EFFECTS OF FEEDING RUMINANTS NON-PROTEIN
NITROGEN AS THE ONLY NITROGEN SOURCE 1

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Several excellent reviews on nitrogen utilization by ruminants (McLaren, 1964; Phillipson, 1964; Blackburn, 1965; Hungate, 1966; Briggs, 1967; Waldo, 1968; Chalupa, 1968) have been written recently. In general, these reviews have been concerned with results obtained mainly from feeding limited to moderate quantities of non-protein nitrogen (NPN) to ruminants.

Since Loosli et al. (1949) and Duncan et al. (1953) found that all of the essential amino acids were synthesized by the rumen microbial population of sheep and goats fed a purified diet containing urea as the only source of dietary nitrogen, there has been considerable academic interest in various aspects of feeding large quantities of NPN to ruminants. It is the purpose of the present review to summarize the results obtained when NPN comprised all, or was the primary source of nitrogen in diets for ruminants. A special attempt has been made to include work pertaining to the effect of NPN on the host animal as well as to review the ruminal aspects of NPN utilization. Most of the manuscripts reviewed were concerned with urea as the nitrogen source but other NPN sources are also discussed. This review will be concerned mainly with nitrogen metabolism and many aspects of the influence of carbohydrates and minerals on NPN utilization will not be stressed.

In most of the reports which have been reviewed semi-purified and purified diets were used to test a NPN source against a purified protein source. The purified diet technique is very useful in studies of this nature because the NPN source can supply essentially all of the nitrogen in the diet and it can be compared directly to the protein source without altering other nutrients in the diet. In the purified diet studies conducted at Beltsville during the past few years, various NPN sources have supplied 98.0% of the total nitrogen in the diet (Oltjen et al., 1969a) while Virtanen (1966) has used a purified diet in which urea and ammonium salts supplied 94.5% of the total nitrogen in the diet.

Growth Studies. The growth rates of ruminants fed purified diets ad libitum which contained all of the dietary nitrogen from NPN have been lower than those of ruminants fed protein containing purified diets. Oltjen et al. (1962a, b) reported gains and feed efficiencies of lambs fed urea to be 65% of that of lambs fed isolated soy protein. A combination of 30% wood pulp and 6.5% of an alkaline mineral mixture promoted the highest gains of lambs fed the urea purified diet in which 30, 40 and 50% wood pulp and 3.5, 5.0 and 6.5% minerals were compared (Oltjen et al., 1962c). McDonald (1966) reported that a purified diet containing 25% cellulose was optimal for urea nitrogen utilization, and that gains and feed efficiency of lambs was 73% as good with urea as with isolated soy protein. Meacham et al. (1961) reported that gains of rams fed a urea containing purified diet were 57% as good as that of rams fed a purified diet containing isolated soy protein. Clifford and Tillman (1968) reported growth of sheep fed the urea purified diet to be 70% as good as growth of sheep fed an isolated soy protein purified diet. Matrone et al. (1964) reported that gains and feed efficiency were about 50% as good when urea was compared to casein in purified diets for sheep. Goodrich and Tillman (1968) reported that growth of lambs fed urea was about 80% of those fed isolated soy protein in a 60-day purified diet study. Oltjen et al. (1969a) reported gains and feed efficiency to be 50% as good with urea as with isolated soy protein with calves which had been weaned onto the diets at 84 days of age.

The results from these studies indicate that when NPN completely replaces the protein in purified diets, growth rate and feed efficiency are about 65% as good on the NPN diet as on the protein containing diets. Furthermore, there appears to be a trend for a further reduction in animal performance in extended growth trials (Matrone et al., 1964; Oltjen et al., 1969a). This could possibly be related...
to the nutritional inadequacy of the purified diets as well as NPN effect per se. In general, feed intake has been similar with both types of nitrogen sources in the purified diet contrary to the slight depression which is often found when urea is used in natural diets. Perhaps the lowered palatability of the purified diets has tended to nullify this response. It is surprising that research has not been conducted to determine the optimum level of NPN which is needed in the purified diet for the greatest animal performance. The currently used crude protein equivalent levels are based on protein requirements which were established with natural diets. These results may not be applicable when NPN comprises all of the nitrogen in purified diets.

