UREA UTILIZATION STUDIES WITH RUMINANTS

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Submitted to the faculty of the Graduate College of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
July, 1967

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ACKNOWLEDGEMENTS

The author expresses his appreciation to Dr. A. D. Tillman, Professor of Animal Science, during the course of this study and in the preparation of this thesis.

Appreciation is also extended to Dr. I. T. Omtvedt, Associate Professor of Animal Science, Dr. L. J. Bush, Associate Professor of Dairy Science, Dr. D. D. Goetsch, Professor of Physiology and Pharmacology, and Dr. E. C. Nelson, Assistant Professor of Biochemistry, for their suggestions in the preparation of this thesis.

Further acknowledgement is due to Dr. E. I. Williams, Assistant Professor of Veterinary Medicine and Surgery; Mrs. Jane Witte, Laboratory Technician, and John R. Bourdette, Undergraduate Assistant, for their assistance during this study.

Special recognition is extended to the wife and family of Dr. Allen D. Tillman for the many courtesies during the author's stay in Oklahoma and to Carolyn K. Harman, Instructor, Poultry Science Department, who assisted in preparing and typing this thesis.

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CHAPTER I

INTRODUCTION

It is well established that at least one-third of the preformed protein in ruminant diets can be replaced by non-protein nitrogen such as urea. Indeed, many investigators have used urea as the sole source of dietary nitrogen and obtained very promising growth performance and milk production. However, when urea served as the sole source of nitrogen growth performance and milk production were approximately 70 percent as great as when preformed proteins were fed. Possible explanations for this reduced performance concern (a) the inability of rumen microbes to synthesize adequate quantities of branched-chain fatty acids for maximum microbial protein synthesis when urea is fed, and (b) the rapidity with which urea is hydrolyzed to ammonia in the rumen relative to the rate of microbial incorporation of ammonia into protein. Since urea is destined to play a major role in supplying dietary nitrogen for ruminant animals, it is important to learn if diets containing urea as the sole source of nitrogen could promote growth and milk production equal to, or greater than, that obtained with preformed protein. This report describes attempts to investigate the possibilities (a) that branched-chain fatty acids are deficient when urea-containing diets are fed, and (b) of reducing the rate of urea hydrolysis to a level commensurate with microbial ability to incorporate the ammonia released into protein.

CHAPTER II

LITERATURE REVIEW

Properties of Urease

Urea amidohydrolase (urease) which catalyses the hydrolysis of urea to ammonia and CO_2 was isolated from the jackbean by Sumner (1926) and has since been found to occur in bacteria, yeast, fungi and many higher plants (Sumner and Somers, 1953). Urease, a 19.6 S globulin (Sumner and Somers, 1953; Gorin and Chin, 1967); has a molecular weight of 483,000 (Sumner et al., 1938); an isoelectric point at pH 5.0 (Hand, 1939); and optimum pH of 7.0 (Sumner and Somers, 1953). The urease molecule can be dissociated into 6 subunits of 83,000 molecular weight which are held together by covalent bonds (Creeth and Nichol, 1960; Reithel et al., 1964). Hand (1939) reported the dissociation of urease into 16 subunits of 17,000 molecular weight which may have ureolytic activity. Gorin and Chin (1967) dissociated urease into two biologically active subunits using 0.1M phosphate buffer at pH 3.5. Each subunit was a 9.8 S protein and had a molecular weight of 240,000. These investigators also reported the mechanism of action of urease was different at pH 7.0 from that at pH 3.5, activity at the latter pH having less stringent requirements with respect to the integrity of the tertiary molecular structure. Classically urease was thought to be specific for urea, however, Fishbein et al. (1965) and Fishbein (1967) reported that hyd-

roxyurea and dihydroxyurea were hydrolyzed by urease, although at a 100-fold slower rate than urea, to give ammonia, CO_2 and hydroxylamine. The reaction mechanism for the hydrolysis of urea has been worked out experimentally by Gorin (1959) using jackbean urease. These investigators consider that urea was hydrolyzed by urease to form an intermediate ammonium carbamate which then dissociated into ammonia and CO_2. Chemically urea hydrolysis may be represented as follows:

$$H_2NCONH_2 + H_2O \longrightarrow H_2NCOOH + NH_3 \longrightarrow CO_2 + NH_3$$

Sumner and Somers (1953) reported that urease was produced by at least 200 different species of bacteria while Jones et al. (1964a) reported that about 35 percent of the viable bacteria in strained rumen fluid were associated with urease production. Davies and Kornberg (1950), Kornberg and Davies (1952, 1955), Levenson et al. (1959), and Dintzis and Hastings (1953) have concluded that gastric urease is of bacterial origin, whereas Conway et al. (1959) reported that bacteria did not account for all the urease in the gastric mucosa of the mouse. Cardin (1933) discovered fetal gastric urease and reported that bacteria may not be concerned with its formation. Mackay and Oxford (1954) and Huhtanen and Gall (1955) suggested that rumen urease was primarily of microbial origin. Pearson and Smith (1943) concluded that ruminal and jackbean urease were similar from kinetic studies of the properties of both these enzymes. However, Jones et al. (1964b) obtained a stimulation of rumen urease but an inhibition of jackbean urease by addition of cations. In later studies Jones et al. (1964) used mixed cultures of rumen microbes and obtained no differences between jackbean and microbial urease.

Fermentation of Amino Acids

Under anaerobic conditions bacteria ferment mixtures of amino acids to form cell material, ammonia, carbon dioxide and acids (carbon skeletons). The fatty acids formed during the fermentative deamination of ruminal amino acids was first reported by el-Shazly (1952a) who identified propionic, n-butyric, iso-butyric, n-valeric and alpha-methylbutyric acids in rumen liquor of sheep. He also observed that although the concentrations of the branched-chain C_4 and C_5 acids were small, they increased after sheep had eaten and greater increases occurred when lambs were fed protein rich rather than unsupplemented rations. Annison (1956) provided further evidence that isomers of C_4 and C_5 acids were produced by the fermentative deamination of amino acids.

The fermentation of leucine and valine to isobutyrate and isovalerate, respectively (Menahan and Schultz, 1964a), isoleucine to 2-methyl butyrate (Hungate, 1966), lysine to delta-amino valeric (Lewis and Emery, 1962a), proline to delta-amino valeric, glutamic acid and histidine to acetic, propionic and butyric acids (Van Den Hende et al., 1963a,b,c) support the suggestion made by el-Shazly (1952) and Oltjen and Putnam (1966) that the main function of amino acids in the rumen was to supply carbon skeletons rather than amino nitrogen for microbial protein synthesis. Since some amino acids are more readily reduced (hydrogen acceptors) and others more readily oxidized (hydrogen donors), fermentation of mixtures of amino acids proceeds more rapidly than fermentation of single acids (Strickland, 1934). In fact if both types of amino acids are present a fermentation may ensue, even though the acids singly are not fermented.

The foregoing account of the hydrolysis of urea and the fermentation of amino acids by rumen contents indicates that ammonia is always formed during the process. Ammonia is also universally produced during the utilization of proteins as an energy source. Ruminal ammonia levels vary from 0 to 130 mg/100 ml (Johns, 1955b). An important aspect of non-protein nitrogen utilization in ruminant animals is the capacity of the rumen flora (Annison, 1956) and mucosa (Hoshino et al., 1966) to utilize ammonia.

Ammonia Utilization

As reviewed by Reid (1953) various workers have demonstrated the incorporation of ammonia into microbial protein in animals with well developed rumenae. Not only could the rumen microbes utilize ammonia for the synthesis of microbial protein but also Bryant (1963) and Hungate (1966) indicated that ammonia was an essential nutrient for the growth of Bacteroides succinogenes, Ruminococcus flavefaciens, Ruminococcus albus, Bacteroides amylophilus, Methanobacterium ruminantium and Eubacterium ruminantium even when preformed organic nitrogen was present in the media. In addition el-Shazly et al. (1961) concluded that competition for nitrogen between cellulolytic and amylolytic groups of bacteria was the primary factor involved in the inhibition of cellulose digestion by starch. Urea rapidly overcame this inhibition. Burchall et al. (1964), Palmquist and Baldwin (1966) and Hoshino et al. (1966) suggested that the initial step in the fixation of ammonia appeared to be an amination reaction since they observed that NAD-and NADP-linked glutamic acid dehydrogenase was present in rumen microorganisms. Lewis (1965) suggested a consideration of additional mechanisms

such as a glutamine synthetase reaction or some of the steps involved in the urea cycle that will effectively fix ammonia into organic compounds. Synthesis of amino acids and protein from ammonia by rumen microorganisms requires the presence in the rumen of the necessary carbon skeletons. Incorporation of carbon from carbohydrate (Annison and Lewis, 1959), from carbon dioxide (Hutanen et al., 1954; Otagaki et al., 1955), and from acetate (Hoover et al., 1963) into amino acids indicate that carbon from many sources can be used for synthesis of the necessary carbon skeletons. On the other hand, synthesis by carboxylation reactions of leucine from isovalerate (Allison et al., 1966), isoleucine from 2-methyl butyrate (Hungate, 1966), valine from isobutyrate (Allison and Bryant, 1963), phenylalanine from phenylacetate (Allison, 1965) and tryptophan from indole-3-acetate (Allison et al., 1966) suggest a requirement for specific precursors for the synthesis of certain amino acids from ammonia and carbon fragments. Thus the possibility exists of a deficiency of certain amino acids or branched-chain carbon fragments for maximum microbial growth when urea serves as the sole source of dietary nitrogen for ruminants.

Daily essential amino acid requirements for ruminants are not known, except for the suggestion by Black et al. (1952) that amino acids which may be limiting for the dairy cow are those considered essential to the rat. Microbial synthesis of methionine and tryptophan (Ellis et al., 1959), tryptophan and histidine (Virtanen, 1966), tryptophan (Preston, 1961) was reported to be inadequate for maximum growth and milk production. Lower ruminal levels of histidine (Duncan et al., 1953) and lysine and methionine (Richardson and Tsein, 1963) were reported when urea replaced soy protein in purified and natural diets. Little et al. (1966)

observed an 8 percent reduction in plasma amino acids when natural diets were supplemented with urea. In the bovine lower plasma concentrations of leucine, isoleucine, valine and phenylalanine (Oltjen and Putnam, 1966), histidine (Virtanen, 1966), leucine, isoleucine, valine, phenylalanine, lysine, alanine, tyrosine, and glutamic acid (Freitag et al., 1966), isoleucine, valine, proline, and methionine (Little et al., 1966) were observed upon substitution of urea for soybean protein in the diet. Cline et al. (1966) reported the presence of iso-butyric and iso-valeric acids in minimal concentrations in rumen fluid of lambs fed urea-containing purified diets devoid of intact protein. However, despite these minimal rumen levels of branched-chain fatty acids and the fact that the major function of ruminal amino acids was to provide carbon skeletons, as previously indicated, these same workers observed improved nitrogen retention and digestibility when a mixture of iso-butyric, iso-valeric and n-valeric acids were added to a purified diet containing 39 percent cellulose but no improvement was observed when the diet contained 59 percent cellulose. In addition data reported by Hemsley and Moir (1963) showed that adding a mixture of iso-butyric, iso-valeric and n-valeric acids did not improve performance of lambs above that obtained from additions of molasses to diets high in fiber.

Supplementing urea-containing diets with amino acids has not always yielded beneficial results. Additions of methionine, lysine, alanine, or glutamic acid to urea-rich diets did not improve animal performance (Oltjen et al., 1962, 1964; Harbers et al., 1961). On the other hand Gossett et al. (1962) obtained improved growth performance with supplements of lysine and methionine while McLaren et al. (1965a) obtained improved nitrogen utilization when 11 and 17 percent of the urea nitrogen

was replaced with isonitrogenous levels of methionine and tryptophan. It is of interest to note that the addition of amino acids singly did not improve performance; however, when a mixture was added performance was improved. As previously noted this phenomenon may be due to the fact that mixtures of amino acids are more readily fermented than are single acids (Strickland, 1934).

Anaerobic bacterial growth depends on adenosine triphosphate (ATP) produced from rumen fermentation (Bauchap and Elsden, 1960). Hungate (1965) calculated a maximum of 4 to 5 ATP per hexose molecule under anaerobic conditions. Since this level of ATP represents approximately 10 percent of the energy produced under aerobic conditions, Hungate (1966) concluded that anaerobiosis limited the degree of microbial synthesis from dietary constituents. Hungate (1966) calculated that approximately 100 gm of carbohydrate were required per gram of nitrogen fixed *in vivo* while Bloomfield *et al.* (1964) reported a requirement of approximately 55 gm of carbohydrate per gram of nitrogen fixed under *in vitro* experiments. Since abomasal infusion of intact dietary protein would make total host protein synthesis less dependant on microbial protein synthesis, Chalupa *et al.* (1964b) concluded that intact protein was superior to non-protein nitrogen in growth performance because part of the dietary protein escaped ruminal proteolysis and passed intact to the abomasum where it was not hydrolyzed beyond the amino acid stage. The extent to which this process occurred depended on the solubility of the protein source.

Utilization of ammonia by rumen epithelium has been reported by McLaren *et al.* (1961, 1962b), Hoshino *et al.* (1966). Rumen epithelial synthesis of glutamate favored liver synthesis of urea by providing

intermediates required in the synthesis of urea (McLaren, 1964). Glutamine which is synthesized by rumen mucosa utilizes ammonia and serves as a storage form of ammonia for use in tissue synthesis (Hoshino et al., 1966).

