in the diet that escapes rumen degradation is largely a function of solubility and the rate of passage through the rumen. The high-producing cow is characterized by a high feed intake relative to the steer or dry cow and must have a faster rate of passage. Those feedstuffs with a low rumen solubility will be most useful in diets with added urea, and future studies will undoubtedly relate to this point. Total diet soluble nitrogen should also be considered, because both corn silage (Johnson *et al.*, 1967) and hay crop silage (Waldo, 1968) can have a high level of soluble nitrogen. Unfortunately, at this time solubility factors are not well enough understood to apply them in practical feeding situations, but differences in natural protein solubility have probably contributed to the great variation in response to added urea in many reported studies. Very few experiments have been reported on the use of urea-containing diets that were fed to cows producing 40–50 kg/day of milk in early lactation when such cows are usually in negative energy balance. As production levels continue to increase, it becomes necessary to conduct experiments on cows in this critical production period before the results can be extrapolated to the general population.

EFFECT OF UREA ON THE HEALTH OF DAIRY CATTLE

Ryś (1967) stated that, although urea at a level of 2 percent has been routinely included as an ingredient in concentrates for cattle in Poland, veterinarians have not reported any symptoms of liver or kidney damage, except in cases of acute urea poisoning, which were caused by excessive urea intake. Toxicity problems can arise if hungry cattle are allowed access to large amounts of a feed containing a high level of urea; sometimes a urea supplement not intended for direct feeding is fed or a supplement is top-dressed, and aggressive cows eat large amounts in a short time. Because of the potential toxicity of urea, some have implicated it in reproductive disorders, mastitis, milk fever, and nearly every other ill that afflicts dairy cattle. Archibald (1943), in one of the few long-term studies with urea at 3 percent of the concentrate mixture, found no detrimental effects on health and reproduction. Urea at a level of 2.8 percent of the concentrate (up to 250 g/day) fed to Holstein steers for 7 months caused no differences in carcass quality and histopathology of liver, spleen, kidney, or adrenal and pancreas tissues (Muller *et al.*, 1971). The effects of urea, providing 45 percent of dietary nitrogen, was compared to plant protein in diets for Holstein heifers beginning 2 months prior to breeding (Patton *et al.*, 1970). Daily gains, blood urea, services per conception, and cycle lengths in repeat breeders were not affected by the feeding of urea.

The effect of dietary urea on luteal function was examined by Garverick and others (1971), who fed complete mixed feeds of corn silage concentrates and ground corncobs to 15 Holstein–Friesian heifers at least 60 days prior to estrus. One group received from 145 to 240 g of urea per animal per day; the other group received equivalent nitrogen as SBM. Plasma concentrations of progesterone, luteinizing hormone, corticosterone, and cortisol were nearly the same for the two groups. Twelve corpora lutea from urea-fed heifers were lighter, softer, and more fragile than 10 from SBM-fed heifers, but no histological differences were detectable. However, greater progesterone synthesis by corpus luteum tissue incubated *in vitro* was observed from heifers fed SBM. It is not known whether this difference had any effect on reproductive performance.

The Michigan Dairy Herd Improvement Association records of feeding practices were used by Ryder *et al.* (1972) to determine any relationship of urea feeding to milk production, calving interval, and cows sold for sterility. Amount of urea fed was divided into four levels to aid in the detection of any trends. Data from more than 600 herds during a 5-year interval provided 85,281 individual lactation observations for the study. Although urea was fed in over half the observations, no effect or trend could be seen in any of the parameters measured.

A long-term study on the use of urea in diets for dairy cattle fed corn silage as the only forage has been conducted at the University of Illinois. The first phase related to heifer growth from 20 to 104 weeks of age (Clark, 1971). Fifteen Holstein heifers were assigned to three diets at 12–20 weeks of age. Both the corn–soybean meal concentrate and corn silage were used to carry urea, so that average daily consumption was 0, 70, and 114 g urea and the dry matter contained 0, 1.2, and 2.1 percent urea. Dry matter intake decreased, but not significantly, as the urea level increased. However, no effect was seen on wither height, heart girth, or body weight. A second trial employed five heifers, each on the same treatments, plus a fourth group fed the high level of urea and 0.45 kg of alfalfa meal per heifer per day. No significant differences were observed in intake or body measurements. The total diets contained from 11.2 to 14.6 percent protein, with the urea diets containing the higher level, so that some of the nitrogen was probably unneeded.

