An evaluation of biuret as a nitrogen source in wintering and fattening rations for beef cattle
by William Leo Mies

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
Montana State University

Abstract:
Biuret was evaluated as a source of non-protein nitrogen for cattle in wintering and fattening rations. The first wintering trial utilized 32 steer calves initially weighing 197.6 kg. in four treatments. The control treatment was a 12 percent protein supplement, and the three treatments were 20 percent protein supplements in which 50 percent of the protein equivalent was from biuret (B), urea (U), or soybean oil meal (S). The average ration for the 112-day feeding period was 1.71 kg. of supplement, 0.54 kg. barley, 0.54 kg. pelleted beet pulp, and 2.07 kg. wheat straw. Treatment daily gains and feed per unit gain were as follows; C-0.58, 8.46; B-0.59, 8.41; U-0.59, 8.36; S-0.59, 8.30. Treatment did not significantly affect gains.

These cattle were then fed a fattening ration consisting of cracked corn, 40 percent; beet pulp, 30 percent; 20 percent protein supplements, 30 percent; and wheat straw for 103 days. The control lot was changed to a 20 percent protein supplement containing soybean oil meal with added phosphorus. One-half in each lot were implanted with diethylstilbestrol (DES). Daily gain and feed per unit gain were as follows: S + P-1.30, 8.11; B-1.34, 8.10; U-1.29, 8.40; and S-1.37, 7.92. Gains were not significantly affected by protein treatments. Steers implanted with DES gained significantly more (0.16 kg. /day) than non-implanted steers (P<.01).

Yearling heifers averaging 246 kg. were used in a second fattening trial using a barley and beet pulp ration with grass hay and 0.45 kg. of a 32 percent protein supplement containing 20 percent of the protein equivalent from biuret (B), urea (U), one-half each from urea and biuret (U + B), and soybean oil meal (S). Daily gains arid feed per unit gains were as follows: B-1.36, 6.68; U-1.30, 6.84; U + B-1.30, 7.06; and S-1.31, 6.87.

There were no significant differences among gains.

Trial IV was similar to Trial I except the natural protein content of the control supplement was 10.5 percent and 10 steers per treatment were used. Initial weights averaged 204.3 kg. and the cattle were fed for 112 days. The average daily ration was 2.27 kg. supplement and 2.72 kg. wheat straw. Daily gains and feed per unit gains were as follows: C-0.17, 29.42; B-0.37, 13.19; U-0.33, 14.59; S-0.46, 10.44. The treated cattle gained significantly faster (P<.05).

Trial V was similar to Trial II. The steers used in Trial IV were utilized. The control supplement used in Trial IV was used in place of the soybean oil meal, added phosphorus supplement. One-half of the steers were implanted with DES. Daily gains and feed per unit gain were as follows: C-1.37, 7.71; U-1.31, 8.01; B-1.29, 8.18; S-1.36, 7.73. There were no significant differences in gains due to treatment. The DES implanted steers gained significantly more (P<.05) than non-implanted steers.

174

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by

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGEMENTS	iii
INDEX TO TABLES	vi
INDEX TO APPENDIX	viii
ABSTRACT	x
INTRODUCTION	1
REVIEW OF LITERATURE	3
Early History of Urea Research	3
Urea as a Nitrogen Source for Ruminants	4
Factors Affecting Utilization of Urea by Ruminants	5
Effect of Carbohydrates	5
Influence of Protein on Urea Utilization	7
Influence of Sulfur and Methionine on Urea Utilization	9
Influence of Trace Minerals on Urea Utilization	10
The Value of Urea as a Feedstuff	11
The Utilization of Urea as Affected by Adaptation Response	13
Uses of Urea for Ruminant Animals	14
Rations for Growing Cattle	14
Rations for Growing Sheep	15
Urea in Rations for Fattening Beef Cattle	16
The Toxicity of Urea to Ruminants	18
Biuret as a Nitrogen Source for Ruminant Animals	20
Utilization of Nitrogen from Biuret	20

Page

The Adaptation Response of Biuret 23

Effect of Biuret on Digestibility of Nutrients in the Ration . . 23

METHODS AND PROCEDURES 25

Trial I 25

Trial II 28

Trial III 31

Trial IV 33

Trial V 35

RESULTS AND DISCUSSION 38

Trial I 38

Trial II 40

Trial III 44

Trial IV 48

Trial V 50

SUMMARY 56

APPENDIX 58

LITERATURE CITED 73

TABLES | Page

I. ANALYSIS OF BIURET PRODUCT MA-5 26

II. DESIGN OF TRIAL I 27

III. SPECIFICATION OF SUPPLEMENTS FOR WINTERING CAVLES 28

IV. DESIGN OF TRIAL II 29

V. SPECIFICATION OF SUPPLEMENTS FOR FATTENING STEERS 30

VI. DESIGN OF TRIAL III 32

VII. SPECIFICATION OF SUPPLEMENTS FOR FATTENING HEIFERS 33

VIII. DESIGN OF TRIAL IV 34

IX. COMPOSITION OF SUPPLEMENTS FOR WINTERING CALVES 35

X. DESIGN OF TRIAL V 36

XI. SPECIFICATION OF SUPPLEMENTS FOR FATTENING STEERS 37

XII. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY AND COST OF GAIN OF STEER CALVES FED UREA, BIURET, SOYBEAN OIL MEAL, OR A PROTEIN DEFICIENT CONTROL RATION. (Trial I -- January 28, 1966 to May 20, 1966 -- 112 days) 38