Adaptation to Urea

Progressive improvement in the utilization of non-protein nitrogen with time on non-protein nitrogen rich diets has been observed by (Repp et al., 1955a; Ewan et al., 1958; Smith et al., 1957a; Welch et al., 1957; McLaren et al., 1959; Loosli and Campbell, 1961; Virtanen, 1966). This progressive improvement in nitrogen utilization was referred to as the "adaptation response" by Smith et al. (1960) and McLaren et al. (1965b) who found that nitrogen utilization improved 0.2 and 0.3 percentage units per day, respectively, during a 50-day period. Oltjen and Putnam (1966) reported adaptation to isolated soy protein as well as urea in purified diets. Diethylstilbestrol has been shown to shorten the time required for adaptation to take place (McLaren et al., 1959, 1960; Karr et al., 1965). Many conflicting reports have appeared in the literature concerning the exact location of the adaptation response. Since Browning et al. (1959) reported that diethylstilbestrol had no effect on rumen microbes, the shortening of the period required for adaptation to take place would suggest that adaptation took place in tissues other than in the contents of the digestive tract. On the other hand, Lewis (1960) and Virtanen (1966) reported that adaptation took place in the rumen flora. However, Barth et al. (1961) found no changes in protein synthesis by rumen flora when samples were taken at weekly intervals after starting lambs on diets supplemented with urea.

Holzschuh and Wetterau (1965) suggested the liver to be the site of adaptation. McLaren et al. (1962b) reported that the rumen epithelium was not the site of adaptation and later McLaren (1964) reported that adaptation was due to increased urea reabsorption from the glomerular filtrate by the renal tubules of the kidney.

Control of Urease Activity

Since Bloomfield et al. (1960) reported that the rate of ammonia release from urea exceeded microbial uptake of ammonia by a factor of four, it appears that a major factor limiting urea utilization is the rate of urea hydrolysis.

If urease inhibitors could be used to reduce ureolytic activity to a rate commensurate with microbial protein synthesis, it would be possible to use these in improving urea utilization. Urease inhibitors have been ranked in order of their decreasing effectiveness as follows by Groll (1917, 1918): $SO_4^{=} > Cl^{-} > Br^{-} > NO_3^{-} > S^{=} > I^{-}$, $NH_4^{+} > K^{+} > Na^{+} > Sr^{++} > Ba^{++}$ and by Shaw (1954) $Ag^{++} > Hg^{++} > Cu^{++} > Cd^{++} > Co^{++} > Ni^{++} > Mn^{++}$. Vandevelde (1947) reported that Pb^{++}, Cd^{++}, Fe^{++}, Bi^{++} and Zn^{++} in addition to the sulfate, chloride and nitrite salts of sodium and potassium inhibited urease activity. Sulfites and bisulfites also had inhibitory effects on urease activity (Ambrose et al., 1949).

Microbial urease has been shown to be inhibited by nitrofurazone and trivalent arsenicals (Yall and Green, 1952) whereas intestinal ureolytic activity was inhibited by alloxan, murexide and barbituric acid (Visek et al., 1961). Cyclic urea-related compounds, such as alloxanic acid, dialuric acid, alloxantin and murexide also inhibited urease

activity (Siliprandi and Daghetti, 1950; Gray et al., 1959). Kristiakowsky and Shaw (1953) reported that thiourea was a competitive inhibitor of urease at pH 6.0 but was both a competitive and non-competitive inhibitor at pH 7.6. High concentrations of urea also inhibited urease activity (Deasy, 1947; Merino and Raun, 1964). Anti-bacterial agents have also been reported by many workers to reduce urease activity. Penicillin (Turner et al., 1943; Vargas and Escubas, 1945; and Prescott, 1953), neomycin (Silen et al., 1955), mixtures of sulfaguanidine, terramycin and pencillin (Dintzis and Hasting, 1953) and penicillin, chlortetracycline or arsanilic acid (Visek et al., 1959) inhibited urease activity. Merino and Raun also reported that chlortetracycline per se did not reduce rumen ureolytic activity. Harbers et al. (1963a) and Alvares et al. (1964) decreased ureolytic activity of intestinal contents and significantly increased the growth rate of chicks by supplementing the diets with barbituric acid or chlortetracycline. Harbers et al. (1965) and Glimp and Tillman (1965) found that antibodies developed in ruminants to injected urease and associated with this injection treatment was increased gains. These workers also found that urease injection caused a decrease in ammonia levels of blood plasma obtained from the ruminal, cecal and jugular veins. It is possible that the improved growth resulted from a decreased ureolytic activity.

Although it is well established that rumen microbes can synthesize amino acids from ammonia and carbon fragments the extent to which this synthesis takes place for each amino acid is not clear. Thus the possibility exists that a deficiency of certain amino acids may occur when urea is the only source of dietary nitrogen in ruminant diets. If certain amino acids are deficient in the diet then improved growth perfor-

mance would be expected when these are added to the diet.

Since ammonia production from urea in the rumen proceeds at a faster rate than ammonia absorption by rumen microbes the reduction of rumen ureolytic activity to a level commensurate with microbial ability to synthesize protein appears to offer promise in improving urea utilization in ruminant diets. The following research describes attempts to improve urea utilization by adding amino acids and urease inhibitors to urea rich diets for ruminants.

CHAPTER III

UREA AND ISOLATED SOY PROTEIN IN SHEEP PURIFIED DIETS

Introduction

Inferior growth performance in ruminants has been reported when urea rather than isolated soy protein was the sole source of nitrogen in purified diets (Oltjen et al., 1962; Oltjen and Putnam, 1966). Possible explanations concern (1) the faster rate of ammonia release from urea in the rumen (Pearson and Smith, 1943a,b), (2) lack of adaptation to urea (McLaren et al., 1960; Barth et al., 1961), and (3) deficiency of readily hydrolyzable carbohydrate and/or branched-chain fatty acids (Hemsley and Moir, 1963; Cline et al., 1966). Since Oltjen et al. (1962) and Oltjen and Putnam (1966) compared diets in which urea or isolated soy protein alone were the sole sources of nitrogen, it appeared desirable to repeat these experiments and extend them by studying various combinations of urea and isolated soy protein in a growth and metabolism experiment with lambs fed purified diets.

Experimental Procedure

Twenty lambs were treated with phenothiazine as an antihelminth, allotted at random into five treatment groups, and fed the diets shown in Table I over a 60-day period. All lambs had free access to feed and water at all times and were kept without stanchioning in 90 x 130 cm

TABLE I

PERCENTAGE COMPOSITION OF DIETS

Ingredients	U/S[a]	Diets 1 4/0	2 3/1	3 2/2	4 1/3	5 0/4
Corn starch		34.37	32.82	31.82	30.27	29.02
Glucose[b]		24.37	22.82	21.82	20.27	19.02
Cellulose[c]		30.00	30.00	30.00	30.00	30.00
Corn oil[d]		1.00	1.00	1.00	1.00	1.00
Polyethylene resin[e]		1.00	1.00	1.00	1.00	1.00
Choline chloride		0.10	0.10	0.10	0.10	0.10
Vitamins A and D[f]		0.02	0.02	0.02	0.02	0.02
K_2CO_3		2.21	2.21	2.21	2.21	2.21
$CaHPO_4$		1.32	1.32	1.32	1.32	1.32
$MgSO_4$		0.12	0.12	0.12	0.12	0.12
$MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$		0.27	0.27	0.27	0.27	0.27
Na_2SO_4		0.25	0.25	0.25	0.25	0.25
NaCl		0.62	0.62	0.62	0.62	0.62
Trace minerals[g]		0.10	0.10	0.10	0.10	0.10
Urea[h]		4.20	3.70	2.10	1.60	----
Isolated soy protein[i]		----	3.60	7.20	10.80	14.90

[a]Urea/Soy protein ratio.

[b]Cerelose. Corn Products Co., Argo, Illinois.

[c]Solka-Floc. B-W20. Brown Co., Berlin, New Hampshire.

[d]Mazola. Santoquin added to give 0.0125% in total ration.

[e]Alathon. E. I. du Pont de Nemours, Inc., Wilmington, Delaware.

[f]Containing 20,000 I.U. and 2,500 Units/gm. of vitamins A and D, respectively. Courtesy NOPCO Chemical Co., Harrison, New Jersey.

[g]Composition of the trace mineral mixture: (mg/100 gm. diet) $FeSO_4$, 42.51; $MnSO_4 \cdot H_2O$, 15.37; $Na_2B_4O_7$, 12.56; $ZnSO_4 \cdot 7H_2O$, 26.35; $CuCO_3 \cdot Cu(OH)_2$, 1.97; KI, 0.07; $CoCl_2 \cdot 6H_2O$, 0.05; CaF_2, 0.20; $Na_2MoO_4 \cdot 2H_2O$, 0.50; $Cr_2(SO_4)_3$, 0.04; Na_2SeO_4, 0.02.

[h]Crystalline urea. Courtesy Nipak Chemical Co., Pryor, Oklahoma.

[i]Purina Assay Protein RP-100. Ralston Purina Co., St. Louis, Missouri.

slatted floor growth and metabolism stalls, which were equipped for the separation and quantitative collection of feces and urine. A sixteen hour shrink preceded initial and final weighings. All feces and urine were collected over the 60-day period and composited into successive 10-day intervals. After the final weighing all lambs were fed their respective rations ad libitum for 48 hours and sacrificed without a preceding fast. At this time 500 ml of blood was collected in heparin for amino acid analysis. Also, the rumens were removed, weighed, and samples of rumen fluid taken for chemical analyses. After the contents were removed, the rumens were washed and weighed. The livers, kidneys, and cannon bones were also removed and weighed. The carcasses were chilled and their specific gravities determined. These carcasses were then split in halves with one side being deboned, ground, and sampled for total nitrogen analysis.

Ruminal acetic, propionic, iso-butyric, butyric, iso-valeric + 2 methyl butyric and valeric acids were determined on strained rumen fluid samples after precipitating the protein with 25% metaphosphoric acid (Erwin et al., 1961). The acids were separated on a 10' x 1/8" stainless steel column of 28% carbowax 20M terephthalic acid substrate on 60/80 acid washed chromosorb W. Ammonia-nitrogen concentration was determined by the procedure of Conway (1950) and total nitrogen by standard Kjeldahl procedures (A.O.A.C., 1960). Protein-nitrogen concentration (TCA-N) was determined on strained rumen fluid by the procedure of Cline et al. (1958). Urea-nitrogen was determined by the Hycel (1960) procedure. Plasma samples were prepared for amino acid analyses by the procedure of Oltjen and Putnam (1966). Amino acids and other ninhydrin positive compounds were determined by ion exchange chromatography on an

automatic amino acid analyzer (Spackman et al., 1958). The data were analyzed statistically by analyses of variance and treatment means compared using Duncan's new multiple-range test (Steel and Torrie, 1960)

Results and Discussion

Table II shows growth and carcass data. Although no treatment differences were significant ($P > .05$), there appeared to be a trend toward larger livers, kidneys, and rumens as soybean protein replaced increasingly larger proportions of urea.

Fecal and urinary excretions of nitrogen and the percentage of dietary nitrogen retained over the 60-day period are shown in Table III. As the animals had free access to feed, there were variations in nitrogen intake. Fecal and urinary nitrogen excretions were not affected by diet. Nitrogen retention was lowest in animals fed diet 2 and there were no other differences among the other treatments. No logical explanation for the reduction found in these animals fed diet 2 was apparent, thus the possibility of a chance occurrence exists.

Gain on lambs fed urea as the sole source of nitrogen were approximately 70 percent those obtained on diets 3, 4, and 5 and agree with the 75 percent value found by Oltjen and Putnam (1966). If the nitrogen balance data are converted into protein storage using the factor 6.25 (Jones, 1931) there is a trend toward greater protein storage in animals consuming diets containing soybean protein with the notable exception of diet 2. When the protein storage of animals on diets 1 and 5 were compared, it was found that those fed diet 1 stored approximately 81 percent as much as the latter. This is also in close agreement with the growth data found in this experiment and by Oltjen and Putnam (1966).

TABLE II

EFFECTS OF UREA, SOY PROTEIN AND VARIOUS COMBINATIONS OF UREA-SOY UPON DAILY GAINS AND CARCASS MEASUREMENTS

Item	Diets					
	U/S[a]	4/0	2/1	2/2	1/3	0/4	SE[b]
Daily gain (gm)	69	27	119	88	90	22
Feed intake (kg)	1.08	0.83	1.20	1.02	1.08	0.11
Carcass sp. gr.[c]	1.03	1.04	1.04	1.03	1.04	0.01
Weight of:						
Cold carcass (kg)	18.29	16.33	17.66	18.43	19.50	0.98
Livers (gm)	481	511	595	575	656	57
Kidneys (gm)	85	94	95	83	91	10
Cannon bones (gm)	94	100	101	104	98	5
Empty rumen (gm)	800	809	961	818	1112	121
Carcass protein (kg)	1.71	1.63	1.74	1.91	1.97	0.28

[a]Urea/Soy protein ratio.

[b]Standard error.

[c]Specific gravity. Determined on whole carcass.

It is of further interest to note that these figures are in general agreement with those of Virtanen (1966), who compared urea-containing purified diets to natural diets in bovine lactation studies.