The second phase of the Illinois work (Clark *et al.*, 1973) described the effect of the urea diets on milk production, diet digestibility, and nitrogen balance by cows fed the diets for 1 year (Trial 1) or 2 years (Trial 2) before the study. Six-day digestibility and nitrogen balance trials were carried out with four cows per diet at early and mid-lactation and during the dry period. Corn silage contained 0, 0.4, or 0.75 percent urea; the concentrate mixtures 0, 1.6, and 2.4 percent urea. In Trial 1 the concentrate mixtures were nearly isonitrogenous (ca. 16.5 percent protein), so the higher levels of urea in the silage caused the urea–silage groups to receive more protein than the control group. In Trial 2, the concentrate mixtures were adjusted to make the total diets isonitrogenous. Diets imposed had no measurable effects on dry matter intake, 4 percent fat correct milk (FCM), or milk fat content in either trial. However, in Trial 2 milk protein content was lower on both urea diets. Milk production (FCM) averaged 16.2 to 18.1 kg/day in early lactation and 12.4 to 16.2 kg/day in mid-lactation. Nitrogen balances revealed that productive nitrogen (milk plus retained nitrogen) was 69, 58, and 51 g/day for cows fed the control, low-urea, and high-urea diets in Trial 1. More nitrogen was excreted via urine by cows fed urea, but productive nitrogen was equal in early lactation and lower in mid-lactation and in the dry period. In Trial 2 with isonitrogenous diets, there was no detectable diet effects on nitrogen intake, nitrogen absorbed, or productive nitrogen. Urinary nitrogen was greater, however, in cows fed the high-urea diet than those fed the control. Nitrogen from soybean meal was used more efficiently than from urea. No data on reproductive efficiency or health disorders have yet been reported on this study.

Results of another long-term study on urea feeding have recently been reported from Purdue (Erb *et al.*, 1975). In Trial 1, 81 Holstein heifers were randomly assigned to three isonitrogenous diets in which urea provided 0, 50, and 100 percent supplemental protein for diets 1, 2, and 3, respectively. Corn silage plus alfalfa grass silage was blended with the concentrate premix to provide a complete ration. The heifer diets were 12 percent protein and were changed 2 weeks before calving to lactation diets of 54 percent silage and 46 percent concentrate (dry basis), which contained urea at 0, 18, and 36 percent of the total diet nitrogen. The lower urea level during lactation was about 1 percent of diet dry matter and the higher level 2 percent. In Trial 2, 18 heifers were each assigned to diets 1 and 3 just prior to calving. No problems of diet acceptance were encountered. Milk production (FCM) was 3.2 percent and 4.5 percent lower for cows on diet 3 compared to those on diet 1 during lactation one in both trials (nonsignificant). No significant diet effects were seen for incidence of infectious disease, days post-partum to first estrus and breeding, or in services per conception. Number of abortions for cows on diet 3 in Trial 1 was higher than for cows on diets 1 and 2, but this effect was not observed in Trial 2. In Trial 2, cows on diet 3 had more retained placentas compared to the other two groups, but this effect was not observed in Trial 1. Nothing in this study, carried on for four lactations in Trial 1 and two lactations in Trial 2, showed any cause for alarm at a urea feeding level of 1 percent of the total diet, or about 200 g/day. These data do suggest caution in moving to higher levels for a sustained continuous time.

UREA PLUS NITRATE

Both urea and nitrate, which liberate NH_3 upon hydrolysis, can be used by rumen microbes for protein synthesis. It has been postulated that adding urea to diets that have nitrate-containing forages may decrease the reduction of nitrate to ammonia and increase nitrite toxicity. However, no interrelationship between sodium nitrate, included at 2.5 percent of the total diet, and urea at 1 percent was found (Hoar *et al.*, 1968) with fattening lambs. Elliot *et al.* (1968) found that a combination of urea plus nitrates did not affect feed intake, milk production, level of hemoglobin, or level of methemoglobin in dairy cows. The results of Sebaugh *et al.* (1970) are in close agreement.

SUMMARY AND CONCLUSIONS

Two major factors are related to urea usage by ruminants: (1) acceptance of urea-containing diets and (2) effective utilization of the consumed urea.

Acceptance of the urea-containing diet is favored by:

- Multi-ingredient concentrate mixtures that include industrial by-products such as distillers' grains (a protein supplement) and molasses (a carbohydrate supplement).
- Feeding conditions that permit cows to eat small amounts throughout the day.
- Thorough mixing of urea into the diet.
- Diluting urea into the total diet, i.e., use of complete diets.
- Avoidance of ingredients such as raw soybeans that contain urease capable of releasing ammonia from urea and wet mangers that favor fermentation of the feed.
- The use of products that slow ammonia release. Consumption, however, does not necessarily mean effective use.

Utilization of urea is improved by:

- A level of protein in the basal diet that is below the requirement.
- A continuous supply of soluble carbohydrates such as starch. Conrad *et al.* (1969) suggest about 1 kg of readily fermentable carbohydrate is needed per 100 g of urea in the diet.
- *Ad libitum* feeding that will result in small amounts of feed eaten

throughout the day. This approach should ensure that ammonia release and soluble carbohydrate presence more nearly coincide.