XIII. NRC REQUIREMENTS, CALCULATED PROTEIN LEVEL, ACTUAL PROTEIN LEVEL AND AVERAGE DAILY GAIN OF 227 KG. WEANLING CALVES FED TO GAIN 0.45 KG. PER DAY 39

XIV. PROXIMATE CHEMICAL ANALYSIS OF FEED UTILIZED IN WINTERING CALVES IN TRIAL I 40

XV. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY, AND FINANCIAL RETURNS FOR STEER CALVES FED UREA, BIURET, SOYBEAN OIL MEAL, AND SOYBEAN OIL MEAL-HIGH PHOSPHORUS. (Trial II -- June 27, 1966 to October 9, 1966 -- 104 days) 41

XVI. SUMMARY OF WEIGHT AND AVERAGE DAILY GAINS OF STEERS IMPLANTED AND NOT IMPLANTED WITH DIETHYLSTILBESTROL 42

XVII. PROXIMATE ANALYSIS OF FEEDS FED IN TRIAL II 43

TABLES Page

XVIII. CARCASS DATA FOR STEERS FED SOYBEAN OIL MEAL, WITH AND WITHOUT HIGH PHOSPHORUS, UREA, AND BIURET. (Trial II -- June 27, 1966 to October 9, 1966 -- 104 days) 44

XIX. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY AND FINANCIAL RETURNS FOR HEIFERS FED UREA, BIURET, UREA-BIURET, AND SOYBEAN OIL MEAL. (Trial III -- July 5, 1966 to October 16, 1966 -- 103 days) 46

XX. PROXIMATE ANALYSIS OF FEEDS FED IN TRIAL III 47

XXI. CARCASS DATA FOR HEIFERS FED UREA, BIURET, UREA PLUS BIURET, AND SOYBEAN OIL MEAL. (Trial III -- July 5, 1966 to October 16, 1966 -- 103 days) 48

XXII. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY, AND COST OF GAIN FOR STEER CALVES FED A PROTEIN-DEFICIENT CONTROL, UREA, BIURET, AND SOYBEAN OIL MEAL RATION. (Trial IV -- November 28, 1966 to March 20, 1967 -- 112 days) 49

XXIII. PROXIMATE ANALYSIS OF FEED FED STEER CALVES ON WINTERING TRIAL IV 50

XXIV. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY AND FINANCIAL RETURNS FOR STEER CALVES FED UREA, BIURET, SOYBEAN OIL MEAL OR A LOW-PROTEIN CONTROL. (Trial V -- March 20, 1967 to August 7, 1967 -- 140 days) 51

XXV. SUMMARY OF WEIGHTS AND AVERAGE DAILY GAINS OF STEERS IMPLANTED AND NOT IMPLANTED WITH DIETHYLSTILBESTROL 52

XXVI. PROXIMATE ANALYSIS OF FEED FED IN TRIAL V 53

XXVII. CARCASS DATA FOR STEERS FED UREA, BIURET, SOYBEAN OIL MEAL, OR A LOW-PROTEIN CONTROL. (Trial V -- March 20, 1967 to August 9, 1967 -- 140 days) 54

XXVIII. SUMMARY OF WEIGHTS AND AVERAGE DAILY GAINS OF STEERS FED CONTROL, UREA, BIURET, OR SOYBEAN OIL MEAL SUPPLEMENTS ON BOTH WINTERING AND FATTENING TRIALS. (Trial IV and V -- November 28, 1966 to August 7, 1967 -- 252 days) 55

INDEX TO APPENDIX

TABLES Page

I. INITIAL AND FINAL WEIGHTS OF INDIVIDUAL STEERS FED UREA, BIURET, SOYBEAN OIL MEAL (SBOM) OR A PROTEIN DEFICIENT CONTROL. (Trial I -- January 28, 1966 to May 20, 1966 -- 112 days) 59

II. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY AND COST OF GAIN OF STEER CALVES FED UREA, BIURET, SOYBEAN OIL MEAL (SBOM) OR PROTEIN DEFICIENT CONTROL SUPPLEMENTS. (Trial I -- January 28, 1966 to May 20, 1966 -- 112 days) 60

III. INITIAL AND FINAL WEIGHTS OF STEER CALVES FED SOYBEAN OIL MEAL-HIGH PHOSPHORUS, UREA, BIURET, OR SOYBEAN OIL MEAL ON A FATTENING DIET. (Trial II -- June 27, 1966 to October 9, 1966 -- 104 days) 61

IV. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY AND FINANCIAL RETURN FOR STEERS FED SOYBEAN OIL MEAL-HIGH PHOSPHORUS, UREA, BIURET, OR SOYBEAN OIL MEAL. (Trial II -- June 27, 1966 to October 9, 1966 -- 104 days) 62

V. CARCASS DATA OF STEERS IN TRIAL II 63

VI. INITIAL AND FINAL WEIGHTS OF HEIFERS FED UREA, BIURET, 1/2 UREA 1/2 BIURET, OR SOYBEAN OIL MEAL SUPPLEMENTS. (Trial III -- July 5, 1966 to October 16, 1966 -- 103 days) 64

VII. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY AND FINANCIAL RETURN OF HEIFERS FED UREA, BIURET, 1/2 UREA 1/2 BIURET, OR SOYBEAN OIL MEAL SUPPLEMENT. (Trial III -- July 5, 1966 to October 16, 1966 -- 103 days) 66

