EFFECT OF LYSINE INFUSION PER ABOMASUM OF STEERS FED CONTINUOUSLY OR TWICE A DAY

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Roy John Boila

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ABSTRACT

Two 4 x 4 Latin Square Designs using two methods of feeding, continuous (Part A) or twice a day (Part B) were conducted with eight dairy steers. Within each square four levels of lysine (0, 3, 6 or 9 grams) were infused daily.

Nitrogen retention was significantly ($P<0.05$) lower for steers infused with 9 grams lysine daily in Part A. Urinary excretion of nitrogen (percent of intake nitrogen) was higher ($P<0.05$) and plasma free lysine was elevated ($P<0.05$). A significantly higher ($P<0.05$) rumen ammonia level, a slightly higher organic matter digestibility in the rumen (mainly as non nitrogenous dietary components) and a reduced rumen output of nitrogen to the abomasum (as a percent of intake nitrogen) were observed at 3 grams lysine infusion per day. The possible interrelationships at the 3 and 9 gram lysine infusions were discussed.

Nitrogen retention (percent of absorbed nitrogen retained) was not significantly different in Part B. However, a significant ($P<0.05$) increase in fecal nitrogen (percent of intake nitrogen) was found concomitant with plasma amino acids arginine, methionine, leucine, phenylalanine and tyrosine (plasma amino acids were significant P 0.15) peaking at 6 grams lysine infusion per

day. Amino acid interactions at absorption sites in the small intestine or metabolism sites in body tissue were indicated.

For both Parts A. and B. abomasal amino acids as a molar percent of total amino acids were similar except for lysine which was significantly higher ($P<0.05$) at the higher levels of lysine infusion in Part A. Total essential amino acids as a molar percent of total amino acids present, increased from diet (37.2%), to abomasum (averaging 44%), to plasma (50% in Part A., 55% in Part B.) over all treatment combinations. Abomasal lysine levels were two to three times dietary lysine levels.

Differences in rumen fermentation were observed when comparing the feeding schedules in Part A. with Part B. The effect of feeding on rumen fermentation, and microbial population variation could have affected subsequent amino acids available for absorption in the small intestine. Consequently the animals would have had different responses to lysine infusion within squares as has been shown to have occurred.

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INTRODUCTION

The ruminant animal contains a population of microflora and microfauna in the rumen. Consequently much of the protein available to the ruminant is of microbial origin and dietary deficiences of amino acids are more difficult to define.

In specifying the amino acid requirement of a ruminant, the dietary amino acid content is of little help in determining a deficiency in body tissue. A high level of dietary protein conversion (range 25-90%) to microbial protein (McDonald and Hall, 1957; Ely et al., 1966) has been shown to occur. The deficiency therefore must be related to the bacterial and protozoal protein contribution to the dietary protein in the lower gastrointestinal tract. The levels of amino acids in the abomasum and their availability, release from proteins by means of proteolytic enzyme degradation, have been studied in order to resolve an amino acid deficiency in the rumen digesta.

Dietary supplementation of rumen digesta with amino acids could have been undertaken if the protein or amino acid was protected from degradation in the rumen. Chemical treatment of proteins (formaldehyde or tannin) and coating proteins or amino acids with a rumen non digestible compound are two ways to prevent or slow down the microbial action on the α-amino nitrogen source. Experimentally, abomasal cannulation has allowed the

infusion of amino acids per abomasum (Schelling and Hatfield, 1968; Nimrick et al., 1970a, b; Reis and Schinkel, 1963, 1964). Pure proteins (casein mainly) were infused as well as amino acids singly or in combination. The sulphur amino acids, methionine and cysteine, as well as lysine have been investigated quite extensively. Methionine was indicated first limiting, and lysine second limiting in rumen digesta (microflora dominated) from a urea diet fed to sheep (Nimrick et al., 1970b). A corn based diet has been shown to be limiting in lysine for monogastric animals. Infusion of lysine per abomasum has increased nitrogen retention in steers (Devlin and Woods, 1964).

The present experiment was designed to study the effect of lysine infusion per abomasum in steers on nitrogen balance and other nitrogen parameters such as blood urea nitrogen, rumen ammonia, the nitrogen levels in the abomasum, plasma amino acids, plus digestibilities of dietary components other than nitrogen in the rumen, under feeding regimes of continuous or twice a day at twelve hour intervals. The two feeding methods were used to determine if continuous feeding would result in less total variation in factors such as nitrogen balance, plasma amino acids and rumen fermentation characteristics than twice a day feeding.

LITERATURE REVIEW

Nitrogen metabolism in the rumen

Domestic ruminating animals, cattle and sheep, have maintained an environment within their digestive tracts in which a population of microflora and microfauna has been established. The rumen microbial population metabolized the animal's dietary components, proteins, fats and carbohydrates and the resulting products passed to the lower gastrointestinal tract in proportions which differ from the original dietary intake. Certain fermentation products such as ammonia and volatile fatty acids are absorbed across the rumen epithelium. The microbial population is specifically effective in changing amino acid levels and proportions in the digesta leaving the rumen relative to the diet. The microbial breakdown of fiber components, cellulose, hemicellulose and to some extent lignin, is presented in a more readily available form of carbohydrate to the host animal.

The ruminant animal is able to utilize organic nitrogen sources such as true protein and non protein sources such as urea and nitrates. In each case the nitrogen components are eventually degraded to the level of ammonia (McDonald, 1952) and used in the synthesis of microbial protein (McDonald, 1954; Ely, Little, Woolfolk and Mitchell, 1967). With reference to proteins, proteolytic activity of the microbial population yields

the constituent amino acids and the amino acids are immediately deaminated to yield ammonia and the corresponding carbon chain (Lewis, 1955). Very little free amino nitrogen (Lewis, 1955) or amino acids as such (Leibholz, 1965) are found in the rumen liquor. Therefore, the most available source of nitrogen in the rumen is ammonia, while the remainder of the nitrogen component would be protein nitrogen from dietary or microbial sources.

Bryant and Robinson (1962) cultured 89 freshly isolated strains of bacteria for their ability to grow on certain nitrogen sources. The data indicated 13% grew poorly or not at all on the defined medium plus casein hydrolysate, 6% required casein hydrolysate, 56% grew either on ammonium or casein hydrolysate and 25% grew on ammonium but not casein hydrolysate. Approximately 81% of the strains of rumen bacteria studied were able to utilize ammonia as the ammonium ion for the synthesis of protein. The bacterial population had adapted itself to the type of nitrogen source available.

Proteolytic activity in the rumen (Blackburn and Hobson, 1960a) has been associated with the microbial population, specifically protozoa and large bacteria with less activity in small bacteria and very little in the supernatant. The end products of casein degradation (Abou Akkada and Blackburn, 1963) were mainly amino acids and polypeptides, but no dipeptides. The majority of the

proteolysis was by way of endopeptidase activity, which was totally in agreement with Blackburn and Hobson (1960a). Ammonia was preferentially used for the synthesis of protein by bacteria as has been shown (Bryant and Robinson, 1962), although according to Wright (1967), peptide nitrogen appeared to be more efficiently utilized by certain bacteria than free amino acids. Peptides may provide a source of amino acids essential for growth of bacteria. These amino acids, as free amino acids in rumen fluid, may otherwise be more readily converted to ammonia by other rumen microbes.

Protozoa, as examplified by ciliates (Coleman, 1963), have not been shown to utilize food protein directly. Their nitrogen requirement could most likely have been met by the intake of bacteria and the utilization of this preformed protein (Abou Akkada, 1965). The breakdown products of protein digestion by Entodinium were amino acids and peptides (Abou Akkada and Howard, 1962) with only 10% of the degradation products having been ammonia.

Ammonia diffused readily across the rumen wall at pH 6.5. Rumen pH of 7.0 was considered normal; however deviations to less than pH 7.0 were common. The concentration gradient at high levels of ammonia production in the rumen was from rumen to plasma according to the Henderson-Hasselbalch equation (Hogan, 1961). As pH was lowered, the ammonia diffusion reversed and

moved freely from plasma to rumen contents. The low pH values (pH 4.5) at which this occurs were not normally encountered in the rumen. Levels of ammonia in the rumen of 0-150 mg per 100 ml of rumen fluid (Hungate, 1966) allowed for diffusion in either direction at usual pH values.

Urea as a nonprotein nitrogen source (Chalupa, 1968) will be degraded easily by urease to ammonia and carbon dioxide in the rumen. Limited use has been made of urea in diets as a high level of ammonia production could result in a toxic effect (Morris and Payne, 1969). Other than the toxicity factor, urea as a dietary source of nitrogen has proved to be very useful in reducing the levels of true protein fed to ruminants.

Urea recycling via saliva and transport across the rumen epithelium into the rumen are important sources of nitrogen to the ruminant. Studies conducted by Somers (1961 a, b, c, d) in sheep, and Bailey and Balch (1961 a, b) in cattle, have reported on the salivary contribution to the nitrogen pool in the rumen. Urea provided 60-70% of the total salivary nitrogen in sheep and approximately 75-80% in cattle. Total salivary nitrogen was correlated with nitrogen intake ($r=0.9940$), and blood urea nitrogen ($r=0.9625$) (Somers, 1961b). Salivary nitrogen levels were maintained at approximately 65% of plasma levels of 4-19 mg. urea nitrogen per 100 ml of plasma (Bailey and Balch, 1961b). The limit to salivary transport was about 30 mg. total nitrogen per

100 ml or 25 mg urea nitrogen per 100 ml of saliva (Somers, 1961d).

Plasma urea was also transported across the ruminal epithelium to the rumen. Early work (Houpt and Houpt, 1964), using washed antibiotic treated rumen pouch studies in the goat, indicated that urea diffused freely across the rumen wall due to the concentration gradient, whether it be from rumen to plasma or vice versa. Subsequent work (Houpt and Houpt, 1968), using essentially non washed and non treated rumen pouches, allowed the postulation of a urea transport system involving bacterial urease. Bacterial urease has been found in rumen epithelium, and has been proved to be of bacterial origin (Rahman and Decker, 1966). Urea diffused from plasma to rumen from a high to a low concentration gradient. This urea was reacted upon by bacterial urease and metabolized to carbon dioxide and ammonia. Ammonia, having a greater diffusability and being more lipid soluble than urea, diffused at a greater rate into the rumen fluid and was utilized by the rumen mocrobial population. More recently, Thorlacius, Dobson and Sellers (1971) stimulated both urea and ammonia transport across the rumen epithelium by the use of a 100% carbon dioxide atmosphere in either isolated caudodorsal or ventral sacs of the rumen. The above mechanisms of urea transport have been studied under artificial situations. In animals on normal diets, varying plasma urea by infusion of urea intravenously, the

limits of urea transport were established to be 16-18 mg. blood urea nitrogen per 100 ml plasma in cattle (Vercoe, 1969) and 10-12 mg. blood urea nitrogen per 100 ml plasma in sheep (Weston and Hogan, 1967). The type of urea transport mechanism actually functioning has not been defined. A true transport of urea across the ruminal epithelium into the rumen has been clearly indicated in the most recent results (Houpt and Houpt, 1968; Thorlacius et al., 1971). Failure has been to coordinate results from all experimental evidence to provide for a firm conclusion.

The transport of urea across the ruminal epithelium contributes to nitrogen conservation within the animal. Ammonia produced in the rumen, absorbed into the blood stream and converted to urea in the liver, provided a source of urea in the body. Rumen derived urea, as well as urea from metabolism of nitrogen components such as amino acids and plasma proteins, was recycled to the rumen for use by the rumen microbial population. Recycling was very critical in an animal on a low nitrogen diet, as the recycled nitrogen maintained a minimal level of rumen fermentation to support the host animal through the microbial protein contribution in the abomasum.

The most important factor of nitrogen metabolism in the rumen was the utilization of basic nitrogen sources by the microbial population for the synthesis of microbial protein. This microbial synthesized protein, as well as

unfermented protein of dietary origin, in total constituted the protein fraction reaching the lower gastrointestinal tract. Urea recycling via saliva or the rumen epithelium at times contributed to the nitrogen pool in the rumen to such an extent as to yield total nitrogen per day greater than the nitrogen intake (Clarke, Ellinger and Phillipson, 1966). The next factors to be considered will be the proportion of the microbial protein reaching the intestines, the quality of this protein as per rat feeding trials and various amino acid deficiencies that one could possibly encounter.