- A minimum adjustment period of 2–4 weeks. In practice, a stepwise introduction of urea into the rations should be considered.
- Formulation of diets to contain appropriate levels of all known required minerals.
- Nitrogen compounds in the basal diet that have low rumen solubility. It is now known that some feedstuffs contain high levels of natural NPN and/or high levels of rumen soluble-true proteins; consequently, there may be dietary conditions (e.g., those with two or three fermented feeds) under which no added NPN would be used.

In terms of actual amounts, 1.5–2.0 percent urea in the grain mixture under general conditions or 1 percent of the total diet dry matter are good general guidelines for lactating cows, or 200–250 g/day. When high-moisture hay crop silage is the primary forage, and/or when this forage is fed with fermented grain, these levels should be reduced by at least one-half.

SHEEP

Many of the principles of urea use discussed in earlier sections were obtained using the sheep as a model for ruminants. Therefore, much of the research and applications discussed in the beef and dairy cattle sections apply also to sheep. Specific studies that relate to sheep include those by Harris and Mitchell (1941b), Johnson *et al.* (1942), Hamilton *et al.* (1948), Stangel (1967), Hume *et al.* (1970), and Braman *et al.* (1973); general guidelines are found in the National Research Council (1975) publication on sheep. Because the following factors are specifically critical, they are repeated for emphasis:

1. Because urea is very soluble in aqueous solutions and rapidly hydrolyzed to ammonia and carbon dioxide in the presence of urease, periodic feeding of high levels should be avoided to prevent acute ammonia toxicity and inefficient use.
2. Adequate levels of fermentable carbohydrates enriched with sufficient sources and levels of minerals enhance microbial use of urea.
3. Feeding management that insures frequent or continuous intake of urea-containing diets will tend to maximize microbial growth.

The ultimate value of urea in sheep diets is fundamentally based upon the quality (levels and patterns of essential amino acids) and quantity of microbial protein. Hall (1972) demonstrated that rumen microbial protein was a relatively good protein compared to soybean meal, corn gluten meal, or crystalline amino acids in test diets for the nonruminant. Nevertheless, Schelling and Hatfield (1968) provided evidence that microbial protein in sheep could be significantly improved by supplementation (postruminally) with specific amino acids or casein. Subsequently, Nimrick *et al*. (1970a) showed that the first three limiting amino acids in microbial protein were methionine, lysine, and threonine as indicated by nitrogen-balance studies with lambs.

The predominant influencing factors that determine the patterns and levels of amino acids at the absorption sites are: (a) the amount of dietary protein that escapes rumen degradation, (b) the amount of microbial protein synthesized, and (c) the completeness of postruminal digestion of residual dietary protein and microbial protein. The complementing effect of one source of protein (residual dietary) to another source of protein (microbial) could partly or completely correct deficiencies from a single-source protein that could enhance the amino acid status of the animal. The application of feeding practices can influence these factors. For example, the selection of ingredients with high rumen solubility (permitting more rumen degradation) and physical characteristics favoring longer rumen retention time (long hay), and low in natural proteins supplemented with liberal levels of NPN, would increase the ratio of microbial protein to residual dietary protein postruminally.

DIETARY LEVELS OF UREA

The supplementary protein needs of sheep diets are influenced by age and function of the animal (animal's requirements) as well as by the contributions of protein by the energy-furnishing forage and grain ingredients of the diet. Is urea an effective ingredient to furnish all or only part of the supplementary protein needs?

Stangel (1967) reported that a series of experiments were conducted in Germany between 1907 and 1924 in which urea satisfactorily replaced 30–40 percent of the nitrogen in sheep diets. Detailed evidence (Harris and Mitchell, 1941a,b) showed that nitrogen equilibrium in sheep could be maintained when 90 percent of the nitrogen was furnished by urea. Also near-normal growth of lambs was obtained in which urea furnished 50 percent of nitrogen in the 11 percent protein diets. Other investigators (Johnson *et al.*, 1942; Hamilton *et al.*, 1948; Braman *et al.*, 1973) have shown that urea is a useful source of nitrogen in diets containing no more than 12 percent protein. With sheep fed purified urea-containing diets (Hume *et al.*, 1970) with protein levels ranging from 3–18 percent, the maximum amount of protein synthesized in the rumen was recorded on diets containing 10.4 percent protein. Other experiments (Sharma *et al.*, 1969) have shown that a maximum amount of alpha amino nitrogen was found in the duodenum of sheep when they were consuming diets containing from 5.8 to 8.3 percent protein.

Data from Braman *et al.* (1973) indicated that lambs receiving urea-supplemented 10 percent protein diets performed better than lambs fed similar soybean meal-supplemented diets. A possible explanation was that the high-concentrate, soybean meal-supplemented, 10 percent protein diet was not sufficiently degraded in the rumen to supply adequate nitrogen for rumen microbial synthesis of necessary nutrients such as B vitamins, while the high-concentrate, urea-supplemented, 10 percent protein diet may have provided sufficient rumen ammonia levels for adequate microbial synthesis. However, at higher protein levels, Braman *et al.* (1973) found that the soybean meal-supplemented, high-concentrate diets were superior to the urea-supplemented diets.