When nitrogen intake, fecal and urinary nitrogen excretions and nitrogen retention were calculated for successive 10-day periods, there were no differences ($P > .05$) between treatments for any given period;

however, there was an apparent improvement in retention with time on all rations. Therefore, all values for retention and excretion were combined over all treatments and these results are shown in Table IV.

TABLE III

EFFECT OF UREA OR SOY PROTEIN ALONE AND UREA-SOY COMBINATIONS ON FECAL AND URINARY EXCRETIONS AND ON NITROGEN BALANCE

		Diets					
Item	U/S[a]	1 4/0	2 3/1	3 2/2	4 1/3	5 0/4	SE[b]
Intake (gm/day)		16.2	14.3	16.6	19.8	18.1	1.8
Feces Nitrogen (%)[c]		25.4	25.4	27.2	25.0	26.4	2.8
Urine Nitrogen (%)[c]		53.8	58.6	52.4	45.7	50.7	3.0
Retention (%)[c]		20.6[d]	14.2[e]	23.8[d]	29.3[d]	22.7[d]	3.6

[a]Urea/Soy protein ratio.

[b]Standard error.

[c]Expressed as a percent of nitrogen intake.

[d,e]Horizontal values with different superscripts differ ($P < .05$).

Fecal nitrogen excretion was not affected by time; however, urinary nitrogen excretion was higher ($P < .01$) during the first two 10-day periods on all treatments (Table IV).

McLaren et al. (1960) and Barth et al. (1961) indicated that nitrogen retention by sheep fed diets containing large amounts of urea increased with time on feed. As a result the present concept of "adaptation" to urea developed. In this experiment, fecal nitrogen did not

change with time on feed but urinary nitrogen excretion decreased dramatically after the first 10-day period. These data indicate that the "adaptation" took place in the body rather than in the contents of the digestive system and was independent of nitrogen source since improved retention occurred with both urea and soy protein.

TABLE IV

EFFECT OF TIME UPON PERCENTAGE OF NITROGEN RETAINED

Time	Feces Nitrogen	Urine Nitrogen	Retention
0-10 days	28.4	82.8[a]	-11.2[c]
10-20 days	33.7	49.7[b]	16.6[d]
20-30 days	26.7	44.9[b]	28.4[e]
30-40 days	21.5	48.1[b]	30.4[e]
40-50 days	25.8	44.9[b]	29.3[e]
50-60 days	20.0	43.2[b]	36.8[e]
Mean	26.0	52.3	21.7
Standard Error	2.8	3.0	3.6

[a-e]Vertical values with different superscripts differ ($P < .01$).

Ruminal fluid pH and volatile fatty acid (VFA) concentrations, with the exception of valeric acid, were not affected by treatments (Table V). Valeric acid was in greater concentrations ($P < .025$) in lambs fed diets 3 and 5 than in those fed diet 1.

When each VFA was expressed as a percentage of the total (Table VI), there was a tendency, though not significant, for reduced acetic and increased propionic and branched-chain acids concentration as urea was

replaced by soy protein. Lambs receiving diets 3 and 5 still had higher levels of valeric acid than those on diet 1; however, the level of significance was not changed over that found when only absolute concentrations (Table V) were compared.

TABLE V

EFFECTS OF UREA, SOY PROTEIN AND VARIOUS COMBINATIONS OF UREA-SOY ON RUMINAL FLUID PH AND FATTY ACID CONCENTRATIONS

	Diets					
Item	U/S[a]	1 4/0	2 3/1	3 2/2	4 1/3	5 0/4	SE[b]
pH		6.41	6.06	5.98	5.81	6.08	0.20
Acetic[c]		43.92	36.51	55.32	48.90	52.68	5.71
Propionic[c]		18.89	25.66	33.20	29.93	29.53	4.60
n-Butyric[c]		6.54	4.08	12.74	3.63	7.83	2.32
iso-Butyric[c]		0.61	1.03	1.07	1.38	1.54	0.28
iso-Valeric + $2CH_3$Butyric[c]		0.71	1.57	1.78	1.61	1.58	0.48
Valeric[c]		2.20[d]	3.24[d]	6.30[e]	3.23[d]	4.56[e]	0.62

[a]Urea/Soy protein ratio.

[b]Standard error.

[c]Expressed as micromoles/ml of rumen fluid.

[d,e]Values with different superscripts differ ($P < .025$).

The slight reduction in ruminal fluid acetic and elevation of propionic acid concentrations when soy protein replaced urea agrees with results by Oltjen and Putnam (1966). However, our concentrations of butyric acid did not follow any consistent trend and thus are not in

TABLE VI

EFFECT OF UREA, SOY PROTEIN, AND VARIOUS UREA-SOY COMBINATIONS ON RUMINAL FLUID FATTY ACID CONCENTRATIONS

Item	U/S[a]	Diets 1 4/0	2 3/1	3 2/2	4 1/3	5 0/4	SE[b]
Acetic[c]		60.17	50.75	50.34	55.25	53.73	6.64
Propionic[c]		25.88	35.67	30.21	33.82	30.12	5.35
n-Butyric[c]		8.96	5.67	11.59	4.10	7.99	2.70
iso-Butyric[c]		0.84	1.43	0.97	1.56	1.57	0.33
iso-Valeric + $2CH_3$ Butyric[c]		0.97	2.18	1.62	1.82	1.61	0.56
Valeric[c]		3.01[d]	4.50[e]	5.73[e]	3.65[d]	4.65[e]	0.72

[a]Urea/Soy protein ratio.

[b]Standard error.

[c]Expressed as a percent of total fatty acids found in the rumens.

[d,e]Horizontal values with different superscripts differ ($P < .05$).

accord with their results. The greater ruminal concentrations of the higher straight- and branched-chain fatty acids found during this experiment, in lambs consuming soy protein diets rather than diets with urea as the sole source of nitrogen agrees with the data reported by Oltjen and Putnam (1966). The fact that branched-chain fatty acids were detected in measurable amounts in rumen fluid from lambs fed a urea-containing purified diet is not in accord with the data reported by Cline et al. (1966) and may explain why no conclusive improvement in digestibility of dietary components was obtained when these branched-chain

fatty acids were added by these investigators. Since Hemsley and Moir (1966) obtained no response from addition of branched-chain acids above that obtained by the addition of molasses or sucrose, these data indicate that ruminal synthesis of branched-chain fatty acids was not the limiting factor when soluble carbohydrates were present in their diets.

Table VII shows the effect of urea, soy protein, and their combinations upon nitrogen fractions in rumen fluid of sheep. Dietary treatment affected neither ammonia-nitrogen nor urea-nitrogen levels. However, protein nitrogen levels (TCA-N) were higher ($P < .01$) in all diets containing soy protein than that found in the diet in which urea was the only nitrogen source; differences between diets containing some or only soy protein were not significant. The effect of TCA-N levels was carried over into the total nitrogen fraction and all diets containing some soy protein had higher ($P < .025$) levels than diet 1, which contained urea as the sole source of dietary nitrogen.

Virtanen (1966) found that dairy cows fed purified diets containing urea as the nitrogen source had a greater proportion of the total nitrogen in rumen fluid as protein nitrogen than those fed a natural diet containing no urea, thus the data of this experiment are not in accord with that observation. In fact, the opposite was found. Possible reasons for this apparent discrepancy concerns the effects of a natural versus a purified diet rather than increased protein synthesis from urea as Virtanen suggested.

Plasma concentrations of aspartic and glutamic acids were greater ($P < .05$) for lambs fed urea rather than soy protein as the sole source of nitrogen (Table VIII). Concentrations of all other amino acids, ammonia and urea were not affected by the dietary nitrogen source ($P > .05$).

TABLE VII

EFFECT OF UREA, SOY PROTEIN, AND VARIOUS UREA-SOY COMBINATIONS ON NITROGEN FRACTIONS IN RUMEN FLUID

Item	Diets					
	U/S[a]	1 4/0	2 3/1	3 2/2	4 1/3	5 0/4	SE[b]
NH_3-N (mg/100 ml)	22.10	35.70	38.80	29.30	26.40	5.80
Urea-N (mg/100 ml)	4.80	4.70	6.62	4.47	3.92	0.70
TCA-N (gm/100 ml)	0.20[c]	0.35[d]	0.38[d]	0.33[d]	0.36[d]	0.03
Total N (gm/100 ml)	0.29[e]	0.38[f]	0.45[f]	0.44[f]	0.42[f]	0.03

[a]Urea/Soy protein ratio.

[b]Standard error.

[c,d]Horizontal values with different superscripts differ ($P < .01$).

[e,f]Horizontal values with different superscripts differ ($P < .025$).

Oltjen and Putnam (1966) indicated that plasma concentrations of leucine, isoleucine, valine and phenylalanine were lower on sheep fed purified diets containing urea rather than soy protein as the sole source of nitrogen. However, our concentrations of these amino acids were not in accord with this observation. In fact, the opposite was found. Possible reasons for this apparent discrepancy concerns different feeding regimes which preceded the taking of blood samples; their samples were taken two hours after feeding while samples in the present experiment were taken at no set time after feeding because the lambs had free access to feed at all times. Leibholtz (1965) found that plasma free amino acid concentrations varied with the length of time after feed-

TABLE VIII

EFFECT OF UREA VS SOY ON FREE PLASMA AMINO ACID CONCENTRATIONS[a,b]

Item	Diets		
	U/S[c]	1 4/0	5 0/4	SE[d]
Arginine		.125	.108	.033
Lysine		.188	.158	.041
Histidine		.048	.053	.045
Threonine		.133	.110	.016
Phenylalanine		.075	.055	.028
Methionine		.015	.013	.001
Leucine		.178	.098	.058
Isoleucine		.115	.075	.033
Valine		.365	.210	.099
Alanine		.238	.173	.032
Glutamic Acid		.245[e]	.130[f]	.028
Proline		.132	.088	.017
Tyrosine		.085	.058	.015
Ornithine		.160	.115	.046
Citrulline		.198	.138	.035
Glycine		.903	.788	.486
Aspartic Acid		.048[g]	.023[h]	.002
Serine		.230	.150	.029
NH_3		1.278	1.213	.124
Urea		3.800	2.668	1.243

[a]Micromoles/ml.

[b]Each value is an average of four sheep.

[c]Urea/Soy protein ratio.

[d]Standard error.

[e-h]Horizontal values with different superscripts differ ($P < .01$).

ing when the plasma samples were collected. These results indicate that sufficient data are not available to conclude that low concentrations of plasma free amino acids would account for the poorer growth obtained when sheep were fed purified diets containing urea rather than soy protein as the sole source of dietary nitrogen.

Summary

Urea (U) and soy protein (S) were incorporated in purified diets in the following proportions of total nitrogen: Diet 1, U; 2, 3/4 U + 1/4 S; 3, 1/2 U + 1/2 S; 4, 1/4 U + 3/4 S; 5, S. These isonitrogenous diets were fed to 20 lambs over a 60-day growth and balance trial. Feces and urine were collected for the 60 days and composited into successive 10-day intervals. Growth performance of lambs fed diets containing urea as the sole source of nitrogen were approximately 70 percent as great as those when soy protein was used; nitrogen retention and carcass data were in accord with this value. Urinary nitrogen output decreased and fecal nitrogen output was constant with time on feed for both dietary nitrogen sources.

Plasma glutamic and aspartic acid concentrations were greater in lambs fed diets containing urea rather than soy protein as the sole nitrogen source. No significant differences were observed in plasma urea or ammonia nitrogen concentrations when urea or soy protein served as the sole source of nitrogen in purified diets.

CHAPTER IV

AMINO ACID SUPPLEMENTATION OF UREA RICH DIETS FOR LAMBS

Introduction

It is generally accepted that complete substitution of soybean protein with urea results in about a 25 percent reduction in growth (Oltjen et al., 1962; Oltjen and Putnam, 1966) and milk production (Virtanen, 1966). In the bovine lower plasma concentrations of leucine, valine and phenylalanine (Oltjen and Putnam, 1966); histidine (Virtanen, 1966); leucine, isoleucine, valine, phenylalanine, lysine, alanine, tyrosine and glutamic acid (Freitag et al., 1966); isoleucine, valine, proline and methionine (Little et al., 1966) were observed when urea rather than soy protein was the source of dietary nitrogen. If plasma levels of leucine, isoleucine, valine and phenylalanine are limiting growth on urea diets, then dietary addition of these amino acids should improve performance when urea is fed. The following describes a test of this hypothesis.

Experimental Procedure

Six pairs of twin lambs, average weight 18 kg., were treated with a thibenzole preparation and allotted at random within twin pairs to two treatment groups for a 41-day growth trial. One group was fed a purified diet (Table IX) while the other group was fed the control diet

TABLE IX

PERCENTAGE COMPOSITION OF THE DIETS

Ingredients	Diets	
	Control	Control + Amino Acids
Corn starch	34.20	33.60
Glucose[a]	24.37	24.37
Cellulose[b]	30.00	30.00
Corn oil[c]	1.00	1.00
Antioxidant[d]	0.01	0.01
Polyethylene resin[e]	1.00	1.00
Choline chloride	0.10	0.10
Vitamins A and D[f]	0.02	0.02
Minerals[g]	5.00	5.00
Trace minerals[h]	0.10	0.10
Urea[i]	4.20	4.02
Leucine[j]	----	0.28
Isoleucine[j]	----	0.16
Valine[j]	----	0.16
Phenylalanine[j]	----	0.18

[a]Cerelose. Corn Products Co., Argo, Illinois.