Various amino acids have been added either singly or in combination to the urea-basal purified diet in an attempt to improve animal performance. In most cases, some of the NPN nitrogen was removed when the amino acids were added in order to keep the diet on an isonitrogenous basis, but in some cases the amino acids were added to the basal diet. Lysine (Harbers et al., 1961; Meacham et al., 1961) methionine and alanine (Oltjen et al., 1962a) and leucine, isoleucine, valine and phenylalanine (Clifford et al., 1967) have been added to the urea diet and, in general, these amino acid additions have not improved animal performance. Possible reasons for the poor response include (1) in most of these studies the DL-forms of the amino acids were used instead of the active L-forms, (2) the amino acids may have been supplied in too low a quantity, (3) the "unprotected" amino acids may have been too readily available in the rumen and were rapidly catabolized instead of being incorporated into microbial protein and (4) the amino acids were not limiting growth.

Bunn et al. (1968) reported that 5% alfalfa meal or an amino acid supplementation of glutamic acid, aspartic acid, isoleucine, leucine, arginine and tryptophan added to the urea purified diet improved growth of lambs but not nitrogen retention. In that study, lamb allotment to the experimental treatments and differing nitrogen levels in the experimental diets made interpretation of the results difficult. However, in a later study, Bunn and Matrone (1968) reported that when 5% alfalfa leaf meal replaced urea nitrogen on an isonitrogenous basis the growth rate of lambs was again improved. Clifford and Tillman (1968) reported that substituting 33% of the total dietary nitrogen with isolated soy protein did not stimulate the gains of lambs, while McDonald (1966) received a significant increase in the gains of lambs from the same substitution of isolated soy protein.

**Milk Production.** Flatt et al. (1967) reported that a cow fed a urea purified diet produced 2,630 kg. of milk, while her monozygotic twin fed a natural diet produced about two times this amount during 305 day lactations. During a second lactation, milk production of the cow receiving the urea purified diet was about 65% of that of her twin. The metabolizable energy in the purified diet was used by the cows similarly to the metabolizable energy obtained from the natural diet. This was true regardless if protein or NPN was the source of dietary nitrogen in the purified diet. Replacing 10% of the dietary urea nitrogen with isolated soy protein appeared to stimulate milk production.

In a series of extensive studies, Virtanen (1966) reported that six cows fed a purified diet which contained urea and ammonium salts produced an average of 2,750 kg. of milk during standard 305 day lactations. The milk was essentially similar to that produced by cows fed natural diets, except for a slight increase in the percentage of protein and fat. Milk production and the percentage of protein in the milk rose considerably when the crude protein equivalent in the diet (from urea and ammonium salts) was raised from 12.8 to 15.0%. The increased fat content in the milk may have been associated with depressed milk production, but Virtanen (1966) indicated it was a dietary effect.

The amino acid composition of casein and total protein in the milk of the cows fed the urea diet was essentially no different from that of cows fed the natural diet. Based on later experimental results in which protein was added to a semi-purified diet, Virtanen (1967) estimates that without dietary protein the cow is able to produce about 4,000 kg. of milk per year. When about 20% of the digestible crude protein is true protein, the milk yield will rise to about 5,000 kg., and with 30% about 6,000 kilograms. Thus, based on these two studies it appears that small amounts of dietary protein stimulate the production of milk from cows fed protein free diets.

**Reproduction.** Bates et al. (1960) reported results concerning growth, reproduction and lactation by two Jersey heifers fed a corn
cob, molasses and urea diet in which urea supplied approximately 80% of the total nitrogen in the diet. Virtanen (1966) placed several mature dairy cows on the purified diet containing urea and ammonium salts when they were either dry or producing a small amount of milk and reported successful reproductions by the cows. The cows required several services to become pregnant. Two cows have each calved three times while on the diet.