Similar trends also favored urea supplementation over soybean supplementation in low-protein, high-concentrate diets for cattle (Braman *et al.*, 1973). However, higher performance in cattle was observed for soybean supplementation over urea supplementation at higher protein levels in high-concentrate diets (Braman *et al.*, 1973; Hatfield and Cantner, 1973).

High biological responses to soybean meal-supplemented diets over urea-supplemented diets of young growing ruminants that have relatively high protein requirements suggest that rumen microbial synthesis is inadequate (quantitatively or qualitatively) to meet the amino acid needs (Hungate, 1966; Sharma *et al.*, 1969; Mathison and Milligan, 1971).

Experimental data and field observations support the general statement that NPN compounds are satisfactory sources of supplementary nitrogen for sheep if they are limited from about one-fourth to one-third of the total dietary nitrogen and if the total dietary protein level does not exceed 12 percent of the diet. Since the effectiveness of urea nitrogen in replacing supplemental protein nitrogen is partially contingent upon other dietary ingredients, supplementary nitrogen can be furnished almost entirely by NPN sources in high-grain fattening diets. However, in maintenance or growing diets containing large amounts of low-quality forages that are low in protein and soluble carbohydrates, the amount of supplemental nitrogen required can exceed 50 percent of the total dietary nitrogen. For such diets the preformed protein should supply some of the supplemental nitrogen, and general nitrogen utilization would likely be improved if some soluble carbohydrates were supplied with any of the NPN sources. At times it may be economically desirable to use NPN for all of the supplemental nitrogen and accept a lower rate and efficiency of gain. Whenever high concentrations of NPN compounds are used, safety precautions as outlined elsewhere in this volume should be observed.

PROTEIN QUALITY

Changing the composition of the diet and/or varying the level of feeding may alter the rate of microbial synthesis, but the amino acid composition of the different strains of microorganisms is fairly constant. Although the biological value of microbial protein is relatively high (Chalupa, 1972), recent evidence is available to show that microbial protein or a combination of microbial protein and residual dietary protein can be improved by exogenous amino acids administered in a manner to prevent rumen degradation and alteration (Schelling and Hatfield, 1968; Al-Rabbat *et al.*, 1971a,b). Methionine, lysine, and threonine are the three first-limiting (and in the order of limitation) amino acids of microbial protein for supporting nitrogen balances in growing lambs. However, there have been conflicting reports regarding the value of free unprotected dietary amino acids. Reported results have varied from favorable responses to no responses or even depressed performances. The reported effects of dietary methionine hydroxy analog on performance have been as variable (Bishop, 1971; Wilson *et al.*, 1971) as the dietary amino acids data. It is likely that any favorable responses in the functional ruminant to dietary amino acids can be attributed to nonspecific nitrogen and/or sulfur responses. At reasonable dietary levels, neither methionine nor its analog increased plasma methionine levels in lambs (Papas *et al.*, 1974).

The recent observations that ruminants do respond favorably to specific protein precursors that reach the absorption sites is a challenge for the development of new practical methods and techniques that will protect dietary constituents from rumen degradation and alteration.

Treating dietary proteins with aldehydes or tannins have significantly improved rate of growth, feed efficiency, and nitrogen balances of growing lambs (Ferguson *et al.*, 1967; Nimrick *et al.*, 1970a,b, 1972; Peter *et al.*, 1971; Driedger and Hatfield, 1972). There are a number of practices presently employed that will reduce protein solubility that permits more rumen bypass of dietary proteins. Specific heat treatment of proteins, which will decrease rumen degradation, may increase dietary amino acid concentration at the absorption sites. However, overtreatment can easily occur that will result in resistance to digestion in the lower tract. In the light of these developments, more research should be conducted to determine the effect of time and intensity of grain-drying temperatures on protein and starch solubility, residual moisture levels, and overall grain quality. Changing feeding methods of ruminants will affect the amount of dietary constituents reaching the absorption sites. High-concentrate diets move more rapidly through the digestive tract than high-forage diets. Both the level of feeding and composition of the diets have some effect on rumen retention time. For example, the favorable responses to high-grain, high-protein diets may be due, in part, to some rumen by-pass of the dietary protein and starch. As indicated earlier, the ideal practical feeding diet might be one containing sufficient NPN to sustain an active rumen microflora that (a) would supply the host with an optimum supply of microbial protein and (b) is fortified with enough "protected" amino acids or with proteins containing high levels of the limiting amino acids, which would elicit maximum performance in the animals. It is especially important to have nonspecific nitrogen available to the microbiota if ruminants are expected to derive energy from forages and other cellulose-containing ingredients, synthesize nutrients not ordinarily added to rumen diets, such as B vitamins, and to detoxify compounds, such as chemical residues or other undesirable dietary contaminants.