Initial work on the conversion of dietary proteins to microbial proteins was done using pure proteins (McDonald, 1954; McDonald and Hall, 1957). McDonald and Hall (1957), feeding casein as 87% of the nitrogen of a purified diet to sheep, recovered 90% as microbial protein. Studies with sheep fed zein exhibited a lower conversion of zein protein to microbial protein. This was related to the insolubility of zein in water and its low level of lysine. McDonald (1954) recovered 40% of zein as microbial protein, while Ely et al.(1967) demonstrated zein protein conversion to be 26.3% on a high cellulose (49.8%) diet and 30.5% on a high starch (42.7%) diet. Starch provided a more readily available source of carbohydrate for the microbial population to utilize in aiding the conversion of zein protein to microbial protein.

Weller, Gray and Pilgrim (1958), using lignin as a marker for dietary nitrogen of wheaten hay and diaminopimelic acid as a marker for bacterial nitrogen, analyzed the contribution in the abomasum of sheep to be 61-82% of nitrogen as microbial nitrogen, 11-27% as plant nitrogen and 5-10% as soluble nitrogen. Using the presence of diaminopimelic acid in bacteria and its absence in protozoa, bacterial nitrogen contributed 40-50% of the total nitrogen. Hutton, Bailey and Annison (1971) were in agreement with the 50% contribution of bacterial nitrogen in the abomasum. In comparing the contribution of microbial protein to total protein in the abomasum, one must take into account several factors that may have affected the results. The insolubility of zein did not make the protein readily available for degradation, nor did a deficiency of lysine allow for adequate microbial growth, unless the amino acid was synthesized by the microorganisms themselves. Casein, on the other hand, was highly water soluble and contained an adequate supply of amino acids. Weller et al. (1958) worked with a diet that more closely duplicated a natural diet. Results were found to be intermediate between 40% and 90%. Difficulties in separation techniques, a variable bacterial content of diaminopimelic acid (Synge, 1953) as well as the fact that lignin was digested to some extent (Porter and Singleton, 1971) could either have underestimated or

overestimated the results. The conversion of dietary protein to microbial protein varied relative to solubility, amino acid content, and general availability of carbohydrate as an energy source. Certain dietary proteins were converted to microbial protein to an extensive degree and subsequently the effect of the rumen was more pronounced relative to the influence of the dietary protein.

Further studies on the microbial contribution to total protein leaving the rumen of sheep were recently published using grams of protein synthesized per 100 grams of organic matter (OM) digested as the standard for conversion. Factors studied were the level of nitrogen intake (Hume, Moir and Somers, 1970a), the response to energy sources such as volatile fatty acids (Hume, 1970b), the effect of dietary protein (Hume, 1970c) and the influence of sulphur (Hume and Bird, 1970d). Hume et al. (1970a) obtained a factor of 9.1 gm. protein per 100 gm. OM digested when nitrogen was most limiting at 2 grams per day and 13.3 gm. per 100 gm. OM digested when nitrogen was in excess at 16 grams per day. An adequate intake level of nitrogen was cited as approximately 9 gm. per day, the level at which maximum conversion of dietary protein to microbial protein occurred. Major factors involved were the recycling and reuse of urea nitrogen as ammonia by the microorganisms on the nitrogen limiting diet and the rumen nitrogen loss as ammonia on the high nitrogen diet.

Soluble carbohydrate energy sources yielded a high level of short chain fatty acids in the rumen. The microbial population has been shown to require a source of long chain and branched chain fatty acids for the synthesis of amino acids. Hume (1970b) increased the yield from 12.5 to 13.4 gm. of microbial protein per 100 gm. OM digested when feeding an additional quantity of isobutyric, 2-methylbutyric, isovaleric and valeric acids. Long chain, rather than short chain fatty acids, stimulated microbial growth. Hume (1970c) corroborated previous data on microbial protein synthesis (McDonald and Hall, 1957; Ely et al., 1967) in relation to the water solubility of protein in the rumen and the availability of the dietary protein to microbial degradation. Preformed proteins may have been important as a supply of peptides (Wright, 1967) or undegraded amino acids. Sulphur as sulphate (Bray and Hemsley, 1969) and as sulphur containing amino acids cysteine and methionine (Hume and Bird, 1970d) increased the level of microbial protein yield in the rumen. The microorganisms could have been growing in a medium deficient in sulphur containing amino acids. Urea containing purified diets have been shown to be first limiting in methionine (Nimrick, Hatfield, Kaminski and Owens, 1970b).

The protein composition of the rumen digesta under various dietary conditions has been studied in relation to the availability of amino acids in the

subfractions of dietary, bacterial and protozoal protein sources. Several workers (McNaught, Owen, Henry, and Kon, 1954; Bergen, Purser and Cline, 1968a) have isolated mixed preparations of bacteria and protozoa and determined the amino acid content, as well as having established deficiences in bacteria and protozoa relative to feeding the preparation to rats. McNaught et al. (1954) determined true digestibility (TD), biological value (BV) and Net Protein Utilization (NPU) by feeding isolated bacterial and protozoal protein to rats. For bacteria TD, BV and NPU were 74, 81 and 60 respectively, while for protozoa TD, BV and NPU were 90, 80 and 73 respectively. This work has since been substantiated by Bergen et al. (1968a). Bacteria and protozoa contained different levels of crude protein calculated at approximately 35% and 50% respectively (Bergen et al., 1968a). Whether it was a difference in nitrogen content or a difference in amino acid proportions, the effect on digestibility differences could not be explained. Amino acids have been demonstrated to interact with respect to availability and competition at absorption sites. A factor that must be taken into account was the amino acid composition of bacterial cell walls and the availability of these cell wall amino acids (Hoogenraad and Hird, 1970). Biological values of bacteria and protozoa were similar, each averaging about 80. It would be interesting to determine if the biological value of a

mixed population was greater than a pure population of bacteria or protozoa. Limiting amino acids in bacterial and protozoal protein for growing rats will be discussed elsewhere.

At about the same time as gross nitrogen parameters of the microbial protein contribution to the host were being studied, the essential amino acids for ruminant tissue had been established (Black Kleiber, Smith and Stewart, 1957; Downes, 1961). According to present knowledge the essential amino acids for the ruminant, at the tissue level, were listed as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, histidine and arginine (Theurer, Woods and Poley, 1966). Tyrosine could possibly have been added to the list as it did not exhibit radioactive ^{14}C due to synthesis by body tissues. Tryptophan and cysteine had not been included as analysis techniques limited estimation of their values.

Taking into account the essential amino acid requirement of the ruminant, several workers have endeavoured to evaluate the protein quality of bacterial and protozoal proteins. The correlation between amino acid analyses (Purser, 1970b) of bacterial protein as determined by Weller (1957) and 22 strains of bacteria by Purser and Buechler (1966) was very high. Even though there was a difference in analytical procedures, as well as diet differences, there was a remarkable agreement. All data

available pointed to a uniformity of analysis of bacterial or protozoal preparations as well as mixed populations (Schelling, Hinds and Hatfield, 1967). Slight differences were shown to exist between the amino acid composition of protozoa and bacteria, protozoa having a higher level of lysine, leucine, phenylalanine and tyrosine. Bergen et al. (1968a) indicated the most likely limiting amino acids in bacterial and protozoal protein when fed to rats. For rumen bacteria, cystine was predicted as the limiting amino acid, whereas arginine, histidine, leucine and lysine were indicated as the next four least available amino acids for rat growth. For protozoal protein, histidine was predicted as the most limiting, whereas cystine, arginine, valine, leucine and threonine were indicated as the next five least available amino acids for rat growth. Thus it can be seen that the contribution of bacteria and protozoa was a relatively homogeneous level of amino acids made available to the host animal. Any differences encountered in the host reaction could possibly have been attributed to the dietary amino acid source (Purser, 1970a).

The protein quality of rumen bacteria (Bergen, Purser and Cline, 1967) was determined using an in vitro pepsin - pancreatin digest system and Essential Amino Acid Indices (EAAI). The pepsin - pancreatin digest index combined essential amino acid factors in both the digested

protein and the undigested protein. Digestibilities were calculated using the amino acid released versus the total amino acid in the protein. A combination of the pepsin - pancreatin digest index and digestibility yielded a high correlation with biological values from feeding trials (Akeson and Stahmann, 1964). Egg protein was used as a standard with a Biological Value of 100 for both the *in vitro* digest system and EAAI. EAAI related essential amino acids in the test protein versus egg values in one calculated index. As with several other workers, egg protein was used as a standard for ruminants. Whether egg protein could have been classed as being the best still remained to be seen. The normal biological value of egg protein in rats was 100, while for ruminants this was reduced to 80 due to the influence of the microbial use of nitrogen in the synthesis of RNA and DNA (Ellis and Pfander, 1965). Rumen bacteria (Bergen *et al.*, 1967) exhibited a wide range of variability for calculated Essential Amino Acid Indices, Biological Value, and digestibility. Amino acids, leucine, phenylalanine, tyrosine, valine, methionine and lysine in combination or alone in the 10 strains of bacteria studied, had a low ratio when compared to egg protein. Cellulolytic bacteria appeared to contain a lower quality protein. Analysis of the digestion mixture revealed a wide range of free amino acids, soluble amino acids and insoluble amino acids as a

percent of total amino acids present. At any time in the rumen no population of bacteria dominated. According to this method of evaluation, several bacterial strains would not have yielded similar results to the host animal. Again, using egg protein as a standard, Bergen (1969) hydrolyzed egg, protozoal and bacterial protein for 30, 60 and 150 minutes in a peptic digest and evaluated the resulting mixtures in vitro for their effect on histidine and methionine uptake by rat jejeunal rings. The amino acids, released as a result of peptic digestion, yielded more realistic results than simulation of dietary protein amino acid proportions. With an amino acid mixture simulating a peptic digest of egg or bacterial protein, uptake of histidine or methionine increased as their levels increased in the mixture, while for the protozoal digest mixture, the absorption of histidine or methionine decreased. Effective transport inhibitors (other amino acids) in protozoal protein had become liberated in increasing quantities as did histidine and methionine. It appeared that despite the similarities of amino acid composition between various microorganisms, the amino acid proportions available to the lower gastrointestinal tract varied according to which organism was prevalent at the time. Variable amino acid proportions have been shown to affect the uptake of amino acids at absorption sites in the small intestine (Bergen, 1969). As a consequence the ruminant would have been affected by

the type of free amino acid spectrum present in the small intestine and as a result the subsequent metabolism of amino acids within the tissues would have been altered.

A further consideration of nitrogen in the rumen was the contribution of nucleotide nitrogen of RNA and DNA. Nitrogen contributed in this manner should have been almost totally of microbial origin (Smith and McAllan, 1970) as dietary sources were readily degraded in the rumen. The level of nitrogen that appeared as polynucleotide nitrogen varied according to the level of microbial activity. Data which denoted the contribution of polynucleotide nitrogen to the abomasum as a percent of total nitrogen present varied from 5% to 14%, (Ellis and Pfander, 1965; Purser and Buechler, 1966; Little, Mitchell and Potter, 1968). This portion of nitrogen reaching the abomasum was almost totally lost to the host animal. An active pancreatic ribonuclease activity in the small intestine of ruminants (Barnard, 1969) has been attributed with the recycling of nucleotide phosphorus in saliva and the subsequent reuse of phosphorus in the rumen. Body metabolites of RNA and DNA were excreted as allantoin and uric acid, the greater proportion having been allantoin (Topps and Elliott, 1965).

The presence of a microbial population of bacteria and protozoa has proven at times to be both detrimental and beneficial to the host animal. Low grade roughages have been improved in protein quantity and quality by microbial

use of recycled urea via saliva and ruminal avenues in the synthesis of microbial protein. High protein diets have resulted in nitrogen losses via ammonia production by the microbial population in the rumen, above that necessary for microbial growth. As a result attempts have been made to limit microbial action under certain conditions, while still having maintained a benefit to the host animal. Much more research must be undertaken in order to gain an understanding of some of the basic reactions within the rumen and their ultimate effect on the ruminant.

Nitrogen fraction in the lower gastrointestinal tract

Nitrogen entering the abomasum could be classified as dietary nitrogen and microbial nitrogen, plus a small soluble fraction. Another basis for classification could be total true protein nitrogen and non protein nitrogen, subdivided into free amino acids, bound amino acids (peptides - non trichloracetic acid soluble) and purine and pyrimidine nitrogen. The various forms of nitrogen entering the lower gastrointestinal tract can be utilized quite adequately by the ruminant.