Successful reproductions of two beef heifers raised from 7 mo. of age on a urea purified diet has been reported (Oltjen and Bond, 1967). One cow has produced two calves and has been on the urea diet for 4 yr. 9 mo. at the present time. The other cow had one calf and was on the diet 4.5 years. These cows also required several services to become pregnant. Milk production was similar, but fat and protein percentages were lower from the cows fed the urea purified diet compared to their twins fed a natural diet in contrast to the results of Virtanen (1966).

Bulls have been determined to be fertile and heifers have produced calves after being weaned onto the urea purified diet at 84 days of age (Oltjen, 1967). The bulls fed urea grew somewhat slower than bulls fed the isolated soy purified diet or bulls fed a natural diet, and reached puberty (measured by semen characteristics) an average of 2 mo. later than bulls fed the protein containing diets. The heifers fed the urea purified diet grew considerably slower than heifers fed the isolated soy protein purified diet and reached puberty (measured by first estrus) approximately 6 mo. later than heifers fed the soy purified diet or the natural diet. The delayed puberty was probably also due to a lowered energy intake by the urea fed heifers. Several heifers fed urea in this study have calved at the present time. Heifers raised on the urea diet and mated to bulls raised on the urea diet produced normal offspring. Several bulls and cows have been on the urea diet 3.5 yr. at the present time. In these studies, bulls have each calved three times while on the diet.

Physiograph studies of bulls and heifers fed the urea containing purified diet for 2 yr. have indicated that the long term feeding of urea as the sole source of dietary nitrogen had no apparent effect on respiratory patterns, heart rate or EKG patterns. These patterns, however, were markedly altered by intraruminal infusions of urea solutions which resulted in acute ammonia toxicity. Normal patterns returned shortly after the physical symptoms of toxicity were no longer apparent (Rumsey et al., 1969).

Cattle fed purified diets containing either urea or isolated soy protein have been sacrificed during the course of several studies at Beltsville. Drs. Samuelson and Migaki (personal communication) in extensive postmortem and histological examinations have not found any significant changes after prolonged feeding of urea (up to 4 yr.) compared to animals fed natural diets.

Nitrogen Balance Studies. Ellis et al. (1956) conducted metabolism trials using purified diets with lambs and reported biological values of 54, 57, 73 and 84 for urea, gelatin, casein and isolated soy protein, respectively.

Oltjen and Putnam (1966) reported that replacing glucose in the purified diet with additional starch resulted in a significant improvement in nitrogen (either urea or isolated soy protein) digestibility, but it also caused a significant increase in urinary nitrogen losses which resulted in little net effect on nitrogen balance. Nitrogen retention of steers fed urea was 60% of that of steers fed the isolated soy protein diet. Clifford and Tillman (1968) reported nitrogen retention of sheep fed the urea containing purified diet was about 70% as good as with sheep fed the isolated soy purified diet. Replacing urea with isolated soy protein in stepwise gradients had no consistent effect on nitrogen balance.

McLaren et al. (1962) fed a semi-purified diet containing 87% of the dietary nitrogen from urea and reported that when 8% of this nitrogen was replaced by enzymatically hydrolyzed casein there was a significant improvement in nitrogen utilization. This was not the case when either 8% intact casein or 8% acid hydrolyzed casein was used. These results suggest the need for peptides to improve nitrogen utilization.

Although Bunn et al. (1968) received a significant growth response with lambs from the addition of 5% alfalfa or a combination of amino acids to the urea purified diet, nitrogen balance studies with the same diets showed no response. McDonald (1966) reported no improvement in the nitrogen retention of sheep when 0.55% DL-methionine, 0.75% L-lysine, 5% dehydrated alfalfa or 5% distiller's dried solubles were included in a urea purified diet. Barth et al. (1959) were able to improve the retention of absorbed nitrogen.
when 2.7% methionine and 1.2% tryptophan replaced urea nitrogen on an isonitrogenous basis in semi-purified diet studies with sheep. Adding 3.0% L-glutamic acid to the urea purified diet has not significantly improved the nitrogen retention of calves (Oltjen et al., 1964).