VIII. CARCASS DATA OF HEIFERS IN TRIAL III 67

IX. INITIAL AND FINAL WEIGHTS OF STEERS FED CONTROL, UREA, BIURET, OR SOYBEAN OIL MEAL SUPPLEMENTS. (Trial IV -- November 28, 1966 to March 20, 1967 -- 112 days) 68

TABLES | Page

X. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY AND COST OF GAIN OF STEER CALVES FED A CONTROL, UREA, BIURET, OR SOYBEAN OIL MEAL SUPPLEMENT. (Trial IV -- November 28, 1966 to March 20, 1967 -- 112 days) 69

XI. INITIAL AND FINAL WEIGHTS OF STEERS FED CONTROL, UREA, BIURET, OR SOYBEAN OIL MEAL SUPPLEMENTS. (Trial V -- March 20, 1967 to August 7, 1967 -- 140 days) 70

XII. SUMMARY OF WEIGHTS, AVERAGE DAILY GAINS, DAILY FEED CONSUMPTION, FEED EFFICIENCY, AND FINANCIAL RETURN FOR STEERS FED CONTROL, UREA, BIURET, OR SOYBEAN OIL MEAL SUPPLEMENTS. (Trial V -- March 20, 1967 to August 7, 1967 -- 140 days) 71

XIII. CARCASS DATA OF STEERS IN TRIAL V 72

ABSTRACT

Biuret was evaluated as a source of non-protein nitrogen for cattle in wintering and fattening rations. The first wintering trial utilized 32 steer calves initially weighing 197.6 kg. in four treatments. The control treatment was a 12 percent protein supplement, and the three treatments were 20 percent protein supplements in which 50 percent of the protein equivalent was from biuret (B), urea (U), or soybean oil meal (S). The average ration for the 112-day feeding period was 1.71 kg. of supplement, 0.54 kg. barley, 0.54 kg. pelleted beet pulp, and 2.07 kg. wheat straw. Treatment daily gains and feed per unit gain were as follows: C-0.58, 8.46; B-0.59, 8.41; U-0.59, 8.36; S-0.59, 8.30. Treatment did not significantly affect gains.

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INTRODUCTION

Urea was the first synthetic organic material ever to be compounded. It had its beginning in the eighteenth century when chemists isolated a nitrogenous substance from animal urine and called it urea. In 1828, a German chemist named Wohler compounded synthetic urea from ammonia and cyanic acid. During World War II, the Germans perfected a process whereby ammonia and carbon dioxide were combined under pressure to form urea. Urea is still made today from these two basic compounds (Cyanamid, 1959).

Since it was first synthesized, urea usage has grown to hold an important place in American agriculture both as a fertilizer for plants and as a nitrogen source for protein synthesis in the ruminant animal. The use of urea for ruminant feeding has grown tremendously in the last ten to fifteen years. Its use is well-recognized as an effective nitrogen source for ruminants. Along with its effectiveness, urea also has certain disadvantages. Urea is hydrolyzed too rapidly in the rumen to permit maximum utilization. If good management is not practiced, urea can be toxic. These two disadvantages limit the use of urea to a recommended level of one-third of the protein equivalent of a ration.

Because of these disadvantages of urea, many other non-protein nitrogen (NPN) compounds have been evaluated for ruminant feeds (Belasco, 1954). One of the compounds which has been evaluated in recent years is biuret. This compound is synthesized by heating urea in the absence of water at a temperature over 271^{o} F. Because of its slow hydrolysis and low toxicity relative to urea, biuret should be a good nitrogen source for beef cattle in wintering and fattening rations. The structural formulas for urea and biuret are shown below.

UREA	BIURET
H-N-H	H-N-H
|	|
C=O	C=O
|	|
H-N-H	N-H
	|
	C=O
	|
	H-N-H

REVIEW OF LITERATURE

Early History of Urea Research

Zuntz (1891) postulated a possible use of non-protein nitrogen by ruminants. Zuntz felt that the mechanism for this utilization might lie in the conversion of organic nitrogen to bacterial nitrogen. The work of Zuntz was confirmed twenty years later by Armsby (1911), when he reported that bacterial protein was utilized for growth, milk production or maintenance. Armsby also raised the question of direct metabolic utilization of ammonia by ruminants. Voltz (1920) found that, when one-half of the nitrogen in the ration was furnished by urea, the digestible nitrogen was increased by 77.7 percent. When all the nitrogen in a ration was provided by urea, a lamb gained 11.91 kg. in a nine-month period. It was postulated that urea was synthesized into bacterial protein in the gastro-intestinal tract and that 80 to 90 percent of this bacterial protein was digested. The results of this work agreed with the theory of Zuntz made twenty-nine years earlier. Voltz also calculated that 350 grams of urea would replace 1000 grams of protein. In later work (Voltz et al., 1922), found that the recommended level of feeding should be 150 grams of urea per head daily for dairy cattle. This level was confirmed by Honcamp et al. (1923), who found that cows could consume 150 to 200 grams daily without ill effects. When urea was included in a carbohydrate-rich, easily digestible ration, the urea caused an increase in the amount of fat and milk produced. In later work, Honcamp and Schneller (1923) found that, when urea was added to an all-roughage ration, the urea was excreted almost quantitatively, while on the carbohydrate-rich ration, a positive nitrogen balance occurred.