The enzymatic aspects of ruminant and monogastric digestive systems have been shown similar in reaction once the rumen was by-passed (Purser, 1970a). At present some small physiological differences have been demonstrated. Amino acid interactions at absorption sites have been judged to be similar, although relative rates of absorption were different (Williams, 1969). The major difference was the composition of the digesta reaching the true stomach, as well as the pH changes along the gastrointestinal tract. As Purser (1970a) has stated - "ruminant nutritionists have been lax in applying available information from work with nonruminant animals

to the ruminant situation".

This section will deal with the fractions of nitrogen entering the abomasum from the rumen, endogenous nitrogen sources, and the subsequent absorption of both endogenous and exogenous nitrogen. The absorption of amino acids will be discussed in so far as to indicate points of absorption and interrelationships within sites of absorption. The effect of the larger intestine will follow as a complementary factor to the microbial nitrogen metabolism of the rumen.

Little et al. (1968) fed soybean, zein, casein and gelatin to sheep with abomasal nitrogen fractions divided into protein nitrogen and non protein nitrogen (free and bound amino acid nitrogen, purine and pyrimidine nitrogen). Zein yielded a level of nitrogen in the abomasum of 104%, as a percent of dietary nitrogen, which was significantly higher ($P<0.05$) than the other protein sources mainly because of its lower water solubility. The type of protein source influenced the quantitative and qualitative aspect of the nigrogen fractions reaching the lower gastrointestinal tract. In order to be of benefit to the animal, the microbial contribution must yield a better balance of the essential amino acids for the animal.

The ruminant animal can utilize a wide spectrum of diets ranging from 100% roughage to 100% concentrate and the various combinations, with protein content varying from near inadequate to more than ample levels of protein intake. A diet such as poor hay (Harris and Phillipson, 1962) resulted in a 50% gain in total nitrogen entering the abomasum, while a high protein diet resulted in a nitrogen loss as ammonia in the rumen (Hogan and Phillipson, 1960). Although secretion of pepsinogen by the abomasum could have masked the dietary plus rumen effect, secretion was relatively constant over a 24 hour period (Ash, 1961) and as a result could have equalled the nitrogen lost as ammonia in the rumen. Several workers (Boyne, Campbell, Davidson and Cuthbertson, 1956; Kay and Phillipson, 1964; Gray, Pilgrim and Weller, 1958) were in agreement with the above principles.

An extensive study on the intake levels of nitrogen and the subsequent quantities reaching the duodenum and ileum and excreted in feces in sheep was undertaken by Clarke, Ellinger and Phillipson (1966). Diets fed were hay and hay supplemented with flaked maize, soybean or both. It was found that when feeding a range of nitrogen levels

(four diets of approximately 5, 7, 16 and 25 grams nitrogen per day) gains in total nitrogen were demonstrated from feed to duodenum with the three lower nitrogen intakes, while nitrogen losses were evident once the absorption sites in the small intestine had been passed. The greatest portion of nitrogen loss on the high nitrogen diet (25 gm. nitrogen intake per day) was as ammonia in the rumen, and the inability for absorption to occur in the small intestine as a more than ample supply of nitrogen flowed past absorption sites. A most striking feature was that once the digesta had reached the abomasum a dietary difference of approximately 20 grams between highest and lowest nitrogen diets was reduced to approximately 10 grams in the abomasum. The microbial effect increased the rumen output of protein on the low protein diet and decreased the protein output on the high protein diet.

A major portion of the study (Clarke et al., 1966) delved into the complexities of amino acid levels and ratios along the digestive tract. Some amino acids such as cystine and valine were in the same proportion in food and duodenum, others such as proline, arginine and leucine formed a smaller fraction in the duodenum while still others such as lysine, threonine and isoleucine formed a greater fraction in the duodenum. Rumen fermentation had increased dietary levels of both essential and non essential amino acids. Data of Clarke et al. (1966)

indicated that the proportions of certain amino acids in the small intestine acquired some of the characteristics of endogenous protein sources.

During the course of a day a large quantity of nitrogen entered the small intestine from endogenous sources and from the sloughed tissue of the intestinal mucosa. Less than one third of the metabolic fecal nitrogen was contributed by endogenous secretions (Hogan and Weston, 1968). The other two thirds appeared to arise in the stomach, possibly as undigested microbial protein which to some extent had been synthesized from recycled nitrogen sources. The importance of endogenous nitrogen has been hypothesized by Nasset and Ju (1961) to provide a constant level of amino acids both in total and in proportion to one another. Analysis of midjejeunal contents of dogs confirmed this relationship. Twombley and Meyer (1961) indicated that the contribution of endogenous nitrogen to the digesta in the intestine was several times greater than the nitrogen intake. Endogenous secretions could have been important in maintaining a steady state of amino acid levels in the intestine. The endogenous protein contribution was expected to have had a masking effect on the identity of the dietary amino acid spectrum. However, this effect did not support the fact that free plasma amino acids reflected the concentrations of amino acids in the diet (Wiseman, 1968). Mettrick (1970) stated that

endogenous nitrogen did not contribute to the nitrogenous homeostasis in the small intestine of rats as an egg and a casein diet produced arrays of amino acids along the small intestine very similar to the dietary array. The addition of endogenous nitrogen into the digesta makes an important contribution to the amino acids available to the animal.

Amino acid transport mechanisms in the small intestine have been demonstrated to be highly competitive (Christensen, 1963; Orten, 1963; Jacobs, 1965; Wiseman, 1968). Definite patterns of absorption exist. Wiseman (1968) listed a possibility of five different functional sites that had affinities for groups of amino acids. Each site had a high affinity for one group of amino acids such as the basic amino acids lysine, arginine and ornithine with cystine included (Hagihira, Lin, Samiy and Wilson, 1961), or for valine, isoleucine and leucine (Hagihira, Ogata, Takedatsu and Suda, 1960). An amino acid imbalance could possibly have been attributed to competition which occurred at absorption sites. Other factors influencing amino acid transport were starvation (Steiner and Gray, 1969), competition by other absorbable compounds such as sugars (Reiser and Christiansen, 1969), as well as any amino acid analogue which may bind the transport site more readily than the amino acid itself.

Amino acid absorption studies have been undertaken using monogastric tissue, and the results have been interpreted in ruminant studies. Not until recently have any species differences been demonstrated. Williams (1969) revealed a difference between the relative rates of absorption of amino acids from the small intestine of sheep. This study was done *in vitro* in a neutral (pH 7.0) solution. However, the pattern of pH in the small intestine was a spectrum from approximately pH2.5 in the abomasum to pH 7.5-8.0 in the ileum in sheep (Kay, 1969). It may have been that the amino acid transport sites in a ruminant had changed in affinity for amino acids according to the pH of the intestinal contents. A lowering of the pH in monogastric tissue had a stimulatory effect on amino acid transport mechanisms (Thompson, Levin and Jackson, 1970). Amino acid absorption affected body metabolism through free plasma amino acid levels, and dietary deficiences were readily established using plasma amino acids levels (Christensen, 1963).

A contributing factor in nitrogen metabolism, which was more important in monogastrics than ruminants, was the presence of an active caecal fermentation. Houpt (1963), using rabbits as an experimental animal, found that urea metabolism in the caecum resembled the rumen mechanism. A certain amount of nitrogen cycling did exist. The amino acid profiles in the caeca of ponies (Reitnour,

Baker, Mitchell, Little and Kratzner, 1970) indicated a definite contribution to the caecal content with caecal molar percentages of leucine and phenylalanine having shown a correlation with serum proportions. The level of caecal fermentation was lower in ruminants (Faichney, 1968) as the greater portion of the carbohydrate intake was fermented in the rumen. As the anatomical placement of the large intestine could only have contributed to a very small portion to the total body pool of nitrogen, except in coprophagic animals (Houpt, 1963), caecal fermentation has proved to be of minor importance in comparison with rumen fermentation.

Infusion of amino acids and proteins into the abomasum

Dietary supplementation of amino acids has not been practical as they are subjected to deamination by the rumen microbes. No data conclusively (Hatfield, 1970) substantiated the need for dietary supplementation. In order to bypass rumen degradation, studies have been conducted on the protection of proteins and amino acids from rumen fermentation (Sibbald, Loughheed, and Linton, 1968) subsequently making the nitrogen source available to the host by means of lower tract acidic action on the protective covering. Chemical treatments by formaldehyde, or tannin have been attempted to reduce the availability of preformed proteins to the rumen population.

Reis (1969) and Reis and Schinkel (1961, 1963, 1964) infused either casein or sulphur containing amino acids into the abomasum of sheep and found an increase in wool growth. Casein (Reis and Schinkel, 1964) infused at a level of 60 grams per day increased wool growth substantially (123-181%). Suspecting the result as a consequence of sulphur containing amino acids, methionine and cystine were infused. A comparable 100% increase in wool growth resulted. Wool was found to contain a high level of the sulphur containing amino acids, cystine and methionine (Broad, Gillespie and Reis, 1970). The

increased wool growth could have been attributed to a greater availability of these two amino acids.

Attention at present has shifted to abomasal infusion of amino acids singly or a combination. Schelling and Hatfield (1968) infusing continuously over a 13 hour period daily (9 AM to 10 PM) found a considerable number of amino acid interactions. The amino acids L-lysine and L-glutamate produced the greatest response when infused singly, while a combination of arginine, histidine, lysine, phenylalanine and methionine produced a similar response to that of lysine alone. An important consideration in this study was the limited presence of a viable protozoal population in the rumen as the result of feeding a semipurified diet.

Nimerick et al. (1970 a,b) fed sheep a semi-purified diet similar to that of Schelling and Hatfield (1968). The amino acids methionine, lysine, threonine, tryptophan, histidine and leucine were infused singly and in combination over a 20 hour period. Nitrogen retention and free plasma amino acid levels were used to evaluate the efficiency of each infusion. As with Schelling and Hatfield (1968) protozoa were not established in the rumen to an extent that the contribution of protozoal protein to the total protein in the rumen was significant. Methionine, lysine and threonine were indicated deficient and limiting in the order mentioned in rumen bacteria.

Lysine infused alone reduced dietary nitrogen retention. Plasma methionine remained low and constant until the requirement was met. Further increases in infusion levels resulted in elevated plasma methionine levels. The same relation applied to infusion of threonine and plasma levels of threonine. This evidence was the first that indicated an order of limitation that existed in the intestinal digesta.

Individual amino acids have been infused by several workers. Infusion of cystine and methionine into sheep (Reis, 1967), lysine at 9 grams per day in steers (Devlin and Woods, 1964), graded levels of lysine both fed and infused in total to yield 6.45 grams lysine per day in sheep (Moore, Little, Scott and Mitchell, 1970), and lysine at 0.0, 2.5, 5.0 and 7.5 grams per day in sheep (Hrytsak, 1970) did not yield any definite patterns of response. In so far as could be explained using the above levels, amino acids, specifically methionine and lysine, could be found to be limiting under varying dietary conditions.

Evaluation of Nitrogen Status

Biological Value

Biological value (BV) of protein denoted the measure of protein quality obtained from animal studies where percent values were calculated. The calculation of BV was as follows:

$$BV = \frac{\text{N intake} - (\text{fecal N} + \text{urinary N})}{\text{N intake} - \text{fecal N}} \times 100$$

This value accounted for growth, while the Thomas-Mitchell Method took into account the maintenance requirements (H.H. Mitchell, 1924). Endogenous urinary nitrogen as well as metabolic fecal nitrogen were considered. Fecal output was corrected for the metabolic fecal nitrogen (MFN) (fecal N-MFN) and the urinary output was corrected for endogenous urinary nitrogen (EUN) (urinary N-EUN).

In the case of a ruminant, calculation of EUN and MFN was difficult as recycling of nitrogen in the body precluded the feeding of a protein free diet to determine the two values. An equation disregarding EUN and MFN gave an indication of the retention of nitrogen fed as calculated from a nitrogen balance trial.