[b]Solka-Floc. B-W20. Brown Co., Berlin, New Hampshire.

[c]Mazola.

[d]Santoquin. 1,2 dihydro-6-ethoxy, 2,2,4, trimethyl quinoline.

[e]Alathon. E. I. du Pont de Nemours, Inc., Wilmington, Delaware.

[f]Containing 20,000 I.U. and 2,500 Units/gm. of vitamins A and D, respectively. Courtesy NOPCO Chemical Co., Harrison, New Jersey.

[g]See Table I.

[h]See footnote g, Table I.

[i]Crystalline urea. Courtesy Nipak Chemical Co., Pryor, Oklahoma.

[j]D-L mixtures.

supplemented with leucine, isoleucine, valine, and phenylalanine at levels of 0.28, 0.16, 0.16, and 0.18 percent of the complete diet, respectively. The level of urea in the supplemented diet was reduced from 4.20 to 4.02 percent to make both diets isonitrogenous. Feed and water were provided ad libitum during the trial and all lambs were individually fed. A sixteen hour fast from feed and water preceded initial and final weighings. All unconsumed feed was removed and weighed at the end of the trial. Jugular blood and rumen fluid samples were collected 2 hours after feeding on the last day of the trial. Chemical analyses were performed by the following procedures: blood hemoglobin (Cannan, 1958); ammonia-nitrogen (Conway, 1957); urea-nitrogen (Hycel, 1960); steam volatile fatty acids (Erwin et al., 1961); and total-nitrogen by standard Kjeldahl procedures (A.O.A.C., 1960). The data were analyzed statistically by analyses of variance and treatment means compared using Duncan's new multiple-range test (Steel and Torrie, 1960).

Results and Discussion

Gain and feed intake are shown in Table X and no significant differences between treatments were apparent. Although differences between treatments were not significant, the control group gained 87 percent as much as the amino acid supplemented group. Oltjen and Putnam (1966) showed that unsupplemented urea fed lambs gained 75 percent as much as lambs fed soy protein in purified diets. Thus, amino acid supplementation does appear to improve growth performance on urea-rich diets. However, the improved growth was not as great as that obtained when soy protein was fed. Hematocrit and hemoglobin levels of whole blood were not affected by dietary treatment (Table XI). This is in contrast with

the data reported by Virtanen (1966) who found reduced hemoglobin values when urea was fed in a purified diet. This apparent discrepancy may be due to the fact that Virtanen's data showed a comparison between urea in a purified diet with preformed protein in a natural diet.

TABLE X

EFFECTS OF AMINO ACID SUPPLEMENTATION OF UREA-CONTAINING PURIFIED DIETS ON THE GROWTH PERFORMANCE OF LAMBS

	Diets		
	Control	Control + Amino Acids	SE[a]
Feed intake (gm/day)	840	889	55
Average daily gain (gm)	90.8	104.4	9.1

[a]Standard error.

Plasma concentrations of ammonia- and total-nitrogen were not significantly ($P > .05$) affected by amino acid supplementation (Table XI). This is in agreement with Oltjen and Putnam (1966) who found no differences in plasma ammonia-nitrogen levels when urea and soy protein purified diets were compared. Amino acid supplementation promoted higher ($P < .05$) plasma urea-nitrogen concentrations when compared with the control group. This is not in agreement with Oltjen and Putnam (1966), but agrees with the data in Chapter V where plasma urea-nitrogen levels were higher in lambs fed soy protein rather than urea. The significance of the higher plasma urea-nitrogen levels is not readily apparent.

Acetic, propionic, and butyric acid concentrations in blood plasma were not affected ($P > .05$) by dietary treatment (Table XII). Amino acid

addition tended to increase plasma n-butyric acid level slightly. Concentrations of plasma branched-chain fatty acids were found in extremely low and variable concentrations.

TABLE XI

EFFECTS OF AMINO ACID SUPPLEMENTATION OF UREA-CONTAINING PURIFIED DIETS ON NITROGEN FRACTIONS IN BLOOD

	Diets		SE[a]
	Control	Control + Amino Acids	
Hematocrit (%)	35.20	32.70	3.86
Hemoglobin (mg/100 ml)	33.70	32.20	2.06
Ammonia-nitrogen (mg/100 ml)	0.15	0.14	0.01
Urea-nitrogen (mg/100 ml)	49.70[b]	53.80[c]	1.69
Total nitrogen (gm/100 ml)	8.74	8.88	0.16

[a]Standard error.

[b,c]Horizontal values with different superscripts differ ($P < .05$).

Ruminal pH, urease activity and ammonia-nitrogen levels were similar for both groups (Table XIII). Since ruminal pH on the control diet was closer to the pH range for optimum urease activity than that observed on the supplemented diet, a greater urease activity should be expected on the control diet. Due to the greater rumen ureolytic activity on the control group, the higher levels of ammonia-nitrogen and lower ($P < .05$) levels of urea-nitrogen on the control diet would be expected. Ruminal urea-nitrogen however was higher ($P < .05$) on the control than

TABLE XII

EFFECTS OF AMINO ACID SUPPLEMENTATION OF UREA-CONTAINING PURIFIED DIETS ON PLASMA VOLATILE FATTY ACID CONCENTRATIONS

	Diets		
	Control	Control + Amino Acids	SE[a]
Acetic[b]	2.41	2.45	0.15
Propionic[b]	0.88	0.75	0.06
n-Butyric[b]	0.09	0.18	0.06

[a]Standard error.

[b]Expressed as micromoles/ml of blood plasma.

the supplemented diet (Table XIII), a result, contrary to what was expected from the urease activity and ammonia-nitrogen levels on these diets. In keeping with the tendency of the amino acids to reduce ruminal ammonia-nitrogen levels it was of interest to note that the branched-chain fatty acids derived from these amino acids also lowered ruminal ammonia-nitrogen (Cline et al., 1966). This could be interpreted to mean a faster rate of ammonia assimilation by microorganisms in the presence of amino acids or their branched-chain carbon residues. The greater urea-nitrogen levels on the control diet were attributed, at least in part, to the greater level of urea in the control compared with the supplemented diet. No explanation is readily apparent for the greater ($P < .05$) total-nitrogen levels in rumen fluid from lambs fed the supplemented rather than the control diet.

Table XIV shows ruminal straight- and branched-chain volatile fatty

TABLE XIII

EFFECTS OF AMINO ACID SUPPLEMENTATION OF UREA-CONTAINING PURIFIED DIETS ON NITROGEN COMPONENTS AND PH OF RUMEN FLUID

	Diets		SE[a]
	Control	Control + Amino Acids	
pH	5.80	5.53	0.13
Urease[b]	1.88	1.79	0.06
Ammonia-nitrogen (mg/100 ml)	1.79	0.91	0.29
Urea-nitrogen (mg/100 ml)	97.00[c]	88.50[d]	2.07
Total nitrogen (gm/100 ml)	0.85[e]	1.66[f]	0.20

[a]Standard error.

[b]International Biochemical Units.

[c-f]Horizontal values with different superscripts differ ($P < .05$).

acid levels which were not affected ($P > .05$) by dietary treatments. Although the differences were not significant, addition of the amino acids tended to increase ruminal levels of all the acids measured. The increased levels of iso-butyric, iso-valeric, and 2 methyl butyric was attributed to the greater levels of valine, leucine, and isoleucine in the diet, respectively, since leucine and valine are fermented to iso-butyrate and iso-valerate, respectively, (Menahan and Schultz, 1964) and isoleucine to 2 methyl butyrate (Hungate, 1966). Leucine (Allison et al., 1966), isoleucine (Hungate, 1966), valine (Allison and Bryant, 1963) and phenylalanine (Allison, 1965) synthesis from isovalerate, 2 methyl butyrate, iso-butyrate, and phenylacetate, respectively, indicated a require-

TABLE XIV

EFFECTS OF AMINO ACID SUPPLEMENTATION OF UREA-CONTAINING PURIFIED DIETS ON RUMINAL VOLATILE FATTY ACID CONCENTRATIONS

Acid	Diets		SE[a]
	Control	Control + Amino Acids	
Acetic[b]	13.95	21.75	3.12
Propionic[b]	8.32	14.18	2.94
n-Butyric[b]	4.09	7.12	1.15
iso-Butyric[b]	0.09	0.19	0.11
iso-Valeric + $2CH_3$ Butyric[b]	0.07	0.17	0.08
Valeric[b]	1.05	2.24	0.63

[a]Standard error.

[b]Expressed as micromoles/ml of rumen fluid.

ment of certain specific branched-chain fatty acid precursors for the synthesis of these amino acids. The results of the present trial support the suggestion of Oltjen and Putnam (1966) that the main function of amino acids in the rumen was to provide carbon skeletons for microbial protein synthesis since addition of amino acids tended to increase ruminal branched-chain fatty acid levels. Matrone et al. (1965) also observed lower ruminal levels of iso-valeric acid when sheep were fed urea rather than casein. Indirect evidence of a deficiency of branched-chain fatty acids was provided by Bentley et al. (1955); Bryant and Doetsch (1954, 1955) and Allison et al. (1958) who stimulated cellulolytic activity in vitro by additions of valeric, caproic, iso-

butyric and iso-valeric acids.

In contrast with the above possibilities of branched-chain fatty acid deficiencies addition of leucine, valine and phenylalanine, which gave rise to slightly increased ruminal concentrations of branched-chain fatty acids in the present experiment did not markedly improve the growth performance of the lambs. Also Hemsley and Moir (1963) observed that addition of a mixture of iso-butyric, iso-valeric and n-valeric acids to a high fiber diet failed to promote greater gains in sheep than did addition of molasses to the same diet. In addition, Cline et al. (1966) reported the presence of iso-butyric and iso-valeric acids in minimal concentrations in rumen fluid of lambs fed urea containing purified diets devoid of protein. However, despite these minimal rumen levels of branched-chain fatty acids and that the major function of ruminal amino acids was to provide carbon skeletons, these same workers observed improved nitrogen retention and digestibility when the diet contained 39 percent cellulose. When the cellulose level of the same diet was raised to 59 percent no response was obtained by supplementation with the same acids. These data suggest that the superior growth performance of lambs fed intact protein rather than urea may be more complex than amino acid or branched-chain fatty acid deficiencies per se.

Summary

Six pairs of twin lambs (average weight 18 kg.) were allotted at random within pairs into two treatment groups for a 41-day growth trial. The control group was fed a purified diet while the other group was fed the control diet supplemented with leucine, isoleucine, valine, and phenylalanine at levels of 0.28, 0.16, 0.16, and 0.18 percent, respec-

tively. The level of urea in the supplemented diet was reduced from 4.20 to 4.02 percent to make both diets isonitrogenous. All animals had free access to feed and water at all times and were kept without stanchioning in 90 x 130 cm. slatted floor growth and metabolism stalls, which were equipped for the separation and quantitative collection of feces and urine. Blood and rumen fluid were collected 2 hours after feeding on the last day of the trial. Amino acids did not effect ($P > .05$) the following: (1) daily gain or feed intake; (2) blood hematocrit or hemoglobin levels; (3) plasma concentrations of (a) total- or ammonia-nitrogen; (b) acetic, propionic, or n-butyric acids; (4) ruminal (a) pH or urease activity (b) ammonia-nitrogen concentrations (c) acetic, propionic, n-butyric, iso-butyric, iso-valeric + 2 methyl butyric or valeric acid concentrations ($P > .05$). These results indicate that ruminal synthesis of branched-chain fatty acids was not the major growth-limiting factor in lambs fed a purified diet, in which urea is the sole nitrogen source.

CHAPTER V

STUDIES ON RUMINAL UREASE ACTIVITY

Introduction

When urea is rapidly consumed or placed directly into the paunch of ruminants, it is rapidly hydrolyzed to ammonia and under certain conditions is toxic (Gallup et al., 1953; Davis et al., 1959; Lewis et al., 1960; Oltjen et al., 1963). Cattle or sheep which (1) have never consumed urea, or (2) are in a semi-starved condition, or (3) consume their feed rapidly are most susceptible to urea toxicity (Whitehair et al., 1955). Since urease is a constitutive enzyme (Jensen and Schroder, 1966) and no quantitative values for ruminal urease activity are available on animals subjected to different feeding conditions, it appeared desirable to test the effects of various feeding regimes on urease activity, pH, and volatile fatty acid levels within the rumen of the bovine.

Experimental Procedure

Trials 1, 2, and 3 were switch-back experiments. Four steers, equipped with permanent rumen fistulae, were divided into two treatment groups in each trial. In pre-trial tests urease activity was determined on strained rumen fluid before centrifugation, and on the supernatant after centrifugation at 5,400 and 25,700 times gravity. Since centrifugation had no effect on the ureolytic activity, all urease

assays were determined on the supernatant obtained by centrifuging strained rumen fluid at 5,400 times gravity. The alkalimetric method of Gorin and Chin (1966) was used to determine urease activity and the units are in accord with the recommendations of the International Union of Biochemists (IUB), where one unit is defined as the amount of urease which catalyzes the hydrolysis of 0.5 micromoles of urea and liberates 1 micromole of ammonia. Rumen volumes were determined by the method of Smith (1958) and total ruminal urease activity was calculated and is shown in Table XVI. Standard procedures were used for pH measurements, and VFA determinations were by the procedure described in Chapter III. The data were analyzed statistically by analyses of variance and treatment means compared using Duncan's new multiple-range test (Steel and Torrie, 1960).