EFFECT OF DIETARY UREA ON UTILIZATION OF OTHER INGREDIENTS

Numerous studies have been designed to show the interaction between urea and other nutrients. Urea, in general, did not adversely affect the utilization of other nutrients by sheep. Some studies report favorable effects of certain additives to urea-containing diets. However, studies reporting favorable responses to additives are usually studies that are deficient in the additive. For example, favorable responses to sulfur in urea diets have frequently been observed in diets in which urea replaced preformed protein, which usually supplies most of the dietary sulfur. Whenever urea has been substituted for alfalfa meal, there has appeared to be some favorable response to macro and micro mineral additives. There appears to be no consistent adverse effect of urea on carotene or vitamin A utilization. Sheep producers may often be concerned about urea additions to diets containing forages produced under conditions that could possibly raise the forage nitrate content. Urea-containing diets do not increase chronic or acute nitrate (nitrite) toxicity. Dietary urea is not a precursor to nitrite, thus it will not substantially increase the amount of nitrite absorbed from the rumen. Smith and Hatfield (1967) found that urea tends to counteract adverse effects of dietary nitrate.

Even though urea, added in excess to silages, may buffer acids sufficiently to affect its spoilage rate, it is unlikely that dietary urea properly used will adversely affect the utilization of other dietary additives.

ADAPTATION TO UREA-CONTAINING DIETS

Total composition of the diet has some effect on adaptation responses in sheep: Nitrogen retention in lambs fed urea-containing diets was improved significantly at the rate of 2 percent per 10-day period for 50 days (Smith *et al.*, 1960). In instances where there are abrupt and drastic changes in the chemical composition of the diet, there may be some adaptation time required for the biological system (microbiota and host). There have been some indications that the microbiota adapted within a few days (Barth *et al.*, 1961; Caffrey *et al.*, 1967a), while changes in animal responses may continue over several days (Ewan *et al.*, 1958; Welch *et al.*, 1957). Some data indicated that measurable responses may be attributed to adaptation to the feeding regime or total diet rather than to administered ammonia (Caffrey *et al.*, 1967a,b).

SUMMARY AND CONCLUSIONS

Urea can serve as a useful source of nitrogen in practical sheep diets. The most favorable responses from urea supplementation have occurred in diets containing relatively high concentration of readily fermentable carbohydrates and relatively low dietary levels of total nitrogen—12 percent protein equivalent or less after supplementation. High levels of urea and/or urea fortification of high-nitrogenous diets have often depressed performance by reducing diet acceptability or by reducing nitrogenous utilization. Fortifying forages at ensiling is a satisfactory and convenient method of nitrogen enrichment of materials to be used in silage-containing diets. Supplemental sulfur is needed also with most corn products.

Levels of urea used and methods of feeding to avoid urea toxicity in sheep appear to be managerial rather than nutritional problems.

GOATS

Little research on the use of NPN compounds in the nutrition of goats is reported in the scientific literature; however, there is no apparent reason why the principles governing the use of nonprotein nitrogen by lactating cattle would not apply to lactating goats and why principles that apply to wooled sheep would not apply to Angora goats.

Two specific reports on the use of urea in goat diets were found. Lindahl (1954) reported no significant difference in utilization of NPN-containing diets from that reported for sheep and cattle. However, Carrera and Killian (1970) fed a sorghum grain supplement containing 4 percent urea once daily to goats grazing poor-quality pasture in Mexico and observed some decrease in milk production. This might have been predicted with such a readily soluble compound fed at that level once daily.

Comprehensive publications on the nutrition of Angora goats (Huston *et al.*, 1971) and on milking goats (Colby *et al.*, 1969) are available.

6 Considerations on the Use of NPN Compounds by Nonruminant Species

The problems of formulating a diet for poultry or swine that supplies the needs of the animal for the essential amino acids yet which is deficient in the intact nonessential amino acids or nitrogen for nonessential amino acid synthesis have been discussed (Featherston, 1967; Moran *et al.*, 1967; Manoukas and Young, 1969; Lewis, 1972). The following factors influence the response of the chicken to NPN sources and to nonessential amino acids: (1) intake of total protein, (2) an adequate and balanced supply of essential amino acids, (3) a deficiency in dietary nonessential amino acids, (4) a single source of dietary nonessential amino acids that may not be as efficiently utilized as a mixture, (5) a nonutilizable source of nitrogen or a toxic effect from the NPN source, and (6) the dietary mineral mixture (D. Miller, 1973).

The use of dietary protein to supply the required essential amino acids usually provides an adequate dietary supply of intact nonessential amino acids or nitrogen for nonessential amino acid synthesis. The availability of one or a few essential amino acids or their corresponding alpha-keto acids at a sufficiently low price for practical animal diets does not alter the situation, since intact protein would be necessary to supply the remaining essential amino acids. The anatomical and metabolic limitations to the efficient utilization of NPN by nonruminant species have recently been reviewed by Lewis (1972).