Rumen Ammonia, Blood Urea Nitrogen

Blood urea nitrogen (BUN) has given an indication of the nitrogen status of the body with respect to the

activity of the urea cycle in the liver in utilizing ammonia absorbed from the rumen, or as amino acids metabolized to ammonia. A pattern of BUN in jugular blood over the day closely followed the peaks of ammonia production in the rumen (Lewis, 1958). When BUN was used as a measurement of nitrogen status, the interpretation was related to recycling of urea in apposition to metabolism of excess amino acids absorbed above that required by the body.

Plasma Amino Acids

A portion of the amino acids in the body were found in a free form in the plasma (Christensen, 1964). Levels of these free amino acids were a reflection of the contribution from absorption sites in the small intestines, storage sites in the body such as muscle tissue (Munro, 1970), as well as the metabolism of amino acids in the liver and other body tissues. Using a method such as Plasma Amino Acid Scores (Longenecker and Hause, 1959) the order of limiting amino acids in the diet were determined.

Plasma amino acid levels have been well documented for monogastric animals, and used as a standard of nitrogen status. In ruminants, true plasma amino acid scores could not be determined using a fasted animal as the basis for the calculation. Plasma free amino acid analyses in ruminants have a variety of areas

of study which included a comparison of portal and jugular levels (Theurer, Woods and Poley, 1966), effect of protein digestion (Hogan, Weston and Lindsay, 1968), defaunation effects (Purser, Klopfenstein and Cline, 1966), starvation (Leibholz and Cook, 1967), as well as purified diets (Oltjen and Putnam, 1966) and general considerations as an aid to interpretation of plasma amino acids as a support for other experimental data. Nimrick et al. (1970b) were the first to use plasma amino acid levels as a measure of the availability of amino acids and to determine the order of limitation in the rumen digesta. From the above it can be seen that ground has been broken for the utilization of plasma amino acid data in ruminants. Further work must be undertaken in order to establish guidelines of interpretation.

Diurnal Rhythms

Diurnal patterns of exogenous and endogenous origin influence animals. Exogenous factors of temperature and daylength measured as hours of sunlight follow a circadian (24 hour) rhythm. Several factors influenced the total aspect of endogenous rhythms. These include various enzymes and plasma amino acid patterns as well as times of ingestion of feed. Feed intake has been found to have an effect on flow patterns along the gastrointestinal tract. Interpreting rhythms appeared to be difficult with respect to the determination of phasic patterns and subsequently resolving the source of variation.

Exogenous Rhythms (Bunning, 1964)

Daily environmental temperatures fluctuate both diurnally and seasonally, the higher temperatures usually having been recorded during the daylight period of the light-dark phases of daylength and the summer season. Seasonal daylength variation has been shown to affect breeding and migration cycles. Daylength has affected the wild animal with respect to the control of diurnal rhythm of feed intake and rest. This control has been shown to occur as long as some natural or controlled lighting sequence reached the animal. Cyclical feeding arrangements plus other external cues could alone have

reinforced diurnal rhythms which had previously been induced by daylength and temperature variations. (Minson and Cowper, 1966; Gordon and McAllister, 1970).

Endogenous Rhythms (Wurtman, 1970)

Diurnal rhythmicity within the body was influenced by such factors as hormonal secretions, body temperature rhythms, feeding schedules and to some extent exogenous factors. Certain hepatic enzymes such as drug metabolizing enzymes and amino acid catabolizing enzymes exhibited a circadian cycle.

Plasma amino acid patterns in the blood followed a diurnal pattern (Wurtman, 1970). Shifts in the pattern could have resulted from changes in the sleep cycle, the times of physical activity, the hours of food ingestion, the light-dark cycle or any other cyclic behaviour or environmental input. It was found that cycles remained in evidence when the animals were on a non protein diet. Free plasma amino acid levels were compared in portal and jugular blood (Theurer, Woods and Poley, 1966). Levels in lambs varied over the 24 hour collection period, (animals having been fed only once on the day of blood collection) with the highest concentration of many individual amino acids occurring 24 hours postprandial. Jugular levels at 12 hours postprandial were in closest agreement with portal blood for the nine essential amino acids,

isoleucine, leucine, lysine, valine, phenylalanine, threonine, methionine, histidine and arginine. While having endeavoured to interpret plasma amino acid levels, diurnal variations and time of collection must also be considered.

Food Intake Patterns - Frequency of Feeding

The most significant effect on diurnal or circadian rhythm in animals, especially the ruminant, was the total amount of food intake, and the number of times the animal was fed over the 24 hour period. The most significant economical effect was an increased growth rate (Mochrie, Thomas and Lucas, 1956; Hardison, Rakes, Engel and Graf, 1957) exhibited as a result of a more frequent feeding of animals in comparison with feeding twice daily when identical quantities of feed were offered. Factors that could possibly have contributed to increased frequency of feeding were first introduced by Gordon and Tribe (1952) as to (1) increased surface area for microbial attack, increasing digestibility, (2) maintenance of digesta flow from the rumen to the omasum, reducing fermentation loss, (3) production of an environment more conducive to growth of microorganisms or enabling the animal to use metabolites more readily or (4) differences in times spent ruminating.

Rumen fermentation varied over a 24 hour period, the main influence being the time of feeding and the subsequent level of metabolites produced. Bacteria (Nottle, 1956), as well as protozoa (Moir and Somers, 1957; Purser and Moir, 1959), exhibited distinct responses to ruminal pH, ammonia levels and volatile fatty acid levels. All variations were a result of the time of

feeding. Peaks of fermentation occurred in accordance with the type of diet fed. Increasing the frequency of feeding, while maintaining feed intake, allowed the fermentation level, as exhibited by ammonia and volatile fatty acid levels, to stabilize (Ibrahim, Ingalls and Phillips, 1969).

It was found that different bacterial and protozoal strains exhibited a specific diurnal fluctuation (Warner, 1965). Increasing the frequency of feeding maintained the microbial population at a very steady level, barring dilution by water, or dietary components (Moir and Somers, 1957). Protozoal cycles varied with the pH in the rumen (Purser and Moir, 1959). The lower pH associated with a high level of volatile fatty acid production after feeding reduced the protozoal population expressed as counts per millilitre of rumen fluid. Achieving a pH of approximate neutrality maintained a stable and active protozoal population (Ibrahim, Ingalls and Stanger, 1970). One can therefore conclude from the above data that reducing diurnal rhythms in the rumen resulted in a steady level of digesta entering the lower gastrointestinal tract.

A seasonal variation has been shown to exist in the rumen bacterial population (Nottle, 1956). This response could have been due in part to the type and availability of forage and its digestibility. Daylength

and daily temperatures might have had some effect. It was found that the bacterial population fluctuated in synchronous cycles with seasonal variations of average monthly daylengths and temperatures.

Flow along the lower gastrointestinal tract varied over the 24 hour period in response to feeding schedules. The flow from abomasum to duodenum increased a short time previous to feeding (Harris and Phillipson, 1962; Dyck, 1963). This could possibly have been associated with a cephalic phase of gastric (abomasal) secretion in readiness for the intake of digesta (McLeay and Titchen, 1970). Abomasal secretion was relatively continuous over the day with respect to pepsin and acid concentration. Increasing the frequency of feeding from once daily to once every hour reduced diurnal variation in the output of fecal dry matter, fecal dry matter concentration, total urine flow, urine specific gravity, urine nitrogen concentration and total nitrogen content (Minson and Cowper, 1966). A reduction in the diurnal variation in the factors listed would most likely have indicated that a continuous feeding mechanism could possibly have reduced normal circadian rhythms in the ruminant body as well as the rumen itself.

Control of Food Intake in the Ruminant

The presence of the rumen has changed to some extent the food intake controls of the ruminant in comparison with the monogastric animal. Theories were interpreted in accordance with rumen fill phenomena, chemostatic control by way of acetic acid levels instead of glucose, and the long term lipostatic control (Baile, 1968). Intake of roughage diets was affected by rumen fill factors while intake of concentrates came under chemostatic control. Rumen fill factors did not control the low level intake of a high concentrate diet. The total aspect of food intake control in ruminants must be studied more extensively in accordance with the unique situation of the ruminant animal and the rumen microbial population.

MATERIALS AND METHODS

Eight dairy steers were used in two 4 x 4 Latin Square designs, animals within each square having been subjected to either continuous or twice a day feeding. Each animal was fitted with an abomasal cannula (pyloric region of the abomasum) made of molded plastic manufactured from plastisol[1]. An attempt was made to make each square as uniform as possible both within and between squares for breed, age and weight of animals (Appendix Table 1).

The diet (composition (Table 1) and proximate analysis (Table 2)) fed during the experimental period contained corn (rolled) 63.6%, corn gluten meal 4.9%, (60% crude protein) and ground (one inch sieve) barley straw 28.4% with minerals and vitamins added plus 2% molasses. The diet was formulated in accordance with National Research Council standards (1963) for young growing steers. Lysine hydrochloride (15% nitrogen, 75% lysine by weight) was infused at levels of 0.00, 3.75, 7.50 and 11.25 grams per day (0.0, 3.0, 6.0 and 9.0 grams of lysine per day respectively).

The experiment was to compare the effect of lysine infusion per abomasum and either continuous fed or twice a day fed. Part A. reports on continuous feeding and Part B. reports on twice a day feeding.

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[1]Plastisol (Polyvinyl chloride)-Norton International Inc., Akron, Ohio.

Table 1 Ration Ingredients

Ingredient	Percent Composition	
Corn (rolled)	63.6	
Corn gluten meal (60% Crude Protein)	4.9	
Ground Barley Straw (1 inch sieve)	28.4	
Molasses	2.0	
Dicalcium Phosphate	0.1	
Calcium Carbonate	0.64	
Sodium Chloride *	0.50	
Vitamin A **	0.5	gm./100 lb.
Vitamin D ***	1.0	gm./100 lb.

* Trace mineralized: sodium chloride 96.5%, Iodine 0.010%, cobalt 0.004%, iron 0.160%, copper 0.330%, Manganese 0.120%, Zinc 0.400%.
** 325,000 I.U. per gram
*** 100,000 I.U. per gram

Table 2 Ration - Proximate Analysis

Dry Matter	91.2%	
	% Dry Matter Basis	
Nitrogen	2.00	
Crude Protein	12.50	
Ether Extract	3.02	
Crude Fiber	12.58	
Ash	4.07	
Calcium	0.33	
Phosphorus	0.28	
Nitrogen Free Extract (by difference)	67.83	
Energy (gross)	4513	cal./gm. (approx 2 Mcal/lb)

Part A. Continuous Feeding

Four steers were used in a 4 x 4 Latin Square design, utilizing a continuous feeding apparatus (Ibrahim, 1970; Ibrahim et al., 1969), in order to be able to meter out feed individually over a 24 hour period. Time intervals between feed presentations were ten minutes. For Period I, an intake of 18 lb. feed per day was maintained, but with difficulty as feed began to gather in the trough at the close of the digestibility trial. For subsequent Periods II to IV, 16 lb. of feed was fed per day except for Animal 10 which was fed 12 lb. per day. Lysine hydrochloride infusion treatments were as previously described. Water was available free choice.

The animals were stanchioned during each collection period. The infusate was pumped through Jayon[1] tubing (O.D.$\frac{1}{4}$", I.D.$\frac{1}{8}$") at a rate of 3000 ml. per day over 24 hours (22-24 hour range) for Period I using a Micropump[2]. For Periods II to IV inclusive, a Harvard pump[3] (non variable speed) was used, with the infusate volume increased to 3600 ml. per day in order to maintain infusion of the solution over 24 hours.

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[1] Johnston Plastics, Winnipeg, Manitoba.
[2] Buechler Instruments Inc., Fort Lee, New York.
[3] Harvard Apparatus Company, Dover, Massachusetts.

For each period a specific program was followed:

Day 1 - 14 - adjustment period.

Day 15 - collection of chromic oxide free abomasal samples obtained at 6 AM and 6 PM for amino acid analysis.

Day 16 - placement of feces collection bags.

Day 17 - placement of urine collection apparatus.

Day 17 - 23 - collection of feces and urine for digestibilities and nitrogen balance.

Day 18 - began feeding ground chromic oxide paper (one gram per pound of feed intake) five days before end of feces and urine collection.

Day 24 - collection of abomasal samples containing chromic oxide at 6 AM and 6 PM.

Day 25 - collection of blood samples, and rumen fluid at 9 AM; then steers off trial, one day rest unstanchioned, and then started on next adjustment period.