Anderson et al. (1959) fed a semi-purified diet containing 67% of the dietary nitrogen from test sources and reported that uramite, a urea-formaldehyde condensation compound, and pure biuret were inefficiently utilized compared to soybean meal. McLaren et al. (1965a) reported that the retention of absorbed nitrogen was significantly increased when 1/3 of the urea nitrogen (which supplied 75% of the total nitrogen in the diet) was replaced by an isonitrogenous amount of creatine. Vitamin B₁₂, folic acid or both in combination did not affect nitrogen utilization.

Urea, biuret, ura phosphate and uric acid were compared in metabolism studies with steers (Oltjen et al., 1968) and nitrogen retention values (% of intake) were 18.4, 16.9, 12.3 and 23.1%, respectively, for these NPN sources. Although the nitrogen retention of the above NPN sources did not differ significantly, uric acid appeared to be a good source of nitrogen for the ruminant animal. Uric acid and other structurally similar compounds should be further tested as potential NPN sources for ruminants.

A significant increase in the digestibility and retention of nitrogen by lambs was observed by Cline et al. (1966) when a urea purified diet containing 39% cellulose was supplemented with isovaleric, isobutyric and n-valeric acids. The dietary addition of the acid mixture decreased ruminal ammonia concentrations which was interpreted to indicate that more ammonia was being utilized by the bacteria. A similar addition of the acids to a 59% cellulose diet, however, resulted in no improvement in nitrogen retention. These workers postulated that adding the iso-acids to the diet markedly increased the rate of feed consumption which resulted in a faster ammonia release which, consequently, was less well utilized as the proportion of cellulose was increased.

Adaptation Response. McLaren et al. (1959, 1960) reported that the utilization of absorbed nitrogen was increased by prolonging the preliminary period to which sheep received NPN or by the feeding of diethylstilbestrol. They reported that diethylstilbestrol and time influence the retention of absorbed nitrogen through direct action on the tissues to promote better utilization of non-protein nitrogen. McLaren et al. (1965b) summarized the effects of level of readily available carbohydrates and length of time of urea feeding on nitrogen retention by lambs fed semi-purified diets containing 75% of their nitrogen from urea. Regression analysis indicated that the retention of absorbed nitrogen was improved 3 percentage units with each 10-day period of urea feeding and 2% for each 100 kcal. of readily available carbohydrates in the diets.

An increased nitrogen retention with time by sheep fed either isolated soy protein or urea was reported by Clifford and Tillman (1968). The improved retention was the result of a decreased urinary nitrogen loss and maximum retention was noted after 30 days on test. Oltjen and Putnam (1966) reported a significant decrease in urinary nitrogen losses with time by cattle fed urea and soy purified diets. This study was of a Latin square design and suggested an adaptation to the purified diet components per se. Virtanen (1966) stressed the need for an adaptation of dairy cows to the purified diet containing urea and ammonium salts before maximum milk production was attained. However, part of this “prolonged adaptation” undoubtedly was due to the purified diet components rather than NPN per se.

Caffrey et al. (1967a) studied the adjustment of sheep to a urea rich diet and reported that sheep fed the urea-diet had lower ruminal urease activity than sheep receiving a similar soybean containing diet. This has also been reported by Chalupa et al. (1968). Caffrey et al. (1967a) reported that an abrupt change from urea to soybean meal and vice versa indicated little adaptation to urea, since nitrogen balance at 4, 20 and 41 days after the abrupt change was similar. The time required for ruminal microorganisms to assimilate ammonia at a maximum rate was 19 days after the change. In further studies, Caffrey et al. (1967b) studied the utilization of, and possible adaptation to, intravenously infused urea and ammonia and reported that lambs receiving an infusion of urea retained 5.2 gm. of nitrogen daily more than the controls receiving the saline solution. The sheep infused with ammonium chloride retained 1.4 gm. more daily than the control animals receiving saline infusions. There was a progressive increase in nitrogen balance as the ammonia infusion
was extended, but there was also a comparable increase in lambs receiving saline. There appeared to be an adaptation to a poor dietary regimen rather than to ammonia *per se*. Miller and Morrison (1942) have reported that the apparent retention of absorbed nitrogen was significantly influenced by the pre-experimental period. Thus, some experimental results showing an adaptation response may well be (1) a reflection of animal adjustment to the purified diet components other than NPN alone and (2) animal adaptation from an adequate protein diet to a lower protein diet.