Urea as a Nitrogen Source for Ruminants

The first work done in America using urea as a nitrogen source for ruminants was done by Hart et al. (1938). They fed growing dairy calves four rations; basal (6 percent protein), basal plus ammonium carbonate, basal plus casein, and basal plus urea. The three treated groups had a protein equivalent of 18 percent. The gains in weight for the four groups were 15.9 kg., 25 kg., 39.1 kg., and 28.0 kg., respectively. This work showed actual utilization of urea by ruminants on a growing ration. This work was confirmed by Bartlett and Cotton (1938), who stated that urea-fed calves showed no difference in average daily gain from calves fed natural protein; however, urea did not appear to be utilized as effectively as natural protein. Hart et al. (1939), working with dairy heifers in a comparison of three levels of urea, ammonium bicarbonate, and casein, found urea to be utilized for growth; however, if more than 50 percent of the total nitrogen was supplied by urea, kidney damage resulted. Work and Henke (1939) concluded that conventional natural protein supplements were better than urea as a protein source. However, had the authors fed a lower protein deficient control ration, more advantage might have been shown for urea. Kammlade et al. (1940) found no difference in average daily gains of lambs fed urea-supplemented rations or rations from natural protein. There also was no difference in final carcass grades. This work indicated that urea could be utilized by ruminant animals and converted to body protein. The results of these trials stimulated studies to evaluate urea as a feedstuff.

Factors Affecting Utilization of Urea by Ruminants

Much experimental work has been done to determine the value of feeding urea in ruminant rations. This work has shown that urea can be of considerable value in replacing natural protein in a ruminant ration. The amount of urea which can be utilized depends on the following factors:

(a) amount and kind of carbohydrates in the ration;
(b) amount and kind of true protein contributed by the ingredients of the ration;
(c) level of urea causing toxicity.

Other factors such as age of animal, mineral elements, roughages, and concentrates in the ration also have a bearing on the utilization of urea (Reid, 1953). The above-mentioned nutrients as well as sulfur, methinonine, and trace minerals have been shown to affect utilization of urea.

Effect of Carbohydrates

The effect of carbohydrates on urea utilization was first studied by Mills *et al*. (1942). They found that, when timothy hay was fed with urea, the level of protein in the rumen contents was the same as when timothy hay was fed alone or in combination with starch. However, when timothy and urea were fed, the rumen ammonia nitrogen was higher than when timothy was fed alone. The addition of starch to the timothy-urea mixture raised the level of protein in the rumen ingesta. They concluded that timothy hay did not supply a suitable medium for bacterial synthesis of protein from urea. Mills *et al*. (1944) reported that, by combining molasses and timothy, the protein content of the rumen ingesta was 7 percent. When urea was added to this ration, the protein content of the rumen contents was raised to 9.3 percent. However, the addition of starch to the timothy, molasses, urea ration gave a protein content of 11 percent in rumen contents. These results indicated that starch improved urea utilization to a greater degree than did molasses. A further study was conducted with growing heifers. A ration of timothy hay, molasses, and urea, in which the urea provided 60 percent of the total nitrogen in the ration, gave a daily gain of 0.31 kg. An addition of 0.14 kg. of starch per head per day to the above ration gave a daily gain of 0.64 kg. When casein was added at the same rate as starch, the daily gain was 0.73 kg. The conclusion reached was that cellulose in a ration is not broken down rapidly enough to provide an energy source for urea utilization. Therefore, a readily soluble carbohydrate is needed for maximum utilization.

Bell et al. (1951) reported that calves fed a combination of ground corn and urea had a higher nitrogen retention than did calves fed urea and molasses. Thirty percent of the nitrogen in the ration was provided by urea. Arias et al. (1951) concluded from in vitro studies that there is a specific need for small amounts of readily available energy such as dextrose, sucrose, or starch when the principal energy source is cellulose, in order to get optimum urea utilization in fermentation. The authors explained that during rapid hydrolysis of urea in the rumen energy was needed in order to convert ammonia to bacterial protein. When cellulose was the only energy source, it was not broken down quickly enough to aid in the utilization process. These findings were substantiated by Pearson and Smith (1943b), when they found starch to be superior to glucose, maltose, galactose, and many other similar carbohydrate sources in promoting protein synthesis. These results coincided with the earlier work done by Wenger _et al_. (1940), who reported cellulose to be a poor energy source for protein synthesis. Belasco (1956) found that urea utilization _in vitro_ was determined by the amount and type of carbohydrate used as an energy source. Urea utilization was greater with starch than with cellulose. Similar results were found by Drori and Loosli (1961) and Bloomfield _et al_. (1958). Bloomfield concluded that perhaps the amylose to amylopectin ratio affected urea utilization in the rumen. Fontinot _et al_. (1955) added cerelose to wintering rations where all the protein was provided by conventional protein sources. The cerelose addition increased the biological value of the protein, depressed the digestibility of the crude fiber, increased the digestibility of the nitrogen-free extract, decreased the apparent digestibility of the nitrogen-free extract, decreased the apparent digestibility of the protein, and increased the true digestibility of the protein. It may be concluded that a readily soluble carbohydrate is needed in ruminant rations in order to achieve maximum utilization for both natural protein and non-protein nitrogen utilization in the rumen.