Part B. Twice a day feeding

Four steers were used in a 4 x 4 Latin Square design, and fed twice a day, 7 AM and 7 PM, 9 lb. per feeding, 18 lb. per day during the total experimental period. Lysine hydrochloride infusion treatments were as previously described. Each animal was individually fed, and allowed to move about within a pen of approximately 50 square feet.

The infusion of the lysine solution was carried out by a Sigmamotor peristaltic pump[1]. Jayon flexible plastic tubing (OD$\frac{1}{4}$", ID$\frac{1}{8}$") was suspended between the pump and the animal. An apparatus was rigged to retain the tubing in a fairly taut position. For Periods II to IV electric fencing wire was strung along the tubing to within one foot of the animal's back in order to prevent the animals from chewing the suspended tubing.

As with Part A., barring technical difficulties, a specific program of events was followed during each period:

Day 1 - 14 - adjustment period.

Day 15 - sampling of abomasum with no chromic oxide 6 AM, 12 noon, 6 PM, 12 midnight.

Day 16 - attachment of feces collection bags.

Day 17 - 24 - nitrogen balance and digestibility trial.

Day 18 - start feeding ground chromic oxide paper (one gram per pound feed intake).

Day 25 - 26 - collection of blood and abomasal samples (with chromic oxide) at three hour intervals over two days. Using only two animals per time, sampling was carried out on each animal at six hour intervals. In order to complete the three hour interval schedule, samples

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[1]Sigmamotor Inc., Middleport, New York.

were collected on two consecutive days. After a schedule of eight collections, one collection was skipped in order to maintain no less than a six hour time interval between each collection time, while still collecting time period samples not collected on the first day of sampling.

Day 26 - collection of rumen fluid at 5 PM (ten hours post feeding).

Urine Collection (figure 1)

The urine collecting apparatus consisted of a rubber inner tube catch basin unit, suspended under the prepuce area of the animal in order to retain quantitatively the urine excreted. Tubing (OD$\frac{1}{2}$", ID$\frac{1}{4}$") was connected from this unit to a collecting bottle. A separate length of tubing (OD$\frac{1}{4}$", ID$\frac{1}{8}$") led to a vacuum pump. All four animals were treated identically and were connected to a single vacuum pump with sufficient capacity to maintain a constant vacuum. Urine (figure 1) was initially removed from the catch basin by means of suction, with the help of gravity once the flow from the catch basin had begun.

The collecting bottle contained a small portion of concentrated hydrochloric acid to acidify the urine and prevent the escape of ammonia and maintain an environment unfavourable for bacterial action. Urine was periodically removed from the collecting bottle and the quantity recorded. An aliquot sample was obtained for the daily

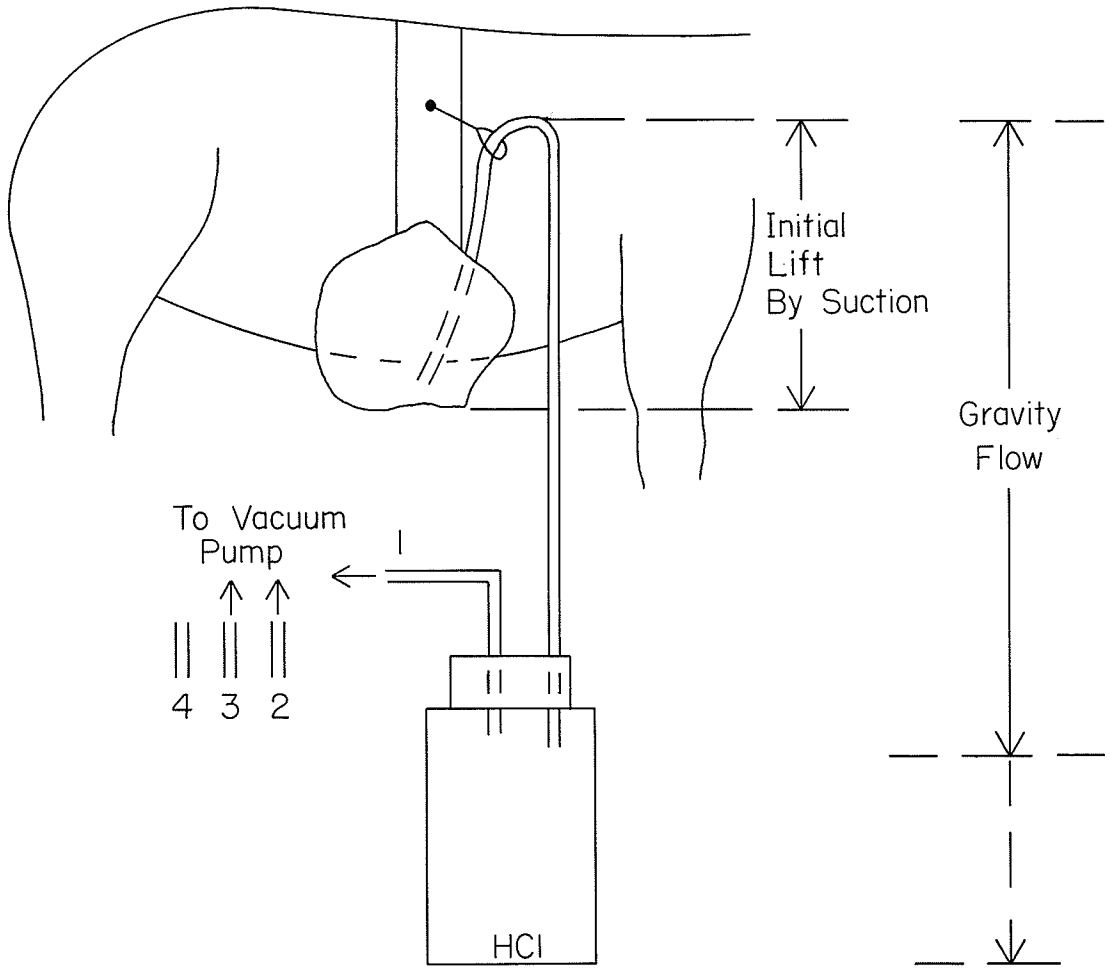

Figure 1. Urine Collection Apparatus.

collection and frozen. After the seven day collection period, the total subsampled urine for each animal was mixed, and a sample retained, which was frozen and kept for subsequent analysis.

The method described for urine collection was used for Periods I to IV inclusive in Part A., and for Periods III and IV in Part B. The animals in Part B. were placed in metabolism crates to collect urine and feces for Periods I and II.

Feces collection

In both Parts A. and B., for all periods, feces was collected using heavy plastic bags attached to the caudal end of the animal by means of bull cement (3M Company[1]) one day prior to the initiation of the collection period. Closure of the lower end of the bag was by means of twine. The feces were weighed daily, mixed, and an aliquot (10%) sample retained. Feces samples were frozen until the end of the seven day collection period, at which time they were thawed, mixed and subsampled. The resulting sample represented approximately one percent of the total seven day output for each animal. These samples were then frozen until analyzed.

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[1]Minnesota Mining & Manufacturing Co. Inc.

Abomasal Sampling

The abomasal cannula was opened and approximately 100 ml. of fluid (not less than 50 ml.) of 2-10% dry matter was collected in a plastic bottle and capped. In order to slow enzymic action, the sample was kept in ice. The abomasal fluid was prepared by taking 25 ml., which was titrated from a pH of approximately 2.0 to a pH of 7.0 in order to inactivate pepsin (pH 4.0) and pepsinogen (pH 6.0) (Hirschowitz, 1967). Noting the volume of 0.5 N. sodium hydroxide required for titration, the sample and pre-weighed sample bottle were weighed and freeze dried (Virtis freeze drier). All abomasal samples, regardless of subsequent analysis, were treated as above.

Blood Collection and Analysis

Blood was collected from the jugular vein (16 gauge needle). Twenty five ml. of blood was collected in tubes previously coated with 2 ml. of a 1.34 gm. per 100 ml. solution of sodium oxalate, the water in the sodium oxalate having been removed by drying in order to exclude volume corrections. Once collected, the blood was centrifuged at 2500 rpm. for ten minutes and the plasma removed. Part of the plasma was set aside for blood urea nitrogen analysis.

In the preparation of plasma for plasma free amino acid analysis, two ml. of plasma was added to thirteen ml.

of a mixture of 8 parts N/12 sulphuric acid and one part 10% sodium tungstate (Oser, 1965). The resulting mixture was centrifuged at 2500 rpm for ten minutes in order to precipitate protein. Seven and one-half ml. of the supernatant (equivalent to one ml. of plasma) was freeze dried and brought up to a volume of two ml. using a sodium citrate buffer solution (pH 1.8).

Plasma amino acids were analyzed using a Beckman 116 Amino Acid Analyzer with a modified version of the procedure by Benson and Patterson (1965). Total running time for the analysis was 310 minutes. Buffers used were pH 3.17 (0.2N.Na^+) at zero time, pH 4.25 (0.2N.Na^+) at 80 minutes and pH 6.30 (0.4N.Na^+) at 138 minutes. The last buffer (pH 6.30) was the modification included in order to allow acids more basic than phenylalanine to be eluted. Arginine alone was determined on the short column using a pH 5.8 buffer (0.38N.Na^+) with the total running time having been 50 minutes. Three-quarters of a ml. of sample was used on each column. The limitations of this technique were glutamine and asparagine were eluted with serine as well as citrulline having been eluted with proline. Citrulline was calculated off the 570 mu curve, while proline was read off the 440 mu curve.

Blood urea nitrogen was determined using a Technicon Auto Analyzer II[1], with the standard technique

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[1]Technicon Inc., Tarrytown, New York.

provided by the manufacturer (Blood Urea Nitrogen, Method Number AAII-1).

Amino Acids - Hydrolyzed Samples

Abomasal samples, plus one feed sample were hydrolyzed according to the method of Bragg et al. (1966). Modification included a hydrolyzing period of 15 hours and reconstitution to 100 ml. with sodium citrate buffer at pH2.2. One-half ml. of sample was analyzed using a 116 Beckman Amino Acid Analyzer for both basic and acidic amino acids.

Miscellaneous Analytical Procedures

General chemical methods used throughout the experimental analysis followed standard laboratory procedure for proximate analysis (AOAC). Feed samples were subjected to a total analysis (dry matter, crude protein (nitrogen x 6.25), crude fat, crude fiber, ash, calcium, phosphorus) as well as energy. Urine and feces samples were analyzed for nitrogen by the macrokjeldahl technique (AOAC 1959). Feces were predried at 75°C. to approximately 95% dry matter, as well as being analyzed for dry matter, nitrogen and energy on air dried samples. All energy determinations were done on a Parr Adiabatic Bomb Calorimeter with automatic water temperature controls. All analyses used in the calculation of digestibilities were converted to an oven dried basis.

Abomasal samples were freeze dried and analyzed for nitrogen by the microkjeldahl technique (AOAC 1965) and for ash, samples were placed in the furnace overnight at 500°C. The resulting ashed samples that contained chromic oxide were analyzed for chromium using the technique of Williams, David and Iismaa (1962).

Rumen samples were analyzed for ammonia by the Conway Diffusion Method (1957) and for volatile fatty acids. Volatile fatty acids were prepared according to the method of Erwin, Marco and Emery (1961). Five microlitres of resulting solution was analyzed using a Burrell Corporation gas chromatograph.

Digestibilities and Nitrogen Balance

In the abomasum, digestibilities were calculated using chromic oxide as a marker. Organic matter was estimated by subtraction of the ash content. Total crude fiber, ether extract and nitrogen free extract (CF+EE+NFE) was estimated by subtraction of the protein content (nitrogen x 6.25) from the abomasal organic matter estimation.

Apparent digestibilities of dry matter and nitrogen were calculated using total dietary intake, versus total output in feces. Nitrogen balance was calculated as percent absorbed nitrogen retained.

$$\text{Nitrogen balance} = \frac{\text{total N intake}-(\text{Total feces N}+\text{total urine N})}{\text{total N intake - total feces N}} \times 100$$

Statistical Methods

All data was analyzed using a standard 4 x 4 Latin Square analysis, no data missing. Data collected over a 24 hour period, for eight time intervals, was analyzed individually as well as a Set of Latin Squares (Cochran and Cox, 1966). Differences between treatment means were analyzed using Duncan's Multiple Range Test (Steele and Torrie, 1960).