**Rumen Studies.** Feeding the urea purified diet twice daily results in high ruminal ammonia concentrations which tend to peak at 1 to 1.5 hr. after diet consumption (Chalupa et al., 1964; Oltjen and Putnam, 1966; Oltjen et al., 1968). These values usually average 35–55 mg. NH₃-N/100 ml. ruminal fluid. Although these ammonia concentrations are considerably below the toxic level, they cause high urinary nitrogen losses which account for a large portion of the difference in performance of ruminants fed protein vs. urea containing diets. If ruminants are fed the urea diet on an *ad libitum* basis, ruminal ammonia concentrations are lowered and improved nitrogen utilization usually occurs. Virtanen (1966) raised the crude protein equivalent in his purified diet from 12.8 to 15.0% on the basis of low ruminal and blood ammonia values and the low ammonia and urea concentrations in the milk. In general, ruminal pH values are similar for urea and isolated soy protein fed animals under *ad libitum* fed conditions but, under controlled feeding, ruminal pH may be considerably higher (1 to 2 hr. after feeding) on the urea diet due to the rapid hydrolysis of urea to ammonia by the ruminal microorganisms (Oltjen et al., 1968). The low ruminal pH (5.4 to 6.0) may be beneficial to animals fed the purified diets in terms of nitrogen retention because the transfer of ammonia through the ruminal wall is much less rapid at a low pH (Hogan, 1961) and more of the free ammonia is trapped for microbial protein synthesis.

The average salivary flow rate of steers fed the urea purified diet is slightly lower than the rates of steers fed an equal amount of the isolated soy purified diet (Oltjen et al., 1965) but the flow rates of steers fed urea were considerably lower 2 hr. after feeding than at 6 hr. after feeding. Later studies (Oltjen et al., 1967) indicated that high ruminal ammonia concentrations can markedly reduce salivary flow.

Although the total time spent in rumination is drastically reduced when ruminants are fed purified diets, replacing the isolated soy protein with urea in the purified diet does not appear to affect total rumination time (Oltjen et al., 1962a) or the initiation of rumination in young calves (Oltjen et al., 1969a). Feed consumption patterns of cattle fed the urea purified diet are similar to those of cattle fed the isolated soy purified diet under *ad libitum* conditions (P. A. Putnam and R. R. Oltjen, unpublished data). There was considerably less diurnal variation in the pattern of cattle fed the purified diets compared to cattle fed a natural diet.

Supplying all of the dietary nitrogen as urea in purified diets does not seem to diminish synthesis of the "B" vitamins by the ruminal microorganisms (Oltjen et al., 1962b). In these studies, the amounts of niacin, thiamine and riboflavin in each gram of dry ruminal contents were similar to those found in cattle fed natural diets and the supplementary addition of these vitamins to the urea purified diet has not improved animal performance. Agrawala et al. (1953) reported that there was a considerable synthesis of riboflavin, pantothenic acid and niacin by calves fed a urea purified diet. Buziassy and Tribe (1960), however, reported that ruminal niacin concentration was greatly increased when casein replaced urea in a similar diet. Virtanen (1966) conducted studies on the vitamin content of the milk obtained from cows fed the protein free diet and reported that the concentrations or riboflavin, nicotinic acid and especially pantothenic acid were generally higher in this milk than milk from cows fed natural diets. Choline was not added to the diet. Brüggemann and Giesche (1967) reported that "dietary urea appears to stimulate the production of B-vitamins in the rumen, but in some cases the availability of easily fermentable carbohydrates and certain amino acids may limit vitamin synthesis."