Influence of Protein on Urea Utilization

The nature of the ration which includes urea has a definite effect on urea utilization. The protein content of the ration has been examined in this regard by Wenger _et al_. (1940) in _in vitro_ studies. They found that the level of protein in an inoculum of rumen micro-organisms influenced the rate of conversion as well as the amount of urea converted to protein. As the amount of casein was increased, the conversion of urea to protein decreased. In later work, Wenger et al. (1941a) used a fistulated heifer to study protein synthesis. When roughages were fed, the animal could consume a 20 percent protein ration and still have the 4 percent protein equivalent from urea fully utilized; however, if the protein equivalent from urea was increased to 6 percent, in an 18 percent protein ration its utilization was depressed. The rate of conversion of urea to protein decreased as the protein content of the ration exceeded 12 percent protein. Wenger et al. (1941b) found that when urea made up 1 to 5 percent of the dry matter of the ration, urea disappeared from the rumen in 4 to 6 hours. Pearson and Smith (1943b) found that certain amino acids promoted and others depressed the conversion of urea to protein. They concluded that when a ration contains insoluble protein, the amount of ammonia released from the protein may be small, thus favoring utilization of ammonia from urea. Hamilton et al. (1948) found that urea was used less effectively in a 16 percent protein ration than in an 11.4 percent protein ration. They concluded that at least 25 percent of the total nitrogen in the ration must come from plant protein and that the ration should not exceed 12 percent protein for sheep; however, Burroughs et al. (1951) found that the nitrogen required by ruminal micro-organisms was in the form of ammonia and amino acids were not required. Loosli and Harris (1954) found that the addition of methionine increased urea utilization. Gallup et al. (1952a) found no differences in nitrogen retention when urea was added to a ration containing either cottonseed meal, soybean oil meal, or corn gluten meal. Urea provided 30 percent of the total nitrogen in the ration. From this, one may conclude that the poor utilization of urea shown in many of the early experiments may be attributed to an excessively high level of protein in the basal ration.

Influence of Sulfur and Methionine on Urea Utilization

The first workers to recognize the importance of sulfur in the non-protein nitrogen diets were Harris and Mitchell (1941b). In their work, they tried to maintain a sulfur to non-protein nitrogen ration of 1:15. Loosli and Harris (1945) compared a basal diet of 6.55 percent protein, basal plus urea, basal plus urea and sodium sulfate, basal plus urea and methionine, and basal plus linseed meal. The four treatment rations were calculated to contain 10 percent protein. The average daily gains of lambs were 0.03 kg., 0.08 kg., 0.13 kg., and 0.14 kg. The percent of absorbed nitrogen retained was the same for basal plus urea and methionine and basal plus linseed meal. This indicated that the protein formed from urea was inferior to natural protein when methionine was absent from the ration. The sulfate improved the nitrogen balance but did not improve the average daily gain. Lofgreen et al. (1947) also reported an improved nitrogen retention due to the addition of methionine to a urea ration. This improved retention was found even though they fed only 18 percent as much methionine and 31 percent more urea. Thomas et al. (1951) used a urea ration with and without sulfur added to the diet in lamb rations. In three replications, the lambs without sulfur died between 90 and 146 days unless they were removed from the trial. Those lambs receiving sulfur gained weight and were in a positive nitrogen balance. This work indicates that the lambs utilized inorganic sulfates to improve the quality of the protein synthesized in the rumen. Gallup et al. (1952b) added methionine at the rate of 1.6 grams, 2 grams, and 3 grams per day to a 10.2 percent protein ration in which urea provided 30 percent of the total nitrogen. All three levels of methionine increased the digestibility of the ration nutrients and increased nitrogen utilization; however, these differences were not statistically significant. Barth et al. (1959) used a basal diet in which urea made up 87 percent of the ration nitrogen. Methionine and/or tryptophan were added to replace 17 percent and 11 percent, respectively, of the urea. They found that with either methionine or tryptophan or both, the retention of the absorbed nitrogen was increased. The digestibility of the nutrients in the ration was not affected. Other workers have used elemental sulfur to determine if rumen bacteria can manufacture their own methionine when given the necessary raw materials. Garrigus et al. (1950) found no difference in gain when urea supplements had either elemental sulfur or methionine added. Starks et al. (1952) found that lambs receiving a sulfur supplemented diet retained more nitrogen than did non-supplemented lambs. The data of Albert et al. (1956) suggested that sodium sulfate provided a better source of sulfur for methionine formation than did elemental sulfur.

The data reviewed here suggest that when urea is fed in a ration, sulfur supplementation has little value if the ration contains 0.1 percent sulfur or more, if the sulfur to non-protein nitrogen ratio approximates 1:15. Little work has been done on sulfur requirements and availability of different forms of sulfur.

Influence of Trace Minerals on Urea Utilization

There has been a limited amount of work published on the effect of trace minerals on urea utilization. The work that has been accomplished is somewhat contradictory suggesting that more research needs to be done in this area.

Thomas et al. (1953) discovered that when no trace minerals were provided, animals fed soybean oil meal rations gained more rapidly than animals fed urea supplemented rations. However, when trace minerals were added to the urea supplemented ration, there was no significant difference between daily gains of cattle fed soybean oil meal and urea rations. This indicates that trace minerals improved urea utilization. Similar results were found by Nelson et al. (1957) who reported that with cattle on dry range grass, trace minerals aided in urea utilization. However, when fed as a supplement to prairie hay, no advantage for trace minerals was detected. The latter result may have been due to the trace mineral content of the prairie hay. In opposition to these two findings, Gossett and Riggs (1956) and Gossett et al. (1962) found no benefit from adding trace minerals (Mn, Fe, Cu, Co, I, and Zn) to urea supplemented rations.

The addition of carbohydrates, protein, sulfur and methionine, and trace minerals to urea rations probably improves the biological value of the urea for the ruminant. It is clear that to attain maximum urea utilization, many factors must be in balance and the ration must be properly fed.