RESULTS

Part A. Continuous Feeding

Nitrogen Balance and Digestibilities

Nitrogen balance (Table 3 expressed as percent of absorbed nitrogen retained) was significantly reduced ($P<0.05$) at the 9 gram level of lysine infusion. The reduction in nitrogen retention was concomitant with a significant increase ($P<0.05$) in nitrogen excreted in the urine (urine nitrogen as a percent of nitrogen intake). There were no differences in fecal nitrogen excretion (fecal nitrogen as a percent of nitrogen intake). Fecal nitrogen expressed as a percent of intake nitrogen was significantly ($P<0.05$) different for periods and animals.

There were no significant treatment effects in the apparent digestibilities of dry matter, energy or nitrogen. Apparent nitrogen digestibility was significantly ($P<0.05$) different for periods and animals.

Abomasal Amino Acids

Amino acids in the abomasum (as percent of total abomasal dry matter) were not significantly different among treatments. Abomasal amino acid nitrogen when expressed as a percent of total nitrogen in the abomasum (Appendix Table 5), resulted in no consistent

Table 3 Part A. Nitrogen Balance and digestibilities of dry matter, energy and nitrogen

	Grams lysine infused per day				Standard Error
	0	3	6	9	
Nitrogen Balance *	76.80[a]	76.28[a]	74.04[a]	63.45[b]	2.84
Urine % of Nitrogen intake	17.26[a]	17.41[a]	17.34[a]	27.05[b]	1.95
Feces % of Nitrogen intake	25.56	26.42	27.60	26.20	0.71
Digestibilities (percent)					
Dry matter	74.43	75.35	73.46	73.47	0.50
Energy	74.55	75.39	73.42	73.66	0.54
Nitrogen	74.43	73.57	72.34	73.79	0.69

a, b, c - Treatment means within an item not showing the same superscript are significantly different ($P<0.05$).

$$* \text{ Nitrogen Balance} = \frac{\text{Total Intake N}-(\text{Total Fecal N}+\text{Total Urine N})}{\text{Total Intake N} - \text{Total Fecal N}} \times 100$$

Table 4 Amino Acid analysis of the feed sample

Essential Amino Acids *	Percent of Ration ***	Molar Percent of Total Amino Acids
Lysine	0.281	2.23
Histidine	0.183	1.38
Arginine	0.389	2.60
Threonine	0.399	3.90
Valine	0.450	4.46
Methionine	0.206	1.61
Isoleucine	0.411	3.64
Leucine	1.508	13.38
Phenylalanine	0.564	3.97
Non essential Amino Acids		
Aspartic Acid	0.841	7.97 **
Serine	0.534	-
Glutamic Acid	2.177	18.67
Proline	0.956	10.48
Glycine	0.421	7.10
Alanine	0.872	12.36
Cystine	-	-
Tyrosine	0.434	3.03

* Essential Amino Acids 37.2% of total amino acids

** Serine was not included in the total of amino acids with respect to molar percent of amino acids in the diet.

*** Expressed on a 100% Dry Matter Basis

Table 5 Part A. Amino acids in the abomasum of steers as a molar percent of total amino acids present (excluding serine) sampled at 6 AM.

	grams lysine infused per day				Standard Error
	0	3	6	9	
Lysine ⁎	5.44^{a}	5.86^{a}	6.87^{b}	6.93^{b}	0.25
Histidine	1.49^{a}	1.71^{b}	1.59^{ab}	1.62^{ab}	0.04
Arginine	4.11	3.12	2.91	2.91	0.66
Threonine	5.43	5.45	5.24	5.36	0.36
Valine	5.99	5.92	5.66	5.93	0.22
Methionine	2.24	2.25	2.21	2.12	0.09
Isoleucine	4.66	4.86	4.74	4.78	0.16
Leucine	12.17	10.95	10.63	11.05	0.93
Phenylalanine	3.93	4.11	4.09	4.15	0.09
Aspartic Acid	10.39	10.05	9.49	10.02	0.34
Glutamic Acid	14.87	15.19	14.50	15.36	0.36
Proline	7.14	7.63	7.06	7.06	0.50
Glycine	7.83	8.05	9.66	8.09	0.86
Alanine	10.51	11.18	10.48	11.04	0.35
Cystine	0.23	0.25	0.33	0.27	0.07
Tyrosine	3.46	3.44	3.26	3.33	0.15

a, b - Treatment means within an item not showing the same superscript were significantly different ($P<0.05$).

⁎ Analysis of variance was significant ($P<0.01$) for treatment means; Duncan's Multiple Range Test was significant (P 0.05).

treatment trends. Abomasal amino acids as a molar percent of total abomasal amino acids excluding serine in the total were calculated for comparative purposes.

The molar percent of essential amino acids increased from diet (37.2%) (Table 4) to abomasum (averaging 44.0%) (Table 8). Relative to the dietary molar percent of total dietary amino acids, differences in abomasal amino acids as a molar percent of total amino acids in the abomasum were evident. The largest amino acid increase from diet to abomasum was with lysine, which included the result for the zero level of lysine infusion. Much less of a change was noted for other essential amino acids from diet to abomasum with the exception of leucine. The molar percent of leucine decreased from diet (13.38%) to abomasum (averaging 11.20%). The molar percent of non essential amino acids decreased for all amino acids with the exception of aspartic acid, glycine and tyrosine. The molar percent of proline exhibited the greatest reduction from diet to abomasum. No dietary values for cystine were obtained. Destruction of cystine occurs with hydrolysis in hydrochloric acid. As analytical techniques were the same for diet and abomasal samples, the values reported for abomasal samples probably reflect an increase in molar percent of cystine from diet to abomasum.

Abomasal amino acids as a molar percent of total abomasal amino acids (excluding serine) remained relatively

constant among treatments. The molar percent of abomasal lysine in the abomasum for the 6 and 9 gram lysine infusion per day were significantly higher ($P<0.05$) than the 0 and 3 gram lysine infusions per day which indicated a response to the levels of lysine infusion. The molar percent of abomasal histidine at zero and 3 grams lysine infusion per day were significantly different ($P<0.05$), but each was not significantly different from 6 or 9 grams lysine infusion per day. The molar percent of abomasal histidine exhibited a significant period response ($P<0.01$) which could not be explained in relation to period variations in nitrogen intake (Appendix Table 3). Animal variations for the molar percent of abomasal lysine were possibly due to variations in rate of infusion. Animals 10 and 21 were significantly different ($P<0.05$) from animals 15 and 17. The higher molar percent of abomasal lysine in animal 10 could be associated with the lower level of nitrogen intake for Periods II to IV, while with animal 21, the intermediate value could not be explained. Essential amino acids in the abomasum (Table 8) as a molar percent of total amino acids in the abomasum excluding serine were not significantly ($P<0.05$) different among treatments.

Plasma Amino Acids

Infusion of lysine significantly increased ($P<0.01$) the level of lysine in the plasma from an average of 7.60 u moles per 100 ml. of plasma for 0, 3 and 6 grams of lysine infused per day to 11.58 u moles per 100 ml (Table 6) at the 9 gram daily infusion of lysine. No other plasma amino acid levels were significantly different among treatments. Arginine values for Periods I and II (14.54 and 12.20 u moles per 100 ml. of plasma, respectively) at the 9 gram level of lysine infusion were 5 u moles per 100 ml. plasma higher than arginine values (average of 8 u moles per 100 ml. plasma) for the 0, 3, and 6 gm. lysine per day treatments and Periods III and IV, 9 gm. lysine per day treatment. Glutamic acid was significantly ($P<0.01$) higher in the plasma during Period I (27.59 versus 10.90, 8.64 and 7.76 for Period I versus Periods II, III and IV respectively) and may be related to the higher nitrogen intakes of animals during Period I (Appendix Table 3). Methionine and cystine as well as citrulline were significantly ($P<0.05$) different for Periods. Methionine plasma levels for Period I were significantly ($P<0.05$) lower during th period of high nitrogen intake (0.38, 2.04, 1.37, and 1.96 u moles per 100 ml. plasma for Periods I, II, III and IV, respectively). Cystine and citrulline yielded no consistent response with respect to other plasma amino acids levels or to infusion treatments. Animal effects were negligible for plasma amino acid values and did not reflect the lower nitrogen

Table 6 Part A. Plasma Amino Acids (u moles per 100 ml.plasma)

	grams lysine infused per day				Standard Error
	0	3	6	9	
Essential Amino Acids					
Lysine	7.68[a]	7.15[a]	7.96[a]	11.58[b]	0.70
Histidine	7.30	7.25	7.39	7.69	0.38
Arginine	8.19	8.59	7.76	10.32	0.97
Threonine	7.83	7.26	7.01	7.85	0.83
Valine	17.01	14.66	14.56	16.28	1.12
Methionine	1.60	1.56	1.38	1.23	0.24
Isoleucine	8.28	7.43	7.11	7.84	0.51
Leucine	15.68	15.98	13.96	14.55	0.65
Phenylalanine	5.04	4.24	4.63	5.16	0.26
Non essential Amino Acids					
Aspartic Acid	4.28	3.26	3.31	4.73	0.61
Glutamic Acid	11.73	14.05	15.73	13.42	1.34
Proline	6.11	6.85	6.30	6.40	0.46
Glycine	27.39	25.08	29.31	27.51	2.34
Alanine	16.49	16.13	17.32	17.08	1.31
Cystine	1.37	1.30	1.33	1.32	0.19
Tyrosine	5.04	4.91	4.85	5.03	0.26
Ornithine	4.92	4.55	5.12	5.44	0.38
Citrulline	3.41	4.03	4.00	3.69	0.33
Taurine	4.92	3.62	3.58	5.81	0.89
TOTAL EAA **	78.36	74.41	71.88	82.30	4.50
TOTAL NEAA **	72.39	71.58	78.14	75.38	4.28
TOTAL AA **	150.75	145.99	150.02	157.68	8.12

a, b - Treatment means within an item not showing the same superscript were significantly different ($P<0.05$).

** - AA - Amino Acids; EAA - Essential Amino Acids - lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine; NEAA - Non essential Amino Acids - excluded ornithine, citrulline, taurine.

EAA Theurer, Woods and Poley, 1966.

intake which occurred with Animal 10.

Analysis of total molar levels of essential amino acids in plasma (Table 7), non essential amino acids in plasma or total amino acids (the total excluding ornithine, citrulline and taurine) in plasma, did not result in any significant treatment differences. A significant period effect ($P<0.05$) was noted, however, but no consistent trend was apparent among the essential, non essential or total amino acids in plasma with regard to periods.

Plasma amino acids were calculated as a molar percent of total plasma amino acids, the total excluding ornithine, citrulline and taurine as well as serine which was not analyzed for in plasma. The molar percent of plasma lysine was significantly higher ($P<0.01$) at the 9 gram level of lysine infusion. The molar percent of plasma leucine and isoleucine were significantly different ($P<0.05$) for levels of lysine infusion. The molar percent of plasma isoleucine was significantly higher at 0 grams lysine infusion per day while the molar percent of plasma leucine was significantly higher at the 0 and 3 gram lysine infusion per day. The molar percent of plasma methionine and plasma histidine were significantly ($P<0.01$) lower for Period I. The molar percent of plasma tyrosine was also significantly lower ($P<0.05$) for Period I. The molar percent of plasma glutamic acid was significantly higher ($P<0.01$) in Period I, while the molar percent of plasma aspartic acid tended to be higher ($P<0.10$) for Period I.

Table 7 Part A. Plasma amino acids as a molar percent of total amino acids excluding ornithine, citrulline, taurine (sampled 9 AM).

	grams lysine infused per day				Standard Error
	0	3	6	9	
Lysine	5.08^{A}	4.91^{A}	5.32^{A}	7.45^{B}	0.32
Histidine	4.84	4.98	4.98	5.01	0.12
Arginine	5.44	5.94	5.14	6.42	0.50
Threonine	5.18	4.97	4.67	5.00	0.37
Valine	11.24	10.05	9.70	10.39	0.59
Methionine	1.06	1.08	0.96$_{b}$	0.78$_{b}$	0.13
Isoleucine	5.49^{a}	5.08^{b}	4.75$_{b}$	4.96^{b}	0.11
Leucine	10.40^{a}	10.94^{a}	9.39^{b}	9.28^{b}	0.26
Phenylalanine	3.19	3.10	3.21	3.29	0.19
Aspartic Acid	2.84	2.23	2.26	2.93	0.35
Glutamic Acid	7.83	9.60	10.02	7.89	1.05
Proline	4.06	4.70	4.25	4.11	0.28
Glycine	18.19	17.14	19.62	17.57	0.83
Alanine	10.96	11.04	11.63	10.81	0.56
Cystine	0.92	0.92	0.90	0.89	0.11
Tyrosine	3.35	3.39	3.23	3.22	0.11

A, B - Treatment means within an item not showing the same superscripts were significantly different ($P<0.01$).

a, b - Treatment means within an item not showing the same superscripts were significantly different ($P<0.05$).