Several investigators have reported pronounced depressions in the ruminal concentrations of isobutyric and isovaleric acids (Orskov and Oltjen, 1967; Cline et al., 1966), somewhat depressed levels of these acids (Clifford and Tillman, 1968) or lowered quantities of isobutyric acid (Matrone et al., 1965) when ruminants are fed diets free of protein. These branched-chain VFA's are
present in the ruminal fluid of ruminants fed natural diets mainly through the degradation of dietary protein and the deamination of the branched-chain amino acids (el-Shazly, 1952a, b; Annison, 1954). Thus, it is not surprising that ruminants fed an essentially protein free diet would have decreased amounts of these branched-chain acids. Replacing isolated soy protein by urea has also resulted in lower acetic acid proportions and increased proportions of butyric acid (Ørskov and Oltjen, 1967).

The fact that the nitrogen requirements of bacteria are simple was demonstrated by Bryant (1961) who examined the nitrogen requirement of 44 strains of rumen bacteria and found that 80% could be grown with ammonia as the sole source of nitrogen, 26% could not be grown unless ammonia was present and 56% could use either ammonia or amino nitrogen.

Slyter et al. (1968) studied the microbial population of calves fed purified diets containing either urea or isolated soy protein and reported similar microbial populations, except that the calves fed the isolated soy diet had fewer cocci than calves fed the urea diet. In additional studies, ruminal samples were examined from steers fed purified diets containing urea, biuret, urea phosphate and uric acid as the sole nitrogen source. The concentration of Bacteroides amylophilus was higher in steers fed either urea or urea phosphate than in steers fed biuret or uric acid. The percentage of facultative anaerobic and H2S-producing ruminal bacteria were significantly greater for steers fed biuret than for steers fed the other NPN sources. Steers fed uric acid had significantly more cellulytic bacteria than steers fed urea and urea phosphate. Steers fed urea had significantly more amylolysic bacteria than steers fed urea phosphate.

Gall et al. (1951) reported similar microbial populations of sheep fed urea and casein containing purified diets and that the total bacterial count was similar, but sheep fed casein had mainly Gram-positive cocci in masses whereas urea fed sheep had Gram-positive slender curved rods and large cocci. Chalupa et al. (1964) found differing ruminal redox potentials in sheep fed urea than in sheep fed corn gluten meal, indicating greater microbial activity when urea was the source of dietary nitrogen. Nitrogen retention, however, favored the corn gluten fed animals. Virtanen (1966) found 2.7% \( \alpha, \epsilon \)-diaminopimelic acid in the fecal hydrolysates of cows fed the protein free diet compared to 0.6% of this compound in fecal hydrolysates of cows fed natural diets, indicating that bacterial protein synthesis was greatly enhanced in the rumen of the cows fed the urea-ammonium salts purified diet.

Virtanen (1966) reported a drastic reduction in protozoa numbers accompanied with increased bacterial numbers in the ruminal contents of cows fed the protein free diet. He implied this was due to urea per se and related this to a greater bacterial synthesis of protein, but it has been shown (Oltjen et al., 1966; Giesecke et al., 1966; Slyter et al., 1968) that the ruminal ingesta of cattle fed purified diets are frequently devoid of protozoa and have increased bacterial concentrations. This is true for protein-containing diets as well as NPN containing purified diets.

The disappearance of protozoa from the ruminal contents of ruminants fed purified diets may be partly responsible for lowered performance of ruminants fed purified diets, since protozoal hydrolyzates contain greater quantities of leucine, isoleucine, phenyalanine and lysine (Weller, 1957; Purser and Buechler, 1966) and have a greater true digestibility (McNaught et al., 1950). Abou Akkada and el-Shazly (1965) reported increased nitrogen retention in faunated lambs when compared with defaunated lambs.