In trial I, four steers which had been fed a high grain diet were confined to small pens on slatted floors and individually-fed a mixed ration (Table XV) for 7 days. At this time two animals were continued on feed, while the other two were fasted. Rumen samples were then taken at 0, 24, 48, 72 and 96 hours from both the fed and fasted animals for urease and steam volatile fatty acids. When the last samples were taken, the fasted animals were fed the mixed ration for 7 days while the fed group continued as before. Animals were then switched to opposite treatments and rumen samples taken as before.

Trial 2 was designed to study the effect of cottonseed hulls and the mixed ration upon ruminal urease activity. The fasted steers, from the second phase of trial 1, were fed cottonseed hulls for 7 days while the other two were continued on the grain diet. At the end of this period, rumen samples were taken as before for 96 hours. The animals were then switched, fed the other diets for 7 days and samples taken as

before.

In trial 3, good quality alfalfa hay was compared to poor quality prairie hay. The animals were handled in the same manner as outlined in trial 2.

TABLE XV

PERCENTAGE COMPOSITION OF THE DIETS

Ingredient	Diets			
	1 Mixed Ration[a]	2 Cottonseed hulls	3 Alfalfa hay	4 Prairie hay
Ground milo	63.96	----	----	----
Alfalfa meal	10.00	----	----	----
Cottonseed meal	10.00	----	----	----
Urea[b]	1.00	----	----	----
Salt	0.50	----	----	----
Dicalcium phosphate	0.50	----	----	----
Limestone	0.50	----	----	----
Cottonseed hulls	13.50	100.00	----	----
Vitamins A and D[c]	0.05	----	----	----
Alfalfa hay	----	----	100.00	----
Prairie hay	----	----	----	100.00

[a]Pelleted into 3/8 inch cubes.

[b]Crystalline urea. Courtesy Nipak Chem. Co., Pryor, Oklahoma.

[c]Containing 20,000 I.U. and 2,500 USP units/gm. of vitamins A and D, respectively. Courtesy NOPCO Chem. Co., Harrison, New Jersey.

Six fistulated steers were used in trial 4. Two steers were fed cottonseed hulls for 7 days while the remaining four were fed the mixed ration. At the end of this period, two steers, which had received the mixed ration, were fasted for 96 hours while the other two groups continued on their assigned diets. During the 96 hours rumen samples were taken as in the previous trials for pH and urease determinations.

Results and Discussion

Fasted animals had a lower ($P < .01$) ruminal ureolytic activity than the fed group (Table XVI). Ureolytic activity decreased with time on treatment in both the fasted and the group fed the mixed ration.

TABLE XVI

EFFECT OF FASTING VS FEEDING OF MIXED DIET ON TOTAL RUMEN UREASE ACTIVITY

Time Hours	Treatment	
	Fasted	Fed
0	55.8[a]	111.7[a]
24	22.8	134.4
48	21.1	69.0
72	18.1	63.5
96	11.5	31.0
Mean	25.9[b]	81.9
SE[c]	4.9	4.9

[a]IUB x 10^6 units of total ruminal activity.

[b]Fasted animal lower ($P < .01$) than fed.

[c]Standard error.

Meiske et al. (1958) observed a continuous increase in the pH value of rumen fluid of steers during starvation period to a high of 7.65 and 7.94. Since these pH values are outside the pH range for urease (Sumner and Somers, 1953) the decrease in ureolytic activity of the fasted group should have been expected. However, the decrease in ureolytic activity among the fed group is more difficult to explain since rumen pH would not be expected to change markedly. As the fasting progressed, rumen samples from the fasted group contained considerably less food material and dry matter. Thus, the lowering of rumen dry matter contents perhaps was involved in decreasing the ureolytic activity of rumen fluid from the fasted steers since bacterial counts also probably decreased (Hogan, 1961). Halliwell (1957) and Meiske et al. (1958) observed decreased cellulolytic activity and dry matter content of rumen fluid upon starvation.

The volatile fatty acids were determined 24 and 48 hours after initiation of the experiments. Since time of sampling had no effect on fatty acid levels the values were combined and are shown in Tables XVII, XIX , and XXI. Feeding promoted greater ($P < .01$) concentrations of total fatty acids than did fasting. When expressed as molar percentages, feeding promoted higher ($P < .01$) levels of propionic and butyric but lower ($P < .01$) levels of iso-butyric and iso-valeric acids than did fasting. This change in proportions of fatty acids may represent changes in ruminal flora on starvation as was reported by Kistner (1964).

Table XVIII shows rumen ureolytic activity of steers fed the mixed and cottonseed hulls diets. Treatment differences were not significant ($P > .05$). However, with the exception of samples taken just before feeding (0 hour) the cottonseed hulls fed group tended to have greater

ureolytic activity. Annison and Lewis (1959) reported decreased ruminal pH levels when rations rich in starch were fed to ruminants in comparison to the pH values obtained when diets devoid of or low in starch were fed. Thus, the mixed diet would promote rumen pH values which would deviate to a greater degree from a value of 7.0 reported by Sumner and Somers (1953) to be optimum for urease. However, other factors could be involved here such as mineral and energy levels of the mixed versus the cottonseed hulls diet.

TABLE XVII

EFFECT OF FASTING VS FEEDING ON RUMINAL VOLATILE FATTY ACID CONCENTRATION

Acids	Treatments		SE[a]
	Fasted	Fed	
Total concentration[b]	34.26[d]	75.61[e]	6.38
Acetic[c]	63.12	53.62	6.51
Propionic	18.97[f]	32.48[g]	4.19
n-Butyric	5.98[h]	9.03[i]	0.91
iso-Butyric	2.39[j]	0.04[k]	0.22
Valeric	1.93	3.83	1.09
iso-Valeric	6.71[l]	0.73[m]	0.76

[a]Standard error.

[b]Micromoles/ml.

[c]Molar %.

[d-m]Horizontal values with different superscripts differ ($P < .01$).

TABLE XVIII

EFFECT OF MIXED VS COTTONSEED HULLS DIETS ON RUMINAL UREASE ACTIVITY

Time Hours	Diets	
	Mixed Ration	Cottonseed hulls
0	46.2[a]	42.9[a]
24	47.5	58.8
48	46.5	82.2
72	45.4	71.8
96	46.3	49.6
Mean	46.4	61.1
SE[b]	3.5	3.5

[a] IUB x 10^6 units of total ruminal activity.

[b] Standard error.

The mixed diet promoted greater ($P < .01$) concentrations of total fatty acids than did the cottonseed hulls diet (Table XIX). Thus, a higher ruminal pH and ureolytic activity would be expected when the cottonseed hulls rather than the mixed diet was fed. The cottonseed hulls diet, however, favored greater ($P < .01$) molar percentages of acetic but lower ($P < .01$) percentages of propionic, butyric, and valeric acids than did the mixed diet (Table XIX). These effects of fatty acid levels were as should be expected from the data reported by Phillipson (1952) and Annison and Lewis (1959).

Differences in ruminal urease activity between animals fed alfalfa and prairie hay (Table XX) were not significant ($P > .10$). However,

there was a tendency for greater activity on the prairie hay rather than the alfalfa hay diet. Time on treatment did not affect ureolytic activity when either source of hay was fed.

TABLE XIX

EFFECT OF MIXED VS COTTONSEED HULLS DIETS ON RUMEN VOLATILE FATTY ACIDS

Acids	Diets		SE[a]
	Mixed Ration	Cottonseed hulls	
Total concentration[b]	67.92[d]	54.81[e]	3.82
Acetic[c]	36.58[f]	78.85[g]	3.26
Propionic[c]	32.68[h]	14.85[i]	1.50
n-Butyric[c]	24.38[j]	4.10[k]	4.95
iso-Butyric[c]	0.03	0.51	0.10
Valeric[c]	5.56[l]	0.88[m]	1.50
iso-Valeric[c]	0.66	1.23	0.34

[a]Standard error.

[b]Micromoles/ml.

[c]Molar %.

[d-m]Horizontal values with different superscripts differ ($P < .01$).

Ruminal levels of total volatile fatty acids (Table XXI) were higher ($P < .01$) when alfalfa rather than prairie hay was fed and there were no further differences. After ruminal urease activities and volatile fatty acid levels were determined in trials 1, 2, and 3, it was thought that the higher pH values associated with fasting might account for the re-

duction in ureolytic activity, and since no direct measurements of ruminal pH were made in the first 3 trials, trial 4 was conducted.

TABLE XX

EFFECT OF ALFALFA VS PRAIRIE HAY DIETS ON RUMINAL UREASE ACTIVITY

Time Hours	Diets: Alfalfa hay	Diets: Prairie hay
0	46.8[a]	118.8[a]
24	53.0	107.1
48	60.0	69.5
72	63.5	47.1
96	64.0	43.8
Mean	57.5	75.9
SE[b]	4.4	4.4

[a]IUB x 10^6 units of total ruminal activity.

[b]Standard error.

The results of trial 4 are shown in Table XXII and fasting promoted higher ($P < .01$) pH values than when either cottonseed hulls or the mixed diets were fed. As expected, the pH of rumen fluid from steers fed the mixed diet was lower ($P < .01$) than those consuming the cottonseed hulls diet. Cottonseed hulls also promoted higher ($P < .01$) pH values than the mixed diet. These results should have been expected from the first 3 trials and from the data reported by Phillipson (1952), Annison and Lewis (1959), Meiske et al. (1958). The ruminal fluid pH values

TABLE XXI

EFFECT OF ALFALFA VS PRAIRIE HAY ON RUMEN VOLATILE FATTY ACID CONCENTRATION

Acids	Diets		SE[a]
	Alfalfa hay	Prairie hay	
Total concentration[b]	72.15[d]	52.28[c]	3.04
Acetic[e]	70.66	77.72	4.21
Propionic[e]	16.22	13.39	1.29
n-Butyric[e]	8.63	7.04	1.14
iso-Butyric[e]	1.50	0.57	0.22
Valeric[e]	1.01	0.48	0.11
iso-Valeric[e]	2.01	0.92	0.34

[a]Standard error.

[b]Micromoles/ml.

[c,d]Horizontal values with different superscripts differ ($P < .01$).

[e]Molar %.

associated with fasting were higher at all times than the pH of 7.0 (Sumner and Somers, 1953) which promotes optimum urease activity. If one uses this reasoning to explain the lower ureolytic activity in rumen fluid of fasting animals, he finds that the argument may be weakened by the fact that the cottonseed hulls diet promoted a pH, which should have given the highest urease activity of the three treatments. Instead, cottonseed hulls promoted only slightly greater ureolytic activity than did the grain diet. It is possible that decreased dry matter content in the ruminal fluid was responsible for the decreased activity but this

was not studied.

TABLE XXII

EFFECT OF MIXED AND COTTONSEED HULLS DIETS, AND FASTING ON RUMEN PH

Time Hours	Treatments		
	Mixed Ration	Cottonseed hulls	Fasting
0	5.70	6.63	6.03
24	5.84	6.62	7.07
48	5.54	6.42	7.79
72	5.45	6.44	7.91
96	5.51	6.50	7.96
Mean	5.61[a]	6.52[b]	7.35[c]
SE[d]	0.04	0.04	0.04

[a,b,c]Horizontal values with different superscripts differ ($P < .01$).

[d]Standard error.

Urease activity appears to be relatively independent of feeding regimes and was affected only by fasting. This idea is supported by the results of Jensen and Schroder (1965), who found urease to be constitutive rather than an adaptive enzyme. Since the microbial population concentration probably also decreased, there is a possibility that the decreased urease activity in fasting animals was associated with a lower dry matter in rumen contents.

Summary

Four rumen fistulated steers were used in each of 3 switch-back trials and six rumen fistulated steers in a fourth trial to test the effects of (1) fasting vs feeding a mixed diet, (2) a mixed vs a cottonseed hulls diet, (3) a good quality alfalfa vs a poor quality prairie hay diet on urease activity, volatile fatty acid concentrations, and (4) the effect of fasting and feeding high concentrate and high roughage diets on pH conditions within the rumen. In trial 1, fasting resulted in lower ($P < .01$) ruminal ureolytic activity and volatile fatty acid levels. Ureolytic activity decreased with time on experiment. Fatty acid levels were not affected by time on feed. Differences in ureolytic activity were not significant ($P > .05$) between steers fed the high versus those fed the low concentrate rations (trial 2). Feeding the high concentrate ration promoted greater ($P < .01$) levels of total volatile fatty acids, propionic, butyric, and valeric acids but lower ($P < .01$) levels of acetic acid. No significant differences in ureolytic activity were observed between steers fed good quality alfalfa versus poor quality prairie hay (trial 3). Alfalfa hay promoted higher ($P < .01$) total ruminal concentrations of volatile fatty acids than did prairie hay and had no significant effect on the molar percentages of individual acids. Fasting promoted higher ($P < .01$) ruminal pH values than feeding the high-grain or cottonseed hulls diets. Ruminal pH was lower when the high-grain rather than the cottonseed hulls diet was fed.