Period values for the molar percent of plasma leucine and cystine were significantly different ($P<0.01$) but no trends were evident among treatments. Essential amino acids as a molar percent of total amino acids varied from 48.11% to 52.27% (Table 8).

Rumen Ammonia and Volatile Fatty Acids (VFA)

There was no significant difference in total VFA expressed as m moles per 100 ml. rumen fluid or as a molar percent of each individual VFA (Table 10A). The rumen ammonia level (Table 9) at the 3 gram lysine infusion per day was significantly higher ($P<0.05$) than the other treatment levels. Production of ammonia at the 9 AM sampling for the 3 gram lysine infusion treatment was 6 to 10 mg. ammonia per 100 ml. rumen fluid higher than the other three treatments. The zero and 9 gram treatment levels, 15.9 and 16.2 mg. ammonia per 100 ml. rumen fluid, respectively, were the lowest values. The 6 gram level was intermediate at 19.1 mg. ammonia per 100 ml. rumen fluid, while the 3 gram level at 25.5 mg. ammonia per 100 ml. rumen fluid was significantly higher ($P<0.05$) than the other three treatments. Plasma ammonia levels were low (0.12, 0.15, 0.14 and 0.11 mg./100 ml. plasma for the 0, 3, 6 and 9 gram levels of lysine infusion, respectively) in relation to rumen ammonia levels, although plasma levels of ammonia reflected rumen levels.

Table 8 Part A. Essential amino acids as a molar percent of total amino acids

	grams lysine infused per day				Standard Error
	0	3	6	9	
Plasma *	50.92	51.03	48.11	52.57	1.21
Abomasum **	44.15	44.04	43.93	44.25	0.87

* Excluded ornithine, citrulline, taurine, (serine was not analyzable).

** Excluded serine.

Table 9 Part A. Ammonia levels in the rumen and plasma and blood urea nitrogen (BUN) (9 AM).

mg/100 ml.	grams lysine infused per day				Standard Error
	0	3	6	9	
Ammonia					
rumen *	15.9^{a}	25.5^{b}	19.1^{a}	16.2^{a}	1.42
plasma	0.12	0.15	0.14	0.11	0.02
BUN	7.72	8.20	7.97	8.72	0.39

a, b - Treatment means within an item not showing the same superscript were significantly different ($P<0.05$).

* Analysis of variance was significant ($P<0.01$) Duncan's Multiple Range test was significant ($P<0.05$).

Table 10A Part A. Volatile fatty acid concentrations and molar propertions in the rumen at the 9 AM sampling time.

	grams lysine infused per day				Standard Error
	0	3	6	9	
Total					
mmole/100 ml.	8.12	8.95	8.88	7.32	0.82
molar percent					
acetic	65.20	65.87	65.57	65.39	0.66
propionic	16.06	15.81	15.78	17.71	0.82
isobutyric	1.13	1.19	1.38	1.26	0.11
butyric	15.39	15.00	14.16	13.03	0.71
isovaleric	1.55	1.40	1.40	1.77	0.13
valeric	0.67	0.73	0.88	0.53	0.08

Table 10B Part B. Volatile fatty acid concentrations and molar proportions in the rumen at the 5 PM sampling time.

	grams lysine infused per day				Standard Error
	0	3	6	9	
Total					
mmole/100 ml.	7.34	8.21	6.93	7.48	0.45
molar percent					
acetic	65.74	66.50	65.71	66.02	0.89
propionic	15.07	15.68	15.84	15.04	1.27
isobutyric	1.66	1.26	1.64	1.41	0.78
butyric	15.19	13.95	13.99	15.01	1.03
isovaleric	1.50	1.74	2.02	1.59	0.23
valeric	0.85	0.89	0.76	0.94	0.17

Blood Urea Nitrogen (BUN)

There was no statistically significant difference for BUN among treatments. Minor peaks were apparent at the 3 and 9 gm. levels of lysine infusion. The peak with the 3 gm. lysine infusion could possibly have been a response to the higher ammonia levels in the rumen, the ammonia having been absorbed across the rumen epithelium into the plasma and subsequently converted to urea in the liver. At the 9 gram level of lysine infusion, elevated plasma lysine (9 gram lysine infusion versus 0 gm. lysine infusion was approximately 5 u moles per 100 ml. plasma higher) may have been converted to BUN and excreted in the urine. The urinary nitrogen excretion was also increased at the 9 gram level of lysine infusion.

Rumen Organic Matter Digestibility (Table 11A)

Dry matter levels in the rumen were not a good indication of the quantity of dietary components reaching the abomasum. A high level of ash in the abomasum (range of 13-30% of abomasal dry matter) in comparison with a low dietary level (3% of dry matter) was found. This increased ash level could have been a result of either the loss in total digestible components as ammonia and volatile fatty acids through transepithelial absorption or to the salivary output of ions having reached the abomasum in large quantities. Rumen organic matter digestibility which includes crude protein (N x 6.25), crude fiber,

Table 11 Part A. Contribution to the abomasum - Nitrogen, Organic Matter and a sum of Crude Fiber, Ether Extract and Nitrogen Free Extract

11A. Rumen Organic Matter Digestibility (percent)

	0	3	6	9	S.E.
6 AM	51.00	54.61	45.06	39.38	8.10
6 PM	40.47	54.84	45.19	50.61	5.69
OVERALL	45.74	54.72	45.13	44.95	6.86

11B. Rumen Digestibility of Crude Fiber, Ether Extract and Nitrogen Free Extract in total (excluded Nitrogen) (CF+EE+NFE) (percent)

	0	3	6	9	S.E.
6 AM	61.22	61.39	54.72	51.98	7.41
6 PM	51.75	65.48	55.22	60.80	4.53
OVERALL	56.49	63.44	54.97	56.39	6.00

11C. Nitrogen Reaching the Abomasum as a Percent of Intake Nitrogen

	0	3	6	9	S.E.
6 AM	118.65	91.34	120.73	145.78	15.32
6 PM	136.84	117.62	123.30	118.27	14.58
OVERALL	127.75	104.48	122.02	132.03	10.77

S.E. - Standard Error

ether extract and nitrogen free extract was calculated using chromic oxide as a marker and was not significantly different at 6 AM or 6 PM or when the two time periods were analyzed as a set of two 4 x 4 Latin Squares. Organic matter digestibility was slightly higher at the 3 gram level of lysine infusion as was the rumen ammonia level.

Crude Fiber (CF), Ether Extract (EE) and Nitrogen Free Extract (NFE) Digestibility in the Rumen (Table 11B)

The total quantity of CF+EE+NFE was calculated by subtracting the total protein (N x 6.25) from the organic matter entering the abomasum. Total rumen CF+EE+NFE digestibility was not significantly different among treatments (analyzed at 6 AM, 6 PM or as a set of two 4 x 4 Latin Squares). Subtraction of the nitrogenous component from organic matter yielded a higher level of rumen digestibility of total rumen CF+EE+NFE at the 3 gm. lysine infusion in response to an increased level of ammonia at this treatment level. The dietary non nitrogenous component was utilized by the rumen microbial population to a high degree as a high level of microbial activity was indicated by a high rumen ammonia level.

Abomasal Nitrogen Levels (Table 11C)

Nitrogen reaching the abomasum expressed as a percent of nitrogen intake was not significantly different among treatments. The percent abomasal nitrogen at 3 grams lysine infusion was 104%, and was lower when compared

to the other treatments which averaged 125%. The significantly higher ($P<0.01$) ammonia level in the rumen at 3 grams lysine per day indicated a loss of nitrogen from the rumen in relation to nitrogen reaching the abomasum.

Part B. Twice a Day Feeding

Animals on Part B yielded a totally different response to lysine infusion from animals on Part A. The main interaction with the treatment levels appeared to be the feeding method.

Nitrogen Balance and Digestibilities

There was no significant ($P<0.05$) treatment difference in nitrogen balance for steers fed twice a day. As the intake of nitrogen per day in Part B. was relatively similar (Appendix Table 3), nitrogen balance was expressed either as percent of absorbed nitrogen retained (Table 12), or as grams of nitrogen retained per day (Appendix Table 2). Part B nitrogen balance results were analyzed in an identical manner as Part A. Nitrogen retention, expressed as percent of absorbed nitrogen retained, although non significant, tended to be lower for the steers on the 6 gram level of lysine infusion. This appeared to be a result of a significantly greater ($P<0.05$) nitrogen loss in feces when expressed as a percent of intake nitrogen, with less nitrogen being absorbed. Urinary nitrogen output differences were non significant. The 6 and 9 grams infusion of lysine per day treatments did, however, show a slight increase in urinary output of nitrogen. A combination of less nitrogen absorbed, with a slight increase in urine nitrogen excretion, yielded a lower nitrogen retention for the 6 gram level of lysine infusion.

Table 12 Part B. Nitrogen Balance And Digestibilities Of Dry Matter, Energy And Nitrogen

	Grams lysine infused per day				Standard Error
	0	3	6	9	
Nitrogen Balance *	74.86	76.71	60.12	67.91	5.95
Urine N% of Nitrogen intake	18.47	17.48	28.04	23.75	4.18
Feces N% of Nitrogen intake	26.10[a]	24.40[a]	28.90[b]	26.19[a]	0.70
Digestibilities (percent)					
Dry matter	73.90	75.68	72.25	75.30	0.74
Energy	73.74	75.89	72.37	75.07	0.77
Nitrogen	73.89[a]	75.59[a]	71.10[b]	73.81[a]	0.70

a, b - Treatment means within an item not showing the same superscript were significantly different ($P<0.05$).

* Nitrogen Balance = $\frac{\text{Total Intake N}-(\text{Total Fecal N}+\text{Total Urine N})}{\text{Total Intake N}-\text{Total Fecal N}} \times 100$

A significant ($P<0.05$) period difference was observed for urinary nitrogen output. The period effect could possibly have been attributable to the different method of urine collection used for Periods I and II, versus Periods III and IV. Urine for Periods I and II was collected in the metabolism crates, while urine for Periods III and IV was collected using the urine collection apparatus which had been constructed and used initially for Part A. Another possible explanation could have been an animal age or weight response to the lysine infused, the periods having been different both for age and weight of animals.

Apparent digestibilities of dry matter and energy were similar among treatments and non significant. Apparent nitrogen digestibility was significantly ($P<0.05$) lower at the 6 gram level of lysine infusion.

A significant ($P<0.05$) period effect was observed for dry matter and energy apparent digestibilities. Periods I and II were significantly ($P<0.05$) higher than Periods III and IV. During Periods I and II the animals were kept in metabolism crates to collect feces and urine. For Periods III and IV feces and urine were collected when animals were stanchioned and stood on a raised platform.

Abomasal Amino Acids

As with Part A, each amino acid as a molar percent of total amino acids reaching the abomasum was calculated excluding serine in the amino acid total (Table 13). No significant ($P<0.05$) differences were noted for treatments, periods or animals. The molar percent of abomasal amino acids for Part B. and the molar percent abomasal amino acids for Part A. were similar. Lysine exhibited the greatest increase among the essential amino acids from diet to abomasum. Most of the other essential amino acid as a molar percent in the abomasum had slight increases or decreases relative to the molar percent of dietary amino acids. Non essential amino acids in the abomasum generally exhibited a decrease in molar percent relative to the diet except for aspartic acid, glycine and tyrosine which exhibited an increased molar percent of total amino acids from diet to abomasum. Proline had the greatest reduction in molar percent from diet to abomasum. Explanation for the cystine difference was the same as has been noted for Part A. Nitrogen reaching the abomasum was enriched by a greater molar percent of essential amino acids (43-44%) in the abomasum, (Table 17) relative to the molar percent in the diet (37.2%) (Table 4). Dietary and abomasal calculations were computed using the same amino acids in each total.

Table 13 Part B. Amino acids in the abomasum as a molar percent of total amino acids present (excluding serine) sampled at 6 AM.