Considerations other than economics may also be associated with the utilization of alpha-keto acids in place of essential amino acids in poultry or swine diets. Lysine and threonine would need to be supplied as such, since their carbon skeletons are apparently not easily aminated. With the exception of extensive findings showing good utilization of the hydroxy analog of methionine, little is known about the efficiency of utilization of alpha-keto acids where a large portion of the essential amino acids might be supplied by alpha-keto acids plus a nonspecific nitrogen source. Studies with rats have shown equal growth from the alpha-keto acid as with the amino acid for leucine, isoleucine, valine, and phenylalanine when tested individually in the diet (Meister and White, 1951) or for the alpha-keto acids of leucine, isoleucine, valine, phenylalanine, and methionine when tested together (Wood and Cooley, 1954). However, in both studies growth of only approximately 1 g/day was observed in any of the rats. Cruz *et al.* (1969) observed equal growth of rats fed equimolar amounts of L-valine or sodium alpha-ketoisovaleric acid; however, the levels fed were approximately double the requirement of the rat. Three times the amount of alpha-ketoisovaleric acid as compared to L-valine was necessary for maintenance of nitrogen balance in man (Gallina *et al.*, 1971).

POULTRY

The results of many studies on the utilization of NPN by the young chick have recently been reviewed (Featherston, 1967). Attempts to obtain satisfactory utilization of various sources of NPN by chicks fed diets containing natural feedstuffs have, for the most part, been unsuccessful. The utilization of various sources of NPN, including urea, diammonium citrate, and triammonium phosphate, has been demonstrated by several workers in chicks fed crystalline amino acid diets devoid of nonessential amino acids. The chick, however, lacks the ability to synthesize glycine and proline at a rate commensurate with the needs for rapid growth. Allen and Baker (1972) recently reported the results of studies concerned with the utilization for weight gain and protein retention of various sources of NPN when added to crystalline amino acid diets for chicks. In comparison to L-glutamic acid, diammonium citrate was utilized about 70–90 percent as efficiently. Diammonium phosphate and urea were utilized with an efficiency of less than 40 percent of L-glutamic acid.

Studies on the utilization of NPN by the laying hen indicate greater promise; however, the results have often been conflicting. Johnson and Fisher (1956) observed that the replacement of alanine, aspartic acid, cystine, glycine, proline, and serine by ammonium citrate in synthetic-type diets was found to have no adverse effect on egg production. Improvements of varying degrees in the egg production of hens fed well-balanced, low-protein diets composed of natural feedstuffs and supplemented with NPN have been reported (Young *et al.*, 1965; Chavez *et al.*, 1966; Reid *et al.*, 1972; Fernandez *et al.*, 1973).

Young *et al.* (1965) reported that the addition of 3 percent protein equivalent of nitrogen from diammonium citrate or glutamic acid to a 13 percent protein corn, soybean meal, fish meal diet resulted in improved egg production of hens up to that obtained with a 16 percent protein diet composed of the same natural protein sources. A response in egg production from the addition of diammonium citrate, but not glutamic acid, was noted when a 13 percent protein corn and soybean meal diet was used.

Chavez *et al.* (1966) reported that adding 2 or 3 percent protein equivalents of nitrogen from diammonium phosphate or diammonium citrate, respectively, to a 12.75 percent protein control diet containing adequate amounts of essential amino acids resulted in 4–5 percent increases in egg production. The addition of 3 percent protein equivalent from urea did not increase egg production. Feed conversion was improved by the first two nonprotein sources studied. More recent studies (Reid *et al.*, 1972) have shown increases in egg production in three of four experiments when diammonium citrate, ammonium sulfate, and diammonium phosphate were used as sources of NPN. Results indicate that the nonessential nitrogen requirement appears to be 1.4–1.9 g/day, of which 0.4 g could be furnished by NPN sources. Fernandez *et al.* (1973) showed some improvement in egg production from supplementation of low-protein laying diets with urea but little or no effect from additions of monosodium glutamate, diammonium citrate, or diammonium phosphate.

Other workers, including Moran *et al.* (1967), Akintunde *et al.* (1968), Bornstein and Lipstein (1968), and Kazemi and Balloun (1973), observed no improvement in egg production from dietary additions of urea, diammonium citrate, or diammonium phosphate. Moran and co-workers (1967) found that additions of 5 percent protein equivalent from urea or diammonium citrate to a 10 percent protein basal proved ineffective for both the growing chick and laying hen and that this level of diammonium citrate proved toxic. Reducing the level of diammonium citrate to 2 percent protein equivalent relieved the depression in chick performance. In a third experiment, in which a semipurified diet containing 8 percent protein from soybean meal plus additions of 11 essential amino acids was used, diammonium citrate additions again failed to result in improved laying hen performance. These workers concluded that a dietary inadequacy of nonessential nitrogen for the laying hen was improbable when normal feedstuffs were used. Kazemi and Balloun

(1973) observed that additions of 2 or 4 percent equivalents from urea or diammonium citrate to a 10.14 percent protein basal diet did not support equal performance of laying hens as that noted when equal protein equivalents were added from soybean meal. It was not possible to determine whether any utilization of the NPN sources occurred, since an unsupplemented basal diet was not fed.