CHAPTER VI

EFFECTS OF UREASE INHIBITORS ON SHEEP FED UREA RICH DIETS

Introduction

Urea undergoes rapid ureolytic activity in the rumen (Pearson and Smith, 1943a,b) such that ammonia liberation frequently exceeds protein synthesis resulting in inefficient nitrogen utilization. Reduction of ruminal ureolytic activity to levels commensurate with microbial ability to synthesize protein from ammonia seems to offer promise for increasing the efficiency of urea utilization. Visek et al. (1959), and Harbers et al. (1962) working with non-ruminants reported a reduction of intestinal ureolytic activity with antibiotics and barbituric acid. It is also known that copper (Shaw, 1954) and nitrates (Groll, 1918) will inhibit urease activity. The report described herein was conducted for the purpose of determining the effects of several urease inhibitors on the growth and metabolism of lambs fed urea-rich diets.

Experimental Procedure

Trial 1. A 2 x 2 factorial arrangement of treatments was used to determine the effects of supplemental copper levels, zero versus 15 ppm, and physical form, ground versus pelleted, of an all-concentrate diet containing two percent urea upon the growth of lambs. The composition of the basal diet is shown in Table XXIII. Twenty-four lambs weighing

TABLE XXIII

PERCENTAGE COMPOSITION OF CONTROL DIETS USED IN EACH TRIAL

Trial	1,2,3	4	5,6	
Milo	91.03	78.91	80.98	18.35
Molasses	5.00	3.00	5.00	5.00
Cottonseed hulls	-----	-----	10.00	70.00
Urea[a]	2.00	10.39	1.65	4.12
$CaHPO_4$	0.52	5.00	0.50	1.66
NaCl	0.25	1.50	0.75	0.75
$CaCO_3$	-----	-----	1.00	-----
$MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$	0.11	0.25	-----	-----
$MgSO_4$	0.05	-----	-----	-----
Na_2SO_4	0.10	-----	-----	-----
K_2CO_3	0.90	-----	-----	-----
Trace minerals[b]	-----	0.50	0.10	0.10
Antioxidant[c]	0.02	0.02	0.02	0.02
Vitamins A and D[d]	0.02	0.02	0.02	0.02

[a]Crystalline Urea. Courtesy Nipak Chemical Co., Pryor, Oklahoma.

[b]See footnote g, Table I.

[c]Santoquin. 1,2 dihydro-6-ethoxy, 2,2,4, trimethyl quinoline.

[d]Containing 20,000 I.U. and 2,500 U.S.P. units/gm. vitamins A and D, respectively. Courtesy NOPCO Chemical Co., Harrison, New Jersey.

initially an average of 24 kg. were allotted at random in equal numbers to the four treatments. Feed and water were given free choice to the individually-penned lambs, which were kept on dirt floors, until each attained a weight of approximately 45 kg., at which time each was removed from the experiment. Gain and feed efficiency were response criteria. A 16-hour shrink period, during which time feed and water were not available, preceded initial and final weighings.

Trial 2. As the results of trial 1 indicated that supplemental copper increased performance of sheep fed an all-concentrate diet having a high level of urea, these studies were continued in trial 2. A 3 x 3 factorial arrangement was used to study levels of supplemental copper (zero, 15, and 30 ppm), barbituric acid (0.01, 0.02, and 0.04 percent), and nitrates (0.33, 0.66 and 0.99 percent) upon the utilization of the urea-containing all-concentrate diet (Table XXIII). Nitrate was supplied equally by sodium and potassium nitrate and the level of urea was reduced in the nitrate-containing diets in accord with the nitrogen supplied by nitrate, thus all diets were isonitrogenous. Forty-five lambs averaging 22 kg. initially were allotted at random in equal numbers to the nine treatments and fed their appropriate diets for a 42-day trial. The animals were drenched with a thibenzole preparation prior to the start of the experiment and then placed in individual pens on slatted floors and given water and feed free choice. All other details were as described in the first trial.

Trial 3. In this experiment the control diet and those containing 0.01 percent barbituric acid and 0.66 percent nitrate, of trial 2, were replicated. Thirty lambs weighing 25 kg. initially were allotted at random to the three treatments. All other details were as described in

the second trial.

Trial 4. As the results of the first three trials indicated that copper, barbituric acid and the nitrate ion did not exert any beneficial effect on lamb utilization of an all-concentrate diet containing urea, it was decided to test barbituric acid and nitrate in urea-containing diets, in which there is a shortage of starch or sugars. Thus, a 32 percent crude protein supplement containing 10.39 percent urea (Table XXIII) was fed to sheep at a level of 454 grams per day and the sheep were allowed free access to cottonseed hulls, which contained 3.5 percent crude protein (diet 1). The experimental diets were the same as the control except the protein supplement contained 2.64 percent nitrates or 0.04 percent barbituric acid; these calculations were based on daily intakes of 454 grams of the supplement and an estimated intake of 1,360 grams of cottonseed hulls. Such an intake of both would provide a complete diet containing 0.66 percent nitrates or 0.01 percent barbituric acid. The diets were isonitrogenous. Thirty lambs weighing about 35 kg. initially were allotted at random in equal numbers to the three treatments for a 56-day feeding period. All other details were described in trial 2.

Trial 5. As the results of the previous four growth trials indicated that copper, barbituric acid and nitrates were ineffective in increasing sheep performance on urea-containing diets irregardless of level of starch or sugars, it was decided to repeat some aspects of the previous trials in nitrogen balance studies. Two diets containing different levels of cottonseed hulls were compounded and fed as complete diets, compositions of which are shown in Table XXIII. The trial was a 2 x 4 factorial arrangement of treatments involving two levels of cotton-

seed hulls, each with no supplement or 30 ppm copper or 0.01 percent barbituric acid or 0.66 percent nitrates added. Thirty-two wether sheep weighing an average of 40 kg. initially were randomly divided in equal numbers to the eight treatments and transferred to metabolism stalls, which permitted separation and collection of feces and urine. Each sheep was provided feed ad libitum for 14 days to determine his daily intake. When this was established, he was fed at this level for a 7-day preliminary period and a 10-day collection period. The total collection of feces was frozen daily and ground and mixed while still frozen with aliquots being taken for nitrogen and cellulose analyses. Urine was collected daily, acidified with HCl, and stored until analyzed for nitrogen. Rumen samples were taken from all animals two hours after feeding on the following day after the collection period had ended and analyzed for pH and amylase and urease activities.

Trial 6. Eight steers, equipped with permanent rumen cannulae, were fed the diet containing 70 percent cottonseed hulls (Table XXIII) for 11 days and allotted to four treatment groups: group 1 continued to receive the control diet, while groups 2, 3, and 4, respectively, received 30 ppm of copper, 0.01 percent barbituric acid or 0.66 percent nitrates. All steers were fed a maintenance diet (Garrett et al., 1959). Ruminal samples were then taken 0, 30, 60, 90, and 120 minutes after feeding on 1, 2, and 10 days after the inhibitors were added. Ruminal samples were strained through four layers of cheesecloth and analyzed immediately for pH, urease activity, urea, ammonia, nitrate, and total nitrogen.

Chemical and Statistical Analyses. Urease was determined by the procedure of Gorin and Chin (1966); amylase by the method of Somogyi

(1960); cellulose by the method of Crampton and Maynard (1938); urea-nitrogen by the procedure outlined in Hycel (1960); ammonia-nitrogen by the method of Conway (1957); nitrate-nitrogen by the method of Nicholas and Mason (1957); total nitrogen by the method of the A.O.A.C. (1960); and copper by atomic absorption spectrophotometry, using the procedures outlined by the manufacturer. The amylase unit is defined as the amount of reducing substances in the incubated sample over a non-incubated control equivalent to 1 mg. glucose.

The data were analyzed statistically by analyses of variance and treatment means compared using Duncan's new multiple-range test (Steel and Torrie, 1960).

Results and Discussion

The results of the first three trials in which the sheep were fed an all-concentrate diet containing two percent urea are shown in Table XXIV. The addition of 15 ppm of copper to the basal diet, which already contained 15 ppm, improved gains and feed efficiency in trial 1; there was no interaction between copper level and physical form, thus the results were combined. Results of trial 2 failed to confirm these results. Differences in experimental procedures offer an explanation. The lambs in trial 1 were not given, inadvertently, anti-helminthic treatment prior to the initiation of the trial and were kept on dirt floors, while those used in trial 2 received an anti-helminth and were kept on slatted floors which prevented coprophagy. As copper supplementation has not given increased growth performance in sheep fed a purified diet (Goodrich and Tillman, 1966) which contains a higher level of urea, it is logical to conclude that no beneficial effects result

TABLE XXIV

EFFECT OF UREASE INHIBITION ON SHEEP PERFORMANCE ON UREA-CONTAINING ALL-CONCENTRATE DIETS (TRIALS 1, 2, AND 3)

Item	Copper[a] (ppm)			Barbituric Acid (%)			Nitrates[b] (%)			SE[c]
	0	15	30	0.01	0.02	0.04	0.33	0.66	0.77	
Trial 1										
Daily gain[d] (gm)	176[e]	225[f]								15
Gain/Feed	0.20[g]	0.23[h]								0.003
Trial 2										
Daily gain (gm)	159	132	195	141	204	163	186	222	209	54
Gain/Feed	0.20	0.13	0.28	0.15	0.29	0.19	0.23	0.24	0.23	0.12
Trial 3										
Daily gain (gm)	163			150			160			15
Gain/Feed	0.17			0.16			0.18			0.01

[a]The basal diet in all trials contained 15 ppm of copper.

[b]The nitrate mixture contained equal quantities of KNO_3 and $NaNO_3$.

[c]Standard error.

[d]Main effects as no interaction existed.

[e-h]Horizontal values with different superscripts differ ($P < .01$).

from its use unless helminths are factors. Unfourtunately, the sheep used in the first trial had been discarded before thought was given to measuring helminth infestation. As supplemental copper has given a growth stimulus in swine raised under practical conditions (Carpenter, 1948; Barber et al., 1955, 1957; Bowler et al., 1955, and Beams and Lloyd, 1965), further research on sheep fed copper under applied conditions is needed; however, it must be remembered that copper at too high levels is toxic (Wallace et al., 1960; Underwood, 1962) to sheep.

Neither barbituric acid nor nitrates affected ($P > .05$) sheep performance in either trial 2 or 3. As all-concentrate or high-grain diets contain high levels of starch, it is doubtful (Reid, 1953) if decreased ureolytic activity is actually needed in such rations. It is possible that the non-competitive inhibitors could actually diminish urea utilization by forming insoluble complexes with urease. All-concentrate or high-grain diets promote lower ruminal pH than high-roughage diets (Annison and Lewis, 1959) which is important in urea utilization. Ammonia, released from urea catalysis, has a pKa of 8.8 at 40°C (Bloomfield et al., 1963) and absorption across the rumen wall is low if the pH is acid because the ammonia is converted to the ammonium ion. However, as the ruminal pH becomes increasingly alkaline, the ammonia ion is not converted, and as such can penetrate the lipid layer of the rumen wall in contrast to the relative impermeability of the charged ammonium ion.

In trial 4, nitrates and barbituric acid were tested in a high-roughage diet and these results are shown in Table XXV. Neither of the urease inhibitors affected ($P > .05$) gains or the intake of hulls; however, there were trends toward reduced ($P < .10$) performance with both barbituric acid and nitrates. The results of trial 5, shown in Table

XXVI bear on these results: a level of 0.01 percent barbituric acid or 0.66 percent nitrates had no significant effect ($P > .05$) upon fecal nitrogen excretion in animals fed the high-concentrate diet; however, the trends were toward increased fecal nitrogen loss and decreased digestibility. However, when the high roughage diet was fed, both of these inhibitors increased ($P < .01$) fecal nitrogen loss. Copper did not affect ($P > .05$) fecal nitrogen loss; however, both copper and nitrates increased ($P < .01$) urinary nitrogen losses. Barbituric acid did not affect nitrogen retention when the diet contained 10 percent hulls; however, in the high-roughage diet, nitrogen retention was lower ($P < .01$) than that of the control animals; its main effect being to reduce digestibility of nitrogen. Copper and nitrates reduced nitrogen retention ($P < .01$) which was reflected primarily in the higher urinary nitrogen losses; however, nitrates also caused increased fecal nitrogen output.

TABLE XXV

EFFECT OF BARBITURIC ACID OR NITRATES ON THE PERFORMANCE OF SHEEP FED HIGH ROUGHAGE DIETS (TRIAL 4)

	Control	Nitrates	Barbituric Acid	SE[a]
Daily gain (gm)	-1.59	-13.80	-22.70	8.04
Daily intake of cottonseed hulls (gm)	814	865	788	56

[a]Standard error.

TABLE XXVI

EFFECTS OF DIETARY COPPER, BARBITURIC ACID, AND NITRATES ON RUMINAL PH, UREASE, AMYLASE ACTIVITIES, NITROGEN BALANCE AND CELLULOSE DIGESTIBILITY (TRIAL 5)

Inhibitor	10% Cottonseed Hulls				70% Cottonseed Hulls				SE[a]
	None	Copper	Barbituric Acid	Nitrates	None	Copper	Barbituric Acid	Nitrates	
pH	5.8[e]	6.0[e]	6.2[e]	5.7[e]	6.4[f]	6.4[f]	6.2[f]	6.4[f]	0.2
Urease[b]	1.1[g]	1.3[g]	1.2[g]	1.0[g]	1.4[h]	1.3[h]	1.4[h]	1.3[h]	0.1
Amylase[c]	0.9[i]	0.9[i]	0.9[i]	0.9[i]	0.8[j]	0.7[j]	1.0	0.7[j]	0.1
Cellulose Digestibility[d]	83.7	85.2	84.1	83.0	55.0	69.6	42.7	55.4	3.8
Digestibility[d]	73.4[k]	73.9[k]	71.2[k]	69.8[k]	70.3[k]	74.7[k]	62.5[l]	59.6[l]	2.7
Urine Nitrogen[d]	36.6[m]	48.0[n]	37.2[m]	45.7[n]	38.1[m]	58.2[o]	44.0[n]	62.3[p]	4.1
Nitrogen Retention[d]	39.3[q]	25.9[r]	39.0[q]	24.2[r]	32.2[q]	16.4[r]	18.6[r]	-2.8[s]	5.5

[a]Standard error.