The Value of Urea as a Feedstuff

The value of urea as a feedstuff is a question on which much research has been done. The most common measurement used is a determination of the biological value of urea to the ruminant. Miller and Morrison (1942) reported that, in 325 nitrogen balance trials, there was very little difference in quality of protein of most feedstuffs when fed to ruminants. They also stated that when urea provided more than 50 percent of the total nitrogen in the ration, the nitrogen was used less efficiently than in rations containing an equivalent amount of nitrogen from linseed meal and urea. Johnson et al. (1944) concluded that, in ruminants, non-protein nitrogen was first synthesized by the bacteria into their own cellular proteins and protozoa utilized the bacterial protein to a considerable extent in their growth. Finally, the host digested the protozoa protein and the remaining bacterial protein. Thus, all nitrogen would exhibit a biological value characteristic of the mixed micro-organisms reaching the abomasum and duodenum. This value seems to be about 60. Earlier workers, working without benefit of this knowledge, tried to compute a different biological value for each feedstuff. Harris and Mitchell (1941a) reported a biological value of 62 for urea and 79 for casein at levels just maintaining nitrogen equilibrium in lambs. Harris and Mitchell (1941b) reported a biological value of 60 for corn silage and urea. They observed that the biological value of nitrogen decreased as the amount of urea in the ration increased. Lofgreen et al. (1947) concluded that urea had a higher biological value when methionine was added to urea diets. He reported values of 71 for urea, 74 for urea plus methionine, 76 for linseed meal, and 80 for dried egg. Therefore, the methionine addition improved the quality of the urea protein; however, Hamilton et al. (1948) showed biological values of 45 when 80 percent of the ration nitrogen was provided by urea. Miller and Morrison (1942), Gallup et al. (1954), and

Johnson _et al_. (1944) all reported a biological value of approximately 60 for urea nitrogen.

The Utilization of Urea as Affected by Adaptation Response

The adaptation response of urea is the increased utilization of urea nitrogen as a function of the length of time a ruminant is fed urea. This relationship was first explored by Campbell _et al_. (1956). They found that nitrogen utilization from urea increased the longer it was fed to animals. Diethylstilbestrol was found to increase nitrogen utilization. The factor of time increased nitrogen utilization with and without the use of diethylstilbestrol. Welch _et al_. (1957) found that animals fed urea for 35 days attained their maximum nitrogen utilization. Diethylstilbestrol decreased this time to 10 days. Smith _et al_. (1957) also concluded that diethylstilbestrol decreased the adjustment period for lambs. Lambs retained 7 percent more nitrogen as the adjustment period was increased from 10 to 35 days without the use of diethylstilbestrol. Six percent more absorbed nitrogen was retained following periods of 10 and 15 days when diethylstilbestrol was used. At 35 days, diethylstilbestrol did not increase nitrogen retention. These findings were in agreement with McLaren _et al_. (1959). MaLaren _et al_. (1960) concluded that diethylstilbestrol and time influenced the retention of absorbed nitrogen through direct action on the tissues to promote better utilization of non-protein nitrogen. Smith _et al_. (1960) concluded that nitrogen retention was increased by 2 percent for each 10-day period on feed up to 50 days. Barth _et al_. (1961) used a semipurified diet in an _in vitro_ study. Urea made up 87 percent of the total nitrogen in the ration. They found that protein synthesis did not increase as a function of time, indicating that a change in rumen micro-organisms was not the cause of the adaptation response to urea nitrogen. In these nitrogen metabolism trials, utilization increased from 36 percent to 51 percent from the first to the fifth week using the purified diet from the *in vitro* trials. The response shown was greater than for lambs fed a lower percent urea. The results of the work reviewed here may be used as criteria for interpreting or evaluating many of the trials which showed poor urea utilization, as the animals used in many of the studies reported were not adapted to the urea nitrogen.

Uses of Urea for Ruminant Animals

Rations for Growing Cattle

Bartlett and Cotton (1938) worked with dairy heifers ranging in age from 7 to 17 months. A 142-day feeding trial was used. The low-protein control heifers had an average daily gain of 0.45 kg., whereas the low-protein plus urea-fed heifers had an average daily gain of 0.56 kg. Thus, the addition of 0.06 kg. of urea increased the rate of gain by 0.11 kg. per day. They concluded that heifers fed natural protein showed no difference in gain from urea but that urea may be less efficient than natural protein. Hart *et al*. (1939) and Work and Henke (1939) found that growing calves could utilize urea with no ill effects. They also concluded that conventional supplements were better utilized and more efficient than urea. These experiments encompassed one year each so the adaptation response would not have been a factor. Loosli and McCay (1943) proved that calves two months of age could utilize urea. They used a basal ration containing 4.4 percent protein and the basal ration plus urea to give a ration containing 16.2 percent protein. The calves fed a basal ration were in a negative nitrogen balance. The urea-fed calves were in a positive nitrogen balance and gained 90-95 percent of what is considered to be normal growth. The urea-fed calves utilized 24-36 percent of their dietary nitrogen. When the calves on the basal ration were placed on the basal plus urea ration, they gained 22.8 kg. in 47 days. Briggs et al. (1947) found that feeds containing 25 percent and 50 percent of the total nitrogen from urea were just as effective as cottonseed meal when fed to yearling heifers on range. Gallup et al. (1953) recommended that urea should supply no more than 25 percent of the dietary nitrogen in growing rations for cattle. When urea was used at this level for wintering beef cattle, the steers fed urea supplement gained the same as the steers fed the plant protein supplement. Brown et al. (1956) found no difference in average daily gain or feed efficiency between protein and urea, when each supplied 54 percent of the dietary nitrogen in their respective rations. The rations were fed to young dairy calves from 2 days of age to 86 days of age. The work reviewed here shows that growing cattle can utilize urea effectively.