The amino acid pattern of rumen microbial hydrolyzates from cattle fed the isolated soy purified diet when expressed on a molar percentage basis is very similar to those of cattle fed the urea purified diet 4 hr. after feeding (R. R. Oltjen and L. L. Slyter, unpublished data). The total concentration (\( \mu \)moles/100 mg. of bacteria) of the amino acids were 15% greater, however, from steers fed the soy diet. This increase must have been due to increased microbial protein, since the percentage of \( \alpha, \epsilon \)-diaminopimelic acid concentration was the same (1%) with both nitrogen sources. Although it did not appear to be a factor in this study, the fraction of dietary protein that escapes ruminal fermentation and passes intact to the abomasum may have a large bearing on nitrogen retention of the ruminant. Certainly, this area of research needs greater emphasis in the future.

Cline et al. (1966) reported that the microbial hydrolyzates of sheep fed purified diets containing urea were not altered by supplementary additions of isovaleric, isobutyric and
n-valeric acids. Schelling et al. (1967) compared the amino acid content of the microbial population of sheep fed a urea purified diet to that of whole egg protein and reported lower amounts of histidine and the sulfur amino acids in the ruminal contents. Duncan et al. (1953) compared the amino acid content of mixed ruminal proteins from a calf maintained on a natural diet to those maintained on a urea purified diet and reported that, with the exception of histidine, the pattern was similar for both groups of calves. In general, however, the essential amino acid content was found in lower amounts in the ruminal contents of calves receiving the urea diet. Virtanen (1966) studied the amino acid composition of hydrolyzates of the total protein in the ruminal contents of his cows fed the urea diet and found a higher (1.1 vs. 0.7%) histidine content in the cows fed the urea diet than in cows fed a natural diet. Weller (1957) reported that the amino acid composition of the bacterial hydrolyzates obtained from sheep fed four different diets was remarkably uniform and differed from that for protozoal hydrolyzates in that they contained lower levels of the "essential" amino acids, particularly lysine.

Purser and Buechler (1966) conducted amino acid analyses on 22 strains of rumen bacteria and reported that there was very little variation in the amino acid pattern of each. Bergen et al. (1967) studied the protein quality of several of the individual bacteria using an in vitro enzymatic digest system and reported ratios from 37 to 80 compared to 100 for an egg protein digest. The proportion of amino acids released as free amino acids during the digest varied between 2.5 and 52.6%.

Hungate (1966) has expressed concern that the anaerobic condition that exists in the rumen is a limiting factor in the amount of microbial protein that can be synthesized. This limitation occurs because anaerobic microorganisms are able to metabolize feed substrates to VFA which results in 10-20% efficiency while aerobic microorganisms are able to complete the metabolism to carbon dioxide and water which results in 60-70% efficiency. It would appear that the extent of this limitation may become more important as the percentage of NPN rises in the diet because lesser quantities of the dietary amino acids would be available for microbial protein synthesis and therefore greater quantities of the amino acids would have to be synthesized from carbon skeletons and ammonia.

Blood Plasma Free Amino Acid Patterns. This area of research has recently been studied in an attempt to relate the performance of ruminants fed NPN containing diets to the free amino acid pattern. When isocaloric and isonitrogenous quantities of purified diets containing urea or isolated soy protein were fed to cattle, it was determined that steers fed urea had significantly lower plasma levels of leucine, isoleucine, valine and phenylalanine with significantly increased levels of serine and glycine (Oltjen and Putnam, 1966). A relationship was suggested between the lowered branched-chain VFA found with ruminants fed the NPN diets and the branched-chain amino acids, since Allison and Bryant (1963) have reported that the amino acids can be synthesized from these precursors.

High plasma levels of serine (Oltjen et al., 1968) and glycine (Virtanen, 1966; Oltjen and Bond, 1967; Schelling et al., 1967) have been reported in ruminants fed urea-purified diets compared to control animals fed natural diets. The plasma amino acid patterns of calves fed a urea purified diet and an isolated soy purified diet were similar at 42 days of age while the calves were still receiving milk, but at 84 (after weaning) and 189 days of age the concentrations of threonine, phenylalanine, valine, leucine, isoleucine, cystine, hydroxyproline and tyrosine were especially low, whereas urea and serine concentrations were especially high in urea fed calves (Oltjen et al., 1969b). The absorption of certain amino acids through the ruminal wall (Tsuda, 1956; Cook et al., 1965) and especially glycine may influence the circulating blood plasma levels.