The reason for the differences noted in utilization of NPN by the laying hen remains unsettled. It would appear to date, however, that the extent of utilization of NPN by poultry would be the addition of 2–3 percent protein equivalent to a 12–13 percent laying diet that is well balanced in the essential amino acids and that the results from such a substitution might well be quite variable and of questionable value from a practical standpoint.

SWINE

Hoefer (1967), in an extensive review of the literature involving studies on the addition of urea to swine diets, concluded that the effect of urea on daily gain was either negative or neutral. Even in instances where the diet was deficient in total protein (Hanson and Ferrin, 1955; Hays *et al.*, 1957), the addition of urea to the basal diet had little beneficial effect. However, no clinical evidence of toxicity was noted when pigs were fed urea at 1.5 percent of the diet (Hanson and Ferrin, 1955).

Recent studies by Grimson *et al.* (1971) using urea labeled with ^{15}N agree with the previous findings of Liu *et al.* (1955) in showing that a small but definite amount of the administered urea was incorporated into body protein. Liver, plasma, and intestinal scrappings had markedly higher levels of ^{15}N label in the protein than existed in skeletal muscle. In further studies, Grimson and Bowland (1971) substituted 2 percent urea for herring meal on an isonitrogenous basis and noted depressed feed intake, daily gain, and feed conversion in pigs from 4 to 11 weeks of age when they were limit-fed, but not when they were fed *ad libitum*. Growing pigs that were fed a urea-containing diet from 11 weeks of age to 90 kg live weight gained less weight and required more feed per kg gain than nonurea fed controls. Supplementation of the urea-containing diet with L-lysine or L-lysine plus DL-methionine resulted in improved performance of pigs as compared to those fed the nonsupplemented diet. The performance of pigs fed the urea plus lysine diet was similar to that of pigs fed the control diet, which contained an equivalent amount of nitrogen from soybean and herring meal rather than from 2 percent urea. These studies indicated some utilization of dietary urea nitrogen by pigs in the presence of supplemental L-lysine or L-lysine and DL-methionine; however, Grimson and Bowland (1971) state that the use of urea at a level of 2 percent in swine diets cannot be recommended as having practical value, even when the limiting essential amino acids are supplemented.

Kornegay *et al.* (1970) demonstrated a limited degree of urea utilization in some experiments by growing pigs fed corn–soybean meal diets in which the urea was added at the expense of either corn or soybean meal protein. They noted that the basal diet must supply a proper balance of essential amino acids before a positive response was obtained. In more recent studies, however, Kornegay (1972) observed that urea and ammonium polyphosphate, either singly or in combination, were ineffective in improving weight gain and gain:feed ratios when added to a low-protein diet.

Wehrbein *et al.* (1970) conducted studies to evaluate the utilization of diammonium citrate or diammonium phosphate as sources of dietary nitrogen for growing–finishing pigs. Efforts were made to replace 0, 5, 10, or 20 percent of the protein in a corn–soybean diet for growing swine with nonprotein nitrogen from an equimolar mixture of diammonium citrate and diammonium phosphate. With this experimental design, dietary essential amino acids as well as nonessential amino acids were being reduced. Average daily gain decreased as the increments of supplemented nonprotein nitrogen increased in the diets. Nitrogen retention was also decreased in pigs fed the diet containing NPN equivalent to 20 percent of the protein in the control diet. Supplementation of this diet with 0.05 percent DL-methionine, 0.20 percent L-lysine, and 0.05 percent DL-tryptophan partially alleviated the depressed growth rate and nitrogen retention.

The results of recent studies with the pig therefore give little basis for changing the views of numerous investigators, as summarized by Hoefer (1967), that nonprotein nitrogen cannot be used to any great extent in practical swine diets.

HORSES

Prospects for utilization of nonprotein nitrogen by horses would appear to be better than that observed in poultry and swine as a result of the large functional cecum and possibility of some protein digestion and absorption of amino acids in the colon. Studies by Reitnour *et al.* (1969) with ponies with cecal fistula that were fed diets containing corn, oats, or barley as the protein sources indicated that the apparent protein digestion anterior and posterior to the fistula averaged 11 and 40 percent, respectively. Subsequent studies by these workers, however, showed closer relationships between the amino acid patterns of dietary protein and serum than between those of cecal bacteria or cecal contents and serum (Reitnour *et al.*, 1970). These workers note the problem of defining the precise location of sampling from cecal fistula because of mixing in the cecum. Hintz and Schryver (1972) observed equal nitrogen retention from urea that was either fed or administered by cecal fistula, indicating that absorption of urea, ammonia, and/or amino acids produced in the cecum does occur. Whether the retention is due to nonessential amino acids synthesized by the liver or the absorption of amino acids synthesized by the microflora of the intestine remains unsettled.