Rations for Growing Sheep

Johnson et al. (1942) showed that growing lambs could utilize urea nitrogen, if urea did not provide more than 25 percent of the ration nitrogen and the ration did not exceed 12 percent protein. They found that 16 to 17 percent protein rations, of which 65 or 46 percent of the total nitrogen was provided by urea, would not satisfy the nitrogen requirements for growing lambs. Harris and Mitchell (1941a) reported earlier that casein nitrogen was superior to urea nitrogen for growing lambs. Hamilton et al. (1948) found that urea was more efficiently utilized in a ration containing 11.4 percent protein (urea provided 46 percent of the nitrogen) than a 16.3 percent protein ration in which urea provided 63 percent of the dietary nitrogen. They also found linseed oil to be more efficiently used than urea in a 12 percent protein ration. Their conclusions agreed with Johnson et al. (1942). Gallup et al. (1954) found that a protein ration of 10 percent protein gave maximum nitrogen retention with urea making up 16, 30, or 43 percent of the dietary nitrogen. However, a greater nitrogen retention was achieved with a 12 percent protein ration that consisted of cottonseed meal as the primary protein source. It can be concluded that urea can replace natural protein up to a level of one-fourth of the dietary nitrogen in a ration of 12 percent protein or less.

Urea in Rations for Fattening Beef Cattle

Briggs et al. (1947) conducted two fattening trials in which urea was compared to cottonseed meal. The supplements which contained 25 and 50 percent of their nitrogen from urea were found to be equal to cottonseed meal. The pellets containing one-half their nitrogen from urea were unpalatable toward the end of the trial. In both trials, a protein-deficient control low enough to give urea and cottonseed meal a fair comparison was not used. In each case, the animals on the basal ration gained only slightly less than the animals receiving the experimental treatments. Long et al. (1951) attempted to evaluate urea and cottonseed meal as protein sources. Because of their experimental design, the only conclusion reached was that urea had some value as a protein replacement in fattening rations. Later, Long et al. (1952) used a basal diet of 9.7 percent protein. Urea was added to the basal to give a total protein equivalent of 12.5 percent protein. Cattle fed equivalent cottonseed meal rations gained approximately 1.0 kg. per day, whereas the urea-fed cattle gained on 0.82 kg. per day. The urea-fed cattle consumed about 10 percent less corn and thus had a lower intake of nutrients. This was attributed to urea's unpalatability reported by Briggs et al. (1947). Baker et al. (1944) compared urea, soybean oil meal, and a combination of one-half urea, one-half soybean meal as protein sources. No significant differences in average daily gain were found among the treatments. The basal ration provided 0.62 kg. digestible protein, while the treatments provided 0.69 kg. digestible protein. Watson et al. (1949) conducted feeding, metabolism, and slaughter studies on beef calves weighing approximately 175 kg. A basal ration of 4.3 percent protein was used. Either casein or urea was added to the basal ration to provide enough protein to satisfy the protein requirement. Urea-fed calves gained 70 percent as much weight as did the casein-fed calves. The casein-fed calves gained more protein and less fat than did the urea-fed calves. The gain in energy by urea-fed calves was approximately 81 percent of the gain made by casein-fed calves. Gallup et al. (1953) recommended that urea should supply no more than 30 percent of the dietary nitrogen in a fattening ration. They also observed that steers utilized urea better in fattening rations than did lambs. Urea did not affect the storage or blood level of vitamin A. Smith et al. (1964) found that urea did not affect vitamin

A storage in the liver, although urea did cause liver enlargement.

Thus, one may conclude from this evidence that urea may be used as a replacement for part of the protein in fattening rations for beef cattle. The amount of urea which may be used effectively is dependent upon further experimentation utilizing a low-protein control and adequate amounts of carbohydrates, proteins, sulfur, trace minerals, and adequate time allowance for adaptation response.

The Toxicity of Urea to Ruminants

Dinning et al. (1948) were the first to study urea toxicity in a systematic manner. They orally administered 20 grams of urea per 45 kg. of body weight to steers. They found that one-half to one hour after ingestion, the steers showed uneasiness, staggering, kicking at the flanks, followed by incoordination, tetany, prostration, and subsequently followed by convulsions, slobbering at the mouth, bloating and finally death. These symptoms were attributed to ammonia toxicity, as the steers had a high blood level of ammonia. Repp et al. (1955) found that in 34 kg. lambs, 20 grams of urea gave peak blood values at 30 minutes after treatment. Forty grams of urea produced toxicity symptoms or death. Acetic acid injected into the rumen or intravenously did not reverse the toxicity if the animal had already gone into tetany. The symptoms found were similar to those reported by Dinning et al. (1948). Whitehair et al. (1955) found that 20 grams of urea per 45 kg. body weight produced toxicity in steers only after the steer had been fasted. There were no toxicity symptoms at the same level of urea in adjusted steers. They concluded that conditions that led to possible toxicity were as follows:

(a) starving or fasting cattle before feeding urea;
(b) animals with "hoggish" appetites;
(c) unadjusted animals;
(d) improperly mixed feeds or feeds too high in urea;
(e) high levels of urea fed with poor quality, high roughage rations.