Nitrogen retention was greatest when steers were fed a purified diet containing uric acid than when they received urea, biuret or urea phosphate (Oltjen et al., 1968). Plasma levels of hydroxyproline, alanine, methionine, isoleucine, tyrosine and phenylalanine were higher and a lower serine level was found when steers were fed this diet. In this study, the plasma "essential amino acid" concentration decreased 4 hr. after feeding compared to before feeding.

Virtanen (1966) reported lower levels of the essential amino acids in the blood plasma of lactating cows fed the urea purified diet than lactating cows fed a natural diet. Histidine was especially low in the plasma, whereas it was found in high concentration in microbial hydrolyzates. The concentrations
of histidine and many other amino acids were detected in much higher concentrations in the blood plasma of dry cows and heifers than in lactating cows. Conrad (1968) conducted studies in which the rate of methionine synthesis by ruminal microorganisms was determined, and based on these results indicated that histidine and methionine are apt to be limiting essential amino acids in milk production. Although the plasma amino acid results with growing ruminants fed the urea purified diet appear to be divergent to those reported for lactating cows, it is quite possible that the use being made of the amino acids, i.e., for growth or lactation can explain this difference.

Freitag et al. (1968) reported that substituting urea and corn for soybean meal in a natural diet in which urea supplied 70% of the dietary nitrogen resulted in a depression in the free amino acid content in the plasma. The amino acid levels were depressed after feeding compared to before feeding. Clifford and Tillman (1968) reported a slight increase in the free amino acid content of the plasma of sheep fed the urea purified diet compared to those fed the isolated soy diet, in contrast to other reports.

In many respects the depression in the essential amino acids observed in the blood plasma of ruminants fed the high NPN diets resembles protein malnutrition in humans (Munro and Allison, 1964). However, Freitag et al. (1968) reported that the blood plasma amino acid patterns of lambs fed a 7% protein diet did not resemble the patterns of lambs fed either a 7 or 11% crude protein equivalent diet in which urea was the primary nitrogen source. This indicated that the sheep were not affected by a simple protein deficiency.

Recently, Hepburn and Bradley (1968) reported that dietary additions of serine and glycine caused a reduction in gain and the effects of each were additive with rats. Inclusion of glutamine in the diet greatly reduced the inhibitory effects. A similar mechanism may be involved in the ruminant, and the high circulating levels of serine and glycine may be indicative of depressed growth. Results of plasma amino acid studies conducted so far at Beltsville indicate that "moderate" levels of isoleucine, leucine, valine, phenylalanine, tryosine and "depressed" levels of urea, serine and glycine may reflect good ruminant growth on purified diets.

Summary

The complete substitution of non-protein nitrogen for protein in purified diets for ruminants results in the following:

(1) Growth, feed efficiency and nitrogen retention are reduced by 35% and attempts to improve performance with amino acid supplementation, either singly or in combination, or by the use of other nitrogenous adjuncts have been largely unsuccessful.

(2) Reproduction has been demonstrated by heifers fed protein-free diets and bulls fed these diets are fertile. Several normal offspring have been obtained from bulls and heifers raised since 84 days of age on these diets. Cattle have been maintained for more than 4 yr. on diets without protein.

(3) A moderate milk production is possible when cows are fed diets devoid of protein and the composition of the milk is essentially similar to the milk produced from cows fed natural diets. Small additions of dietary protein seem to stimulate milk production.

(4) Ruminants fed diets free of protein have depressed concentrations of the branched-chain volatile fatty acids, but ruminal synthesis of the "B" vitamins is apparently normal.

(5) The amino acid pattern of ruminal protein hydrolyzates obtained from ruminants fed urea as the sole source of dietary nitrogen appears to be similar to the patterns from ruminants fed protein containing diets.

(6) Ruminants fed protein free diets have depressed free blood plasma concentrations of the "essential amino acids" and increased concentrations of glycine and serine.

Literature Cited


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