[b]IUB units/ml. of rumen fluid.

[c]Units/ml. See text for definition of 1 unit.

[d]Percent of intake.

[e-f]Horizontal values with different superscripts differ ($P < .01$).

[g-h]Horizontal values with different superscripts differ ($P < .05$).

[i-s]Horizontal values with different superscripts differ ($P < .01$).

In evaluating these results, it must be remembered that nitrates replaced urea in both rations in order to keep all diets isonitrogenous, thus fecal nitrogen could represent differences in the digestibility of nitrates and urea. If barbituric acid (Harbers et al., 1962) and nitrates were non-competitive inhibitors of urease, the reduced digestibility of dietary nitrogen would be expected since the action of these inhibitors would be to reduce the amount of dietary nitrogen available to the microflora. As barbituric acid and nitrates exerted a lesser effect on urease activity when the diet higher in carbohydrate was fed, the results may indicate an altered affinity of the inhibitor for the enzyme at the lower ruminal pH values on these diets.

Cellulose digestibility was not affected by treatments when the diet contained only 10 percent hulls; however, in the diet containing 70 percent hulls, copper increased ($P < .01$) and barbituric acid decreased ($P < .01$) the digestibility of cellulose. The 22 percent reduction in cellulose digestibility on addition of barbituric acid to the 70 percent cottonseed hulls diet is in excellent agreement with the data reported by Harbers et al. (1962) who found that barbituric acid reduced cellulose digestibility by 20 percent in vitro and suggested that the effect of barbituric acid could be complex. It is also of interest to note that barbituric acid had no effect on cellulose digestibility when the level of cellulose in the diet was low.

Ruminal fluid was obtained two hours after feeding and showed that only dietary level of cottonseed hulls affected pH values; the high-roughage diets promoted higher ($P < .01$) values. Urease activity of rumen fluid was higher ($P < .01$) in the high-roughage diet than the one containing only 10 percent hulls and at neither level of cottonseed hulls

did the inhibitors affect urease activity. However, there was a slight trend toward lower urease activities in all of the treated group when the higher level of roughage was fed. As variations between animals on the same treatment were large, wide differences would have to be present to obtain significant differences. The fact that ruminal urease activity was not affected by inhibitors but was increased when ruminal fluid pH was raised from 5.92 on the 10 percent cottonseed hulls diet to 6.34 on the 70 percent cottonseed hulls diets might indicate the possibility of regulating rumen urease activity by maintaining rumen fluid pH away from the urease optimum of 7.0 (Sumner and Somers, 1953).

Amylase activity was higher in the low-roughage diet and was not affected by treatments within the two basal diets. No interactions ($P > .05$) existed between level of roughage and ruminal urease and amylase activities or ruminal fluid pH. If we assume that the amylase present in the rumen is the same as pancreatic amylase whose pH for optimum activity is 4.5 (Sherman and Thomas, 1915), then the lower amylase activity at the higher level of cottonseed hulls would be attributed to the greater deviation of ruminal pH on this type diet from the optimum pH value for this enzyme.

A significant interaction between time on feed and urease inhibitors, on ruminal urease activity, was found in trial 6, thus the simple effects are shown in Table XXVII. Addition of copper or barbituric acid increased and nitrates decreased urease activity during the first day; however, copper and nitrates both decreased ureolytic activity during the second day while there were no differences during the tenth day. A significant interaction between days on feed and time after feeding on urease activity was also observed and the simple effects are shown in

Table XXVIII. When rumen samples were collected before feeding and again at 90 minutes after feeding urease activity was higher ($P < .01$) on the second and tenth than the first day on feed. Samples taken 30, 60, and 120 minutes after feeding showed no differences in activity between days 1 and 2, but had lower activity on the tenth day. Urease activity tended to reach a peak at 60 minutes after feeding and then declined except for the samples collected on the second day when urease activity did not reach a peak until 90 minutes after feeding.

TABLE XXVII

EFFECT OF DAYS ON FEED AND UREASE INHIBITORS ON RUMINAL UREASE ACTIVITY (IUB UNITS X 10/ML)[a,b]

Days on Feed	Inhibitors				SE[c]
	Control	Copper	Barbituric Acid	Nitrates	
1	9.2[d,e]	11.7[d]	11.1[d]	7.1[e]	1.1
2	15.6[f]	10.7[g]	15.5[f]	11.1[g]	1.1
10	10.2	10.4	9.8	9.0	1.1

[a]Since there was an interaction between time on feed and inhibitor, only simple effects are shown.

[b]International Biochemical Units.

[c]Standard error.

[d-g]Horizontal values with different superscripts differ ($P < .05$).

Urease inhibitors promoted higher ($P < .01$) ruminal pH values when compared with the control group (Table XXIX) and there was no interaction between inhibitor and days on feed or time after feeding. However, rumen samples taken before feeding and again 120 minutes after

feeding had higher ($P < .05$) values on the second than the first or tenth day on feed, thus the simple effects are shown in Table XXX.

TABLE XXVIII

EFFECTS OF DAYS ON FEED AND TIME AFTER FEEDING ON RUMINAL UREASE ACTIVITY (IUB UNITS X 10/ML)[a,b]

Time After Feeding	Days on Feed 1	2	10	SE[c]
0	5.1[d]	11.3[c]	8.4[f]	1.2
30	12.5[g]	12.6[g]	9.9[h]	1.2
60	13.9[i]	14.0[i]	10.8[j]	1.2
90	7.4[k]	16.2[l]	10.2[m]	1.2
120	9.9[o]	14.7[o]	10.0[n]	1.2

[a]Since there was an interaction between time on feed and time after feeding, the simple effects are shown.

[b]International Biochemical Units.

[c]Standard error.

[d-o]Horizontal values with different superscripts differ ($P < .01$).

Rumen samples taken 30 and 90 minutes after feeding showed a decrease ($P < .05$) in ruminal pH with time on feed while the pH was higher ($P < .05$) on the second than on the first or tenth days when samples were taken 60 minutes after feeding.

When ruminal urea nitrogen was considered there were two significant interactions; inhibitors by days on feed and inhibitors by time after feeding. The simple effects of these treatments are shown in Tables XXXI and XXXII. During the first day nitrates reduced ($P < .01$)

ruminal urea-nitrogen levels; on the second day inhibitors had no effect ($P > .05$) while on the tenth day the barbituric acid-supplemented group had lower ruminal urea-nitrogen levels (Table XXXI). Ruminal urea-nitrogen levels were lower ($P < .01$) 30 minutes after feeding than at all other times (Table XXXII).

TABLE XXIX

MAIN EFFECTS OF UREASE INHIBITORS ON RUMINAL PH

Days on Feed	Treatments				SE[a]
	Control	Copper	Barbituric Acid	Nitrates	
1	6.76[b]	7.07[c]	7.33[d]	7.11[c]	0.06
2	7.05[e]	7.24[f]	7.43[g]	7.10[e]	0.06
10	6.73	6.76	6.82	6.82	0.06
Mean	6.85[h]	7.02[i]	7.19	7.01[i]	0.04

[a]Standard error.

[b-j]Horizontal values with different superscripts differ ($P < .01$).

Copper promoted higher ($P < .01$) and nitrates lower ($P < .01$) ruminal ammonia-nitrogen levels than the control or barbituric acid containing diets and since there was no interaction between the inhibitors and days on feed or time after feeding the main effects of the inhibitors on ruminal ammonia-nitrogen levels are shown in Table XXXIII. A significant interaction between days on feed and time after feeding on ruminal ammonia-nitrogen levels was observed and the simple effects are shown in Table XXXIV, where the levels were higher at 30 minutes after feeding than at all other times, while the levels at 60,

90, and 120 minutes were still higher ($P < .01$) than those before feeding. Ammonia-nitrogen levels decreased ($P < .01$) during the first two days on feed after which there was no further decrease (Table XXXIV).

TABLE XXX

EFFECTS OF DAYS ON FEED AND TIME AFTER FEEDING ON RUMINAL PH[a]

Time Minutes	Days on Feed 1	2	10	SE[b]
0	6.51[c]	6.74[d]	6.51[c]	0.07
30	7.35[e]	7.10[f]	6.76[g]	0.07
60	7.43[h]	7.70[i]	6.82[i]	0.07
90	7.34[k]	7.16[l]	6.91[m]	0.07
120	6.95[n]	7.83[o]	6.93[n]	0.07

[a]Since there was an interaction between time on feed and time after feeding only simple effects are shown.

[b]Standard error.

[c-o]Horizontal values with different superscripts differ ($P < .05$).

TABLE XXXI

EFFECTS OF DAYS ON FEED AND UREASE INHIBITION ON RUMINAL UREA-NITROGEN LEVELS[a,b]

Days on Feed	Treatments				SE[c]
	Control	Copper	Barbituric Acid	Nitrates	
1	51.5	52.6[d]	50.6	46.4[e]	2.5
2	50.4	51.8	53.2	54.4	2.5
10	52.4[f]	53.5[f]	35.1[g]	50.0[f]	2.5

[a]Since there was an interaction between time on feed and urease inhibitors only simple effects are shown.

[b]Mg/100 ml.

[c]Standard error.

[d-g]Horizontal values with different superscripts differ ($P < .01$).

TABLE XXXII

EFFECTS OF TIME AFTER FEEDING ON RUMINAL UREA-NITROGEN CONCENTRATIONS

Time Minutes	Treatments				Mean	SE[b]
	Control	Copper	Barbituric Acid	Nitrates		
0	51.8	51.3	52.8	48.2	51.0[c]	1.6
30	49.6	48.6	33.7	43.6	43.9[d]	1.6
60	50.3	52.8	48.5	53.4	51.3[c]	1.6
90	53.4	54.7	48.1	55.0	52.8[c]	1.6
120	51.3	54.9	47.6	51.8	51.4[c]	1.6

[a]Mg/100 ml.

[b]Standard error.

[c,d]Vertical values with different superscripts differ ($P < .01$).

TABLE XXXIII

MAIN EFFECTS OF UREASE INHIBITORS ON RUMINAL AMMONIA-NITROGEN CONCENTRATIONS[a]

Days on Feed	Treatments				SE[b]
	Control	Copper	Barbituric Acid	Nitrates	
1	41.3[c]	45.1[d]	47.5[e]	26.3[f]	0.92
2	44.4[g]	49.2[h]	43.5[i]	40.2[j]	0.92
10	35.5[k]	42.0[l]	33.8[m]	24.1[n]	0.92
Mean	40.4[o]	45.4[p]	41.6[o]	30.2[q]	0.53

[a]Mg/100 ml.

[b]Standard error.

[c-q]Horizontal values with different superscripts differ ($P < .01$).

TABLE XXXIV

EFFECTS OF DAYS ON FEED AND TIME AFTER FEEDING ON RUMINAL AMMONIA-NITROGEN LEVELS[b]

Time Minutes	Days on Feed 1	2	10	Mean[c]	SE[d]
0	14.6	15.3	16.4	15.4[o]	1.0
30	23.1[e]	22.4	20.6[f]	22.0[p]	1.0
60	22.0[g]	19.0[h]	20.8	20.6[q]	1.0
90	20.5[i]	17.2[j]	18.4	18.7[q]	1.0
120	20.3[h]	17.5[l]	19.5	19.1	1.0
Mean[r]	20.1[m]	18.3[n]	19.1		
SE[s]	0.6	0.6	0.6		

[a]Since there was an interaction between time on feed and time after feeding only simple effects are shown.

[b]Mg/100 ml.

[c]Time means across days.

[d]Standard error on time mean.

[e-n]Horizontal values with different superscripts differ ($P < .01$).

[o-q]Vertical values with different superscripts differ ($P < .01$).

[r]Means for days on treatment across time.

[s]Standard error on means for days.

Urease inhibitors showed no significant interaction with days on feed or time after feeding, thus the main effects of the inhibitors are shown in Table XXXV.

TABLE XXXV

MAIN EFFECTS OF UREASE INHIBITORS ON RUMINAL NITRATE-NITROGEN CONCENTRATIONS[a]

Days on Feed	Treatments				SE[b]
	Control	Copper	Barbituric Acid	Nitrates	
1	33.6[c]	29.2[c]	41.9[d]	45.2[d]	4.3
2	33.7	27.2[e]	41.7[f]	39.6[f]	4.3
10	26.3	28.8	21.4	30.2	4.3
Mean	31.2[g,h]	28.4[g]	35.0[h]	38.3[h]	2.4

[a]Mg/100 ml.

[b]Standard error.

[c-h]Horizontal values with different superscripts differ ($P < .05$).