Studies measuring NPN utilization by the horse have given conflicting results in some cases. Reitnour and Treece (1971) observed that urea was not utilized. Slade *et al.* (1970) observed that nitrogen balance and digestibility were improved as a result of urea additions to diets in which fish meal or corn gluten meal were the major sources of protein. They suggested that the improved nitrogen retention resulted from microbial synthesis of protein or free amino acids from urea nitrogen and their subsequent digestion and absorption. Hintz and Schryver (1972) observed equal increases in nitrogen retention by mature ponies from isonitrogenous additions of either urea, soybean meal, or linseed meal when added to a low-protein basal diet. These workers concluded that equines can utilize urea to increase nitrogen retention when fed low-protein diets, but that in general the efficiency of the utilization of the absorbed nitrogen from urea is considerably less than that of nitrogen from intact protein.

The horse appears to have the capacity to tolerate considerable quantities of urea. Ratliff *et al.* (1963) and Rusoff *et al.* (1965) fed from 0.5 to 0.55 lb of urea per day and did not notice any adverse effects. In the studies by Rusoff *et al.* (1965), horses fed levels of urea up to 0.5 lb/day for a period of 4 weeks exhibited weight gains, glossy coats, and good physical condition. Blood urea values increased from initial levels of 16 mg/100 ml to 90 mg/100 ml in horses fed the highest level of urea.

SUMMARY AND CONCLUSIONS

The following factors influence the response of nonruminants to NPN sources and nonessential amino acids:

1. Intake of total protein.
2. An adequate and balanced supply of essential amino acids.

3. A deficiency in dietary nonessential amino acids.
4. A single source of dietary nonessential amino acid that may not be as efficiently utilized as a mixture.
5. A nonutilizable source of nitrogen or a toxic effect from NPN.
6. The dietary mineral mixture.

Attempts to obtain satisfactory utilization of various sources of NPN by chicks fed diets containing natural feedstuffs have not been successful. More success has been obtained when urea, diammonium citrate, or triammonium phosphate were added to crystalline amino acid diets devoid of nonessential amino acids. However, the practical use of NPN in chick diets awaits future research results.

The inclusion of NPN in diets of laying hens has yielded controversial results. However, present results indicate that the extent of NPN utilization in diets of laying hens would be the substitution of 2–3 percent protein equivalent in diets containing 12–13 percent crude protein in which the balance of dietary essential amino acids would have to be well balanced. The results from such a substitution may be variable and of questionable value from a practical standpoint.

The results of studies in feeding NPN to swine indicate that it cannot be used to any appreciable extent in swine diets.

The horse appears to have the capacity for greater utilization of NPN than chickens or swine; however, the efficiency of nitrogen utilization from NPN is considerably less than that of nitrogen from intact protein.

7 General Conclusions

Despite hundreds of experiments carried out between 1940 and 1970, limited progress and understanding was made toward a more complete characterization of those dietary conditions under which added NPN would be beneficial. Within the past 5 years, new insights and developments, though controversial at this time, are stimulating research that will provide much more definitive guidelines for added NPN use. Already it is apparent that the old guidelines—urea up to (a) one-third of the dietary nitrogen, (b) 1 percent of the total diet dry matter, and (c) 3 percent of the grain mixture—need major modifications. Because high levels of natural NPN and/or high levels of rumen-soluble true proteins occur in some feedstuffs, there may be dietary conditions under which no added NPN would be used.

In the future, to answer the question, "How much NPN can be added to the diet of a ruminant?", it will be necessary to ask: (a) What is the level of protein in the basal diet relative to the animal's requirement? (b) What is the energy level (soluble or available carbohydrate) in the basal diet? (c) What level of soluble nitrogen is in the basal diet? (d) Is the level of other dietary components (especially required mineral elements) high enough to sustain maximum rumen microbial growth? (e) What feeding system will be used? (f) What is the expected level of intake? (g) Does the growth or production requirement for amino acids exceed the capacity of rumen microbial synthesis so that some rumen bypass of natural protein is needed? The answers to these questions and probably others will be needed to define when and how much added

NPN will be useful. Specific suggestions and guidelines are found at the end of each section for individual classes of livestock.

Except by starvation prior to feeding, improper formulation, or use of a supplement not intended for direct feeding, no clear cases of urea toxicity or long-term detrimental effects have been reported.

Research results of the next few years will provide much-needed answers for those questions that must be resolved in order to define those specific conditions under which added NPN will be beneficial.

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