Hale and King (1955) found that the administration of ammonium carbamate either orally or intravenously produced symptoms similar to those of urea toxicity. They suggested that ammonium carbamate was the lethal factor in ammonia toxicity. They further stated that the bloating syndrome was similar to that displayed by cattle consuming immature legume pastures which were naturally high in non-protein nitrogen (ammonium carbamate) and noted that this may have been the toxic factor here, too. Hale (1956) further explained that ammonium carbamate may be formed as an intermediate product in urea hydrolysis by urease, and when ammonium carbamate builds up in the rumen, it may pass to the blood stream and be toxic. Lewis (1960) concluded that toxicity is almost certainly due finally to a direct effect of the circulating ion level, although, in the case of urea, there is an earlier effect that may be of a pharmacological type and less severe.

Therefore, because of the problems involved in the use of urea as a protein replacement in ruminant ration, namely, toxicity and inefficient utilization due to the very rapid hydrolysis by urease, it became necessary to find another non-protein nitrogen compound which would overcome these defects. Biuret has been shown to be non-toxic and slower than urea in ammonia release. Biuret is a condensation product of urea. When urea is heated above 271^{o} F. in the absence of water, ammonia is lost and biuret is formed. The chemical formula for biuret is $NH_2CONHCONH_2$. The molecular weight is 103. The protein equivalent is 237.5.

Biuret as a Nitrogen Source for Ruminant Animals

The first investigations into the use of biuret concerned the possible toxicity of biuret. If biuret was shown to be toxic, it would be of no more value than urea. Repp et al. (1955), working with lambs, found biuret to be non-toxic at levels which urea produced death. Research reported by Meiske et al. (1955), Hatfield et al. (1955), Hatfield et al. (1959), Clark et al. (1965) indicates that biuret is non-toxic. Berry et al. (1956) reported that biuret was non-toxic to rats, poultry, lambs, and steers. There were no acute or chronic toxicity symptoms exhibited. Although the lack of toxicity of biuret has not been disputed, the ability of the ruminant to utilize the nitrogen from biuret has not been clearly established. The investigation of biuret as a nitrogen source was the next logical step.

Utilization of Nitrogen from Biuret

Gaither et al. (1955) found no differences in nitrogen retention from urea and crude biuret. It should be noted that crude biuret was used in many of the early experiments. The crude biuret sometimes assayed as much as 40 percent urea so that some of the results obtained may be attributed to the action of urea. Hatfield et al. (1955) compared urea, biuret, and soybean oil meal when fed to steers in metabolism trials. No difference was observed in dry matter digestibility or nitrogen retention among the three treatments. The nitrogen balance was higher in the urea and soybean oil meal-fed steers than with the biuret-fed steers. The biuret-fed steers ingested 90 grams of biuret per day and excreted 12.8 grams of actual biuret in the urine. Meiske et al. (1955) reported no difference in daily gain in fattening lambs with urea, crude biuret, biuret, and soybean meal as nitrogen sources. The calves fed crude biuret, however, were the least efficient in feed conversion of all four treatments. The conversion rates were 10.52 for crude biuret compared to 9.76, 9.86, and 9.53 for the other three treatments. Gaither et al. (1955) found no difference in nitrogen retention in metabolism trials with lambs when biuret was compared to urea as a supplemental nitrogen source. Meiske et al. (1955) found that crude biuret, when compared to urea, depressed apparent nitrogen digestibility but did not affect nitrogen utilization. Pure biuret, however, reduced the utilization of nitrogen. Smith et al. (1957) found no difference in nitrogen utilization in a comparison of urea and crude biuret. However, one-half urea and one-half biuret depressed utilization. McLaren et al. (1958) conducted an in vitro study in which the production of acid at various times was used as a criterion for growth. No difference was found between crude biuret and urea. McLaren et al. (1959) reported that crude biuret did not affect nitrogen retention when compared to urea. Garrigus et al. (1959) conducted metabolism trials with biuret, urea, and biuret urea comparison. The highest nitrogen balance obtained was with biuret as the sole source of non-protein nitrogen (8.5 grams nitrogen per day). The basal ration with only urea added had a nitrogen balance of 7.6 grams per day. All combinations of urea and biuret had lower nitrogen balances than straight urea. Anderson et al. (1959) noted nitrogen utilization was not significantly changed when crude biuret supplied 50 percent of the supplemental nitrogen. When biuret supplied 100 percent of the supplemental nitrogen, nitrogen utilization was depressed. The lambs fed 100 percent crude biuret had a negative nitrogen balance. Hatfield et al. (1959) concluded that sheep and cattle could maintain a positive nitrogen balance when biuret furnished a major portion of the dietary nitrogen intake. The utilization of biuret appeared to be influenced by the level of feed intake. At high levels of feed, a urea-biuret mixture had the highest nitrogen balance. Campbell et al. (1963) found no difference in nitrogen retention with lambs when comparing urea and biuret. However, urea-fed steers had a higher nitrogen retention than those fed biuret. Karr et al. (1963) reported that biuret had a higher nitrogen retention than urea, but lower than soybean oil meal. Karr et al. (1964a) found that when steers were fed silage to which biuret had been added at ensiling time, a higher nitrogen retention resulted than when steers were fed urea supplemented silage. Clark et al. (1965) found no difference in nitrogen retention when comparing urea, biuret, triuret, and cyanuric acid with sheep.

Berry et al. (1956) suggested that because of the variable response of biuret when fed to ruminants, it would not be a dependable source of non-protein nitrogen. Johnson and McClure (1963) found that biuret had no effect on rumen ammonia or blood urea. Micro-organisms taken from urea- or biuret-adapted animals could not release ammonia from biuret when incubated in vitro. They suggested that if ruminants do utilize biuret, it is not through direct utilization of biuret nitrogen by rumen microflora.