Barbituric acid and nitrate supplementation in the diet increased ($P < .01$) ruminal nitrate-nitrogen levels. The simple effects of a significant ($P < .01$) interaction between days on feed and time after feeding on ruminal nitrate-nitrogen levels are shown in Table XXXVI. Nitrate levels were lower ($P < .01$) on the second and tenth days when samples were taken 30 and 60 minutes after feeding. Samples taken 120 minutes after feeding had lower nitrate-nitrogen concentrations on the tenth than second day but was not different from the first day on feed (Table XXXVI).

TABLE XXXVI

EFFECTS OF DAYS ON FEED AND TIME AFTER FEEDING ON RUMINAL NITRATE-NITROGEN LEVELS[a,b]

Time After Feeding	Days on Feed			SE[c]
	1	2	10	
0	31.7	41.8	33.4	4.8
30	43.2[d]	19.4[e]	20.2[e]	4.8
60	42.6[f]	41.0[f]	22.9[g]	4.8
90	40.1	37.2	31.5	4.8
120	29.6[h,i]	38.1[h]	25.1[i]	4.8

[a]Since there was an interaction between time on feed and time after feeding only simple effects are shown.

[b]Mg/100 ml.

[c]Standard error.

[d-i]Horizontal values with different superscripts differ ($P < .01$).

Total nitrogen in rumen fluid was not affected by inhibitors with the exception of copper which promoted higher ($P < .01$) levels than all other treatments (Table XXXVII). No significant interaction between inhibitors and days on feed was observed on ruminal total-nitrogen levels thus main effects of inhibitors are shown in Table XXXVII. There was a significant ($P < .01$) interaction between inhibitors and time after feeding on ruminal total-nitrogen levels thus the simple effects of inhibitors are shown in Table XXXVIII. Samples collected 30 minutes after feeding had higher ($P < .01$) total-nitrogen values than those taken before, or 90 or 120 minutes after feeding and were not different from

those taken at 60 minutes after feeding. Length of time on feed did not affect levels of total-nitrogen in ruminal fluid.

TABLE XXXVII

MAIN EFFECTS OF UREASE INHIBITORS ON TOTAL-NITROGEN CONCENTRATIONS IN RUMEN FLUID[a]

Days on Feed	Treatments				SE[b]
	Control	Copper	Barbituric Acid	Nitrates	
1	1.45[c]	1.66[d]	1.36[c]	1.30[c]	0.09
2	1.31[e]	1.63[f]	1.11[e]	1.18[e]	0.09
10	1.39[g]	1.60[h]	1.30[g]	1.16[i]	0.09
Mean	1.39[j]	1.63[k]	1.26[j]	1.21[j]	0.05

[a]Gm/100 ml.

[b]Standard error.

[c-k]Horizontal values with different superscripts differ ($P < .01$).

In discussing the results obtained in Trial 6, it might be useful to consider certain interrelationships within the rumen: if ruminal urease activity were high, ammonia and pH should also be high, while ruminal urea should be low; these being modified by absorption of ammonia and urea across the rumen wall (Glimp and Tillman, 1965), synthesis of microbial protein (Annison and Lewis, 1959), posterior passage along the digestive tract and carbohydrate hydrolysis (Phillipson, 1964). Generalizations of the results are made more difficult by the significant interactions; however, relative sizes of mean squares for main effects in comparison to those for interactions justifies discussion of

main effects. Urease activity was not affected greatly by inhibitors, thus ruminal ammonia and pH would not have been affected by urease activity.

TABLE XXXVIII

EFFECTS OF UREASE INHIBITORS AND TIME AFTER FEEDING ON TOTAL NITROGEN CONCENTRATIONS IN RUMEN FLUID[a]

Time Minutes	Treatments				Mean	SE[b]
	Control	Copper	Barbituric Acid	Nitrates		
0	1.29	1.29	0.80	1.03	1.10[c]	0.06
30	1.53	1.84	1.52	1.40	1.57[d]	0.06
60	1.35	1.73	1.54	1.26	1.47	0.06
90	1.25	1.62	1.27	1.21	1.36[e]	0.06
120	1.52	1.66	1.13	1.15	1.37[e]	0.06
Treatment Mean	1.39[f]	1.63[g]	1.26[h]	1.21[h]		
SE	0.05	0.05	0.05	0.05		

[a]Gm/100 ml.

[b]Standard error.

[c-e]Time (vertical) means with different superscripts differ ($P < .01$).

The author, therefore, postulates that the higher ruminal pH values found when the inhibitors were fed were caused by a decreased hydrolysis of the carbohydrate components of the diet resulting in decreased ruminal volatile fatty acid production (Annison and Lewis, 1959). Results of trial 5 and those of Harbers et al. (1962) provide further support for the idea of reduced carbohydrate hydrolysis in the presence of

barbituric acid. The lower ruminal ammonia-nitrogen obtained when nitrates were added to the diet could be caused by a slower rate of nitrate hydrolysis in comparison to that of urea.

Summary

Lambs were used in six trials to determine effects of copper, barbituric acid and nitrates on urea utilization. In trial 1, 24 lambs in a 2 x 2 factorial arrangement were fed 15 or 30 ppm of copper in finely ground or pelleted all-concentrate rations. Copper improved gain ($P < .01$) and gain/feed ($P < .05$). Forty-five lambs were used in trial 2 to study the effects of copper (15, 30 and 45 ppm), nitrates (0, 33, 0.66 and 0.99 percent) and barbituric acid (0.01, 0.02 and 0.04 percent in a milo-urea basal ration. Barbituric acid (0.01 percent) and nitrates (0.66 percent) increased ($P < .05$) gains. Thirty lambs were used in trial 3 to replicate the control, barbituric acid (0.01 percent) and the nitrates (0.66 percent) diets of trial 2. In this trial, barbituric acid (0.01 percent) and nitrates (0.66 percent) did not improve growth performance. In trial 4, a 32 percent crude protein supplement of milo and urea was individually fed to 10 lambs (control) at a level of 454 gm/day; cottonseed hulls were fed free choice. Another 10 lambs received the control diet plus 0.04 percent barbituric acid while another 10 lambs received 2.64 percent nitrates. Neither barbituric acid nor nitrates affected gain or the ad libitum intake of cottonseed hulls. Thirty-two lambs in a 2 x 4 factorial arrangement were fed 10 or 70 percent cottonseed hulls diets each with no supplement; 30 ppm copper; 0.01 percent barbituric acid or 0.66 percent nitrates added and feces and urine collected over a 10-day period. Barbituric acid

and nitrates had no effect on the nitrogen digestibility of the 10 percent cottonseed hulls diets but decreased the digestibility of nitrogen of the 70 percent cottonseed hulls diets. Copper had no effect ($P > .05$) on nitrogen digestibility at any level of cottonseed hulls. Copper and nitrates decreased ($P < .01$) the retention of absorbed nitrogen. Barbituric acid decreased ($P < .01$) retention of nitrogen only on the 70 percent cottonseed hulls diets. In trial 6, 8 rumen fistulated steers were fed the unsupplemented 70 percent cottonseed hulls for 11 days and allotted at random into 4 groups. Group 1 was continued on the same control diet while groups 2, 3, and 4 received 30 ppm copper, 0.01 percent barbituric acid and 0.66 percent nitrates, respectively, added to the control diet. All steers were fed at maintenance and rumen samples collected at 0, 30, 60, 90, and 120 minutes after feeding on 1, 2, and 10 days after the inhibitors were introduced into their diets. Ruminal urease activity, ammonia-, urea- or nitrate-nitrogen were not affected significantly ($P > .05$) by addition of the inhibitors. Inhibitors caused an increase in ruminal pH which reflected a decrease in the rate of carbohydrate hydrolysis rather than increased ruminal ammonia-nitrogen levels. There were no consistent trends in ruminal concentrations of ammonia-, urea-, nitrate-nitrogen or pH levels with time on feed.

CHAPTER VII

GENERAL DISCUSSION

Nitrogen from urea appeared to be utilized only 70 percent as well as nitrogen from soybean protein. Since dietary protein serves as a source of amino acids and branched-chain fatty acids for ruminal microbial protein synthesis many investigators reported the possibility of a deficiency of branched-chain fatty acids or amino acids when urea was the sole source of dietary nitrogen. Although the evidence of a deficiency of certain of these acids appeared quite convincing, addition of the acids did not always yield beneficial results suggesting that a simple deficiency of amino acid and/or branched-chain fatty acids *per se* was not responsible for the poorer performance when urea replaced soy protein.

Data in Chapter IV suggest that addition of a mixture of leucine, isoleucine, valine and phenylalanine to a diet containing urea as the only source of nitrogen only slightly improved growth performance, the improvement, however, was not as great as when soy protein replaced urea (Chapter III). The superiority of the soy protein diet above the diet containing urea supplemented with the mixture of amino acids may be attributed to the higher levels of amino acids present in the soy protein diet, since the urea supplemented diet contained only 25 percent as much leucine, isoleucine, valine and phenylalanine as did the soy protein diet. However, if dietary amino acid or branched-chain fatty acids

per se were limiting, animal performance might be expected to improve when these acids were added to the diet, even in small quantities. Thus it appeared that leucine, isoleucine, valine, or phenylalanine deficiencies per se in the diet were not responsible for the poorer performance when urea rather than soy protein was the source of dietary nitrogen. It is possible that greater quantities of dietary amino acids and peptides passed to the small intestine, when animals were fed soy protein rather than urea and this offers a possible explanation for the apparent improvement in performance with intact protein. If this were true, this makes the animal more independent of ruminal microbial protein synthesis when fed intact protein rather than urea. Greater utilization of intact casein compared with an enzymatic digest of the same casein was reported when casein was infused into the small intestine of sheep.

It is of interest in the present experiments that an adaptation response was observed for soy protein as well as urea. This suggested that the adaptation phenomenon was independent of the dietary nitrogen source. The adaptation phenomenon has also been attributed to changes in the rumen microbial flora, however, the exact nature of these changes is not clearly delineated. Urea would be expected to induce different changes in the rumen microbial flora compared with soy protein yet the same adaptation occurred for both nitrogen sources. Also diethylstilbestrol which has no effect on rumen microbial population was reported to shorten the period required for adaptation to take place. This evidence would suggest that adaptation was independent of changes in the rumen microbial flora. Other possible sites for the adaptation response could be the liver, kidneys or rumen epithelium. Since the exact site of adaptation is unknown, this is a fruitful area for further research.

Since the microbial population concentrations probably decreased, there is a possibility that the decreased urease activity in fasting animals was associated with a lower dry matter in rumen contents (Chapter V). Ruminal urease activity appeared to be relatively independent of various feeding regimes. Thus, the variation among animals in susceptibility to urea toxicity could be a function of the amount and rate of consumption of urea since urease is present in the rumen in sufficient quantities to hydrolyze large amounts of urea. The inability of copper, barbituric acid or nitrates to improve urea utilization may have been due to the fact that the dietary levels of these inhibitors were too low. Higher levels of barbituric, however, were shown by other workers to have a depressing effect on animal performance. Supplementary nitrates were perhaps themselves reduced very rapidly in the rumen before they exerted an inhibitory effect on urease activity. Since many ruminal bacteria produce urease which is very active in small amounts then inhibitors may have to be added in large amounts in the diet in order to have a significant effect at the ruminal level. Under these conditions the urease inhibitors may adversely affect other enzyme systems. Evidence in support of this idea is seen in Chapter VI where barbituric acid and nitrates added to the high roughage diet reduced cellulose digestibility. Thus, inhibitors should be specific for the enzyme urease in order to be effective in reducing rumen ureolytic activity. Copper, barbituric acid or nitrates appeared to be ineffective in improving urea utilization under the conditions used in this experiment. Attempts with other inhibitors such as thiourea and hydroxamic acids would appear to be a very fruitful area for further research.

CHAPTER VIII

GENERAL SUMMARY

A series of four experiments, involving cattle and sheep fed natural and purified diets, were conducted to study urea utilization. In experiment I when urea was substituted for soy protein, growth performance and nitrogen retention of lambs fed diets containing urea as the sole source of nitrogen were approximately 70 percent as great as those obtained when soy protein was used. Nitrogen retention improved with time on feed for both nitrogen sources indicating an adaptation to soy protein as well as to urea. Plasma concentrations of glutamic and aspartic acids were greater when urea rather than soy protein was the nitrogen source. Plasma urea- or ammonia-nitrogen levels were not influenced by dietary nitrogen source. Supplementation of a urea-containing purified diet with a mixture of leucine, isoleucine, valine, and phenylalanine in experiment II did not significantly improve average daily gains or affect the nitrogen or volatile fatty acid components in blood and rumen fluid.

In experiment III fasting steers reduced ruminal ureolytic activity and volatile fatty acid levels. Ureolytic activity progressively decreased as the length of the fasting period increased. Ureolytic activity for steers fed high and low roughage type diets was not significantly different, although there was a tendency for lower levels of activity when the low roughage ration was fed. The low roughage diet promoted

greater ruminal total volatile fatty acid levels. No differences were observed on ureolytic activity of rumen fluid between steers fed good quality alfalfa and poor quality prairie hay. Fasting increased while feeding the high concentrate ration lowered rumen pH relative to that obtained when a low concentrate ration was fed. In experiment IV, addition of urease inhibitors had no dramatic beneficial effects on gain, feed intake, nitrogen digestibility, and retention or on the nitrogen or fatty acid fractions in rumen fluid.

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