	Grams lysine infused per day				Standard Error
	0	3	6	9	
Lysine	6.05	5.50	5.22	5.85	0.74
Histidine	2.00	1.42	1.56	1.48	0.20
Arginine	2.97	2.87	2.85	2.98	0.17
Threonine	5.50	5.70	5.37	5.30	0.17
Valine	6.14	5.88	5.73	5.90	0.15
Methionine	2.24	2.39	2.38	2.33	0.10
Isoleucine	4.83	4.84	4.76	4.88	0.11
Leucine	11.36	10.37	11.32	11.33	0.34
Phenylalanine	4.34	4.21	4.26	4.31	0.08
Aspartic Acid	9.85	9.88	10.32	9.79	0.43
Glutamic Acid	16.19	15.56	15.37	15.48	0.44
Proline	6.88	7.59	7.80	7.01	0.61
Glycine	8.85	8.60	8.22	7.95	0.29
Alanine	11.66	11.49	11.28	11.07	0.28
Cystine	0.36	0.18	0.23	0.27	0.10
Tyrosine	3.38	3.56	3.34	3.44	0.09

Plasma Amino Acids

Treatment means were not significantly different ($P<0.05$) for each plasma amino acid analyzed (Table 14). For the amino acids arginine, methionine, leucine, phenylalanine and tyrosine, the treatment variation was significant ($0.10<P<0.15$) (Table 15). Duncan's Multiple Range test analysis ($P<0.05$) revealed a pattern for the arginine, phenylalanine and tyrosine plasma levels. The 6 gram lysine infusion plasma values for the three amino acids indicated were significantly different ($P<0.05$) from the 9 gram lysine infusion plasma values, while neither the 6 gram or 9 gram lysine treatment levels were significantly different from zero or 3 grams lysine infusion. A possible relationship with the non significant reduction of nitrogen balance at the 6 gram lysine level and significant ($P<0.05$) reduction in nitrogen digestibility was indicated.

Plasma histidine, glutamic acid and cystine were significantly different ($P<0.05$) for periods. For Period IV the histidine level was significantly higher ($P<0.05$) than the other periods (6.25, 6.43, 6.42 and 7.62 u moles per 100 ml. plasma for Periods I, II, III and IV, respectively). Plasma glutamic acid for Period IV was significantly lower ($P<0.05$) than Periods I and II, while not being significantly different from Period III (10.97, 10.94, 8.43 and 7.35 u moles per 100 ml. plasma for Periods

Table 14 Part B. Plasma Amino Acids (umoles per 100 ml plasma)

	Grams lysine infused per day				Standard Error
	0	3	6	9	
Essential Amino Acids					
Lysine	8.87	9.82	8.65	9.06	1.07
Histidine	6.87^{ab}	6.70^{ab}	6.84^{b}	6.31^{a}	0.23
Arginine	8.39^{ab}	9.04^{ab}	9.34^{b}	7.21^{a}	1.05
Threonine	7.81	8.71	9.39	7.72	0.68
Valine	17.35	20.39	21.49	16.04	2.19
Methionine	1.01	1.58	1.90	1.10	0.23
Isoleucine	8.07	9.81	9.90	7.48	0.87
Leucine	15.46^{ab}	18.70^{ab}	21.51^{b}	15.36^{a}	1.74
Phenylalanine	5.09^{ab}	5.80^{ab}	6.77^{b}	4.82^{a}	0.50
Non essential Amino Acids					
Aspartic Acid	4.12	3.91	4.60	3.97	0.50
Glutamic Acid	9.71	10.54	8.91	8.52	0.78
Proline	6.53	6.91	7.70	7.24	0.91
Glycine	23.14	24.90	24.08	21.97	1.48
Alanine	15.22	16.80	17.22	14.66	1.42
Cystine	1.61^{ab}	1.32^{ab}	1.62^{b}	2.06^{a}	0.30
Tyrosine	6.21^{ab}	6.13^{ab}	8.07^{b}	5.92^{a}	0.56
Ornithine	4.73	5.54	4.61	4.59	0.86
Citrulline	3.17	3.41	3.28	4.16	0.54
Taurine	5.35	4.15	4.35	3.77	0.49
TOTAL EAA **	78.90	90.54	95.79	75.00	6.88
TOTAL NEAA **	68.93	70.51	72.20	64.32	3.08
TOTAL AA **	147.83	161.05	167.99	139.32	8.88

a, b - Treatment means within an item not showing the same superscript were significantly different ($P<0.05$) by way of Duncan's Multiple Range Test.

** - AA - Amino Acids; EAA - Essential Amino Acids - lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine; NEAA - Non essential Amino Acids - excluding ornithine, citrulline, taurine.

Table 15 Part B. Plasma amino acid levels (umoles per 100 ml. plasma) having exhibited analysis of variance significant ($0.10<P<0.15$), and Duncan's Multiple Range Test significant ($P<0.05$).

	Grams lysine infused per day				Standard Error	$P\leq$ ⁎
	0	3	6	9		
Arginine	8.39^{ab}	9.04^{ab}	9.34^{b}	7.21^{a}	1.05	0.10
Methionine	1.01	1.58	1.90	1.10	0.23	0.12
Leucine	15.46	18.70	21.51	15.36	1.74	0.15
Phenylalanine	5.09^{ab}	5.80^{ab}	6.77^{b}	4.82^{a}	0.50	0.12
Tyrosine	6.21^{ab}	6.13^{ab}	8.07^{b}	5.92^{a}	0.56	0.12

a, b - Treatment means within an item not showing the same superscripts were significantly different ($P<0.05$) by way of Duncan's Multiple Range Test.

⁎ - F value for the analysis of variance was significant (P x ; $0.10 < x < 0.15$).

I, II, III and IV, respectively). Cystine period variations revealed no consistent response relative to other amino acids found significant. Animal variation for histidine and arginine, although significant ($P<0.05$) did not yield a uniform response.

Analysis of total essential amino acids, non essential amino acids, and total amino acids in plasma yielded no significant treatment differences. Means for animal variation for total non essential amino acids were significantly different ($P<0.05$). Essential amino acids in plasma as a molar percent of total plasma amino acids (excluding ornithine, citrulline and taurine, plus serine which was not analyzed) were not significantly different. The highest values for total essential amino acids (Table 14) were observed with the 3 grams and 6 grams lysine infusions. The molar percentage of essential amino acids in plasma exhibited an increase over the molar percent of essential amino acids in the abomasum (averages of 55% and 44% for plasma and abomasal levels respectively).

The molar percent of plasma amino acids (having excluded ornithine, citrulline and taurine in the total) were significant ($P<0.10$) in several cases. The molar percent of plasma methionine, leucine and cystine were significant ($P<0.10$) by analysis of variance and Duncan's Multiple Range test ($P<0.05$). The molar percent of plasma

Table 16 Part B. Plasma amino acids as a percent of total amino acids excluding ornithine, citrulline, taurine (sampled 9 AM).

	Grams lysine infused per day				Standard Error
	0	3	6	9	
Lysine	6.08	6.07	5.15	6.41	0.63
Histidine	4.62	4.20	4.13	4.51	0.15
Arginine	5.63	5.65	5.63	5.16	0.22
Threonine	5.27	5.36	5.57	5.52	0.16
Valine	11.69^{a}	12.55^{ab}	12.79^{b}	11.51^{ab}	0.79
Methionine *	0.70^{a}	0.97^{ab}	1.14^{b}	0.87^{ab}	0.12
Isoleucine	5.46$_{a}$	6.06$_{ab}$	5.86$_{b}$	5.35$_{ab}$	0.27
Leucine *	10.39^{a}	11.60^{ab}	12.75^{b}	11.01^{ab}	0.57
Phenylalanine	3.47	3.60	4.05	3.45	0.27
Aspartic Acid	2.79	2.43	2.77	2.77	0.37
Glutamic Acid	6.85	6.54	5.22	6.18	0.65
Proline	4.36	4.31	4.65	5.23	0.36
Glycine	15.81	15.55	14.32	15.73	1.03
Alanine	10.32	10.50	10.23	10.57	0.55
Cystine *	1.05^{ab}	0.80^{a}	0.94^{ab}	1.54^{b}	0.18
Tyrosine **	4.21^{a}	3.83^{a}	4.80^{b}	4.26^{ab}	0.17

a, b - Treatment means within an item not showing the same superscripts were significantly different ($P<0.05$)

* - Treatment Analysis of Variance was not significant ($P<0.05$), while Duncan's Multiple Range test was significantly different ($P<0.05$).

** - Treatment Analysis of Variance was significant ($P<0.05$), Duncan's Multiple Range test ($P<0.05$).

methionine and leucine were significantly higher ($P<0.05$) at the 6 gram level of lysine infusion than the zero gram level of lysine infusion. Neither the 3 gram nor the 6 gram level of lysine infusion was significantly different from either the zero gram or 9 gram level of lysine infusion. The molar percent of plasma cystine at the 3 gram level of lysine infusion was significantly lower ($P<0.05$) than the 9 gram level of lysine infusion while neither level was significantly idfferent from the zero or 6 gram level of lysine infusion. Only the molar percent of plasma tyrosine was significant ($P<0.05$). The zero and 3 gram treatments for tyrosine were significantly lower ($P<0.05$) than the 6 grams lysine infusion but not the 9 grams lysine infusion. It should be noted that comparing the molar percent of tyrosine between abomasal and plasma in Parts A. and B., only Part B. had a higher molar percent in plasma relative to abomasal contents. Abomasal and plasma tyrosine in Part A. were 3.46, 3.35; 3.44, 3.37; 3.26, 3.23; and 3.33, 3.22 for abomasal and plasma tyrosine levels as a molar percent of total amino acids, respectively, for 0, 3, 6 and 9 grams lysine infused, respectively. For Part B. abomasal and plasma tyrosine were 3.38, 4.21; 3.56, 8.83; 3.34, 4.80; and 3.44, 4.26 for abomasal and plasma levels as a molar percent of total plasma amino acids for 0, 3, 6 and 9 grams lysine infused, respectively. An interaction with the type of feeding regime could possibly have been indicated.

Significant ($P<0.05$) period differences of plasma amino acids as a molar percent of total amino acids were noted for histidine, arginine and cystine while animal differences were significant ($P<0.05$) for arginine, threonine and tyrosine. No consistent pattern of interrelationship appeared among period and animal value.

Rumen Ammonia and Volatile Fatty Acids (VFA)

There was no significant difference among treatments for total VFA expressed as m moles per 100 ml. of rumen fluid or as a molar percent of each individual VFA (Table 10B). Rumen ammonia levels were significantly ($P<0.10$) different, while Duncan's Multiple Range test indicated a significant ($P<0.05$) response. The highest value, 16.2 mg. ammonia per 100 ml. rumen fluid at 3 grams lysine infusion per day was significantly different ($P<0.05$) from the lowest value of 11.9 mg. ammonia per 100 ml. rumen fluid at the 6 gram lysine infusion, while neither was significantly different from zero or 9 grams lysine infusion (12.7 and 14.7 mg. ammonia per 100 ml. rumen fluid respectively). A pattern similar to that in Part A. was observed, but possibly the time of sampling (10 hours post feeding at 5 PM) resulted in differences in Part B. which were not as marked in response to the lysine infusion in Part A.

Table 17 Part B. Essential amino acids as a molar percent of total amino acids

	Grams lysine infused per day				Standard Error
	0	3	6	9	
Plasma *	53.30	56.05	57.06	54.50	1.63
Abomasum **	43.45	43.16	43.44	45.00	0.71

* Excluded ornithine, citrulline, taurine (serine was not analyzed).

** Excluded serine.

Table 18 Part B. Ammonia levels in the rumen (5 PM) and plasma (9 AM) and blood urea nitrogen (BUN - 24 hour average)

mg/100 ml.	0	3	6	9	Standard Error
Ammonia					
rumen *	12.7^{ab}	16.2^{a}	11.9^{b}	14.1^{ab}	0.98
plasma	0.13	0.10	0.11	0.10	0.01
BUN **	10.07^{A}	9.62^{AB}	9.53^{AB}	9.19^{B}	0.19

* Treatment means were significantly different ($P<0.10$) Duncan's Multiple Range test ($P<0.05$)

** Treatment means were significantly different ($P<0.01$).

Blood Urea Nitrogen (BUN)(Table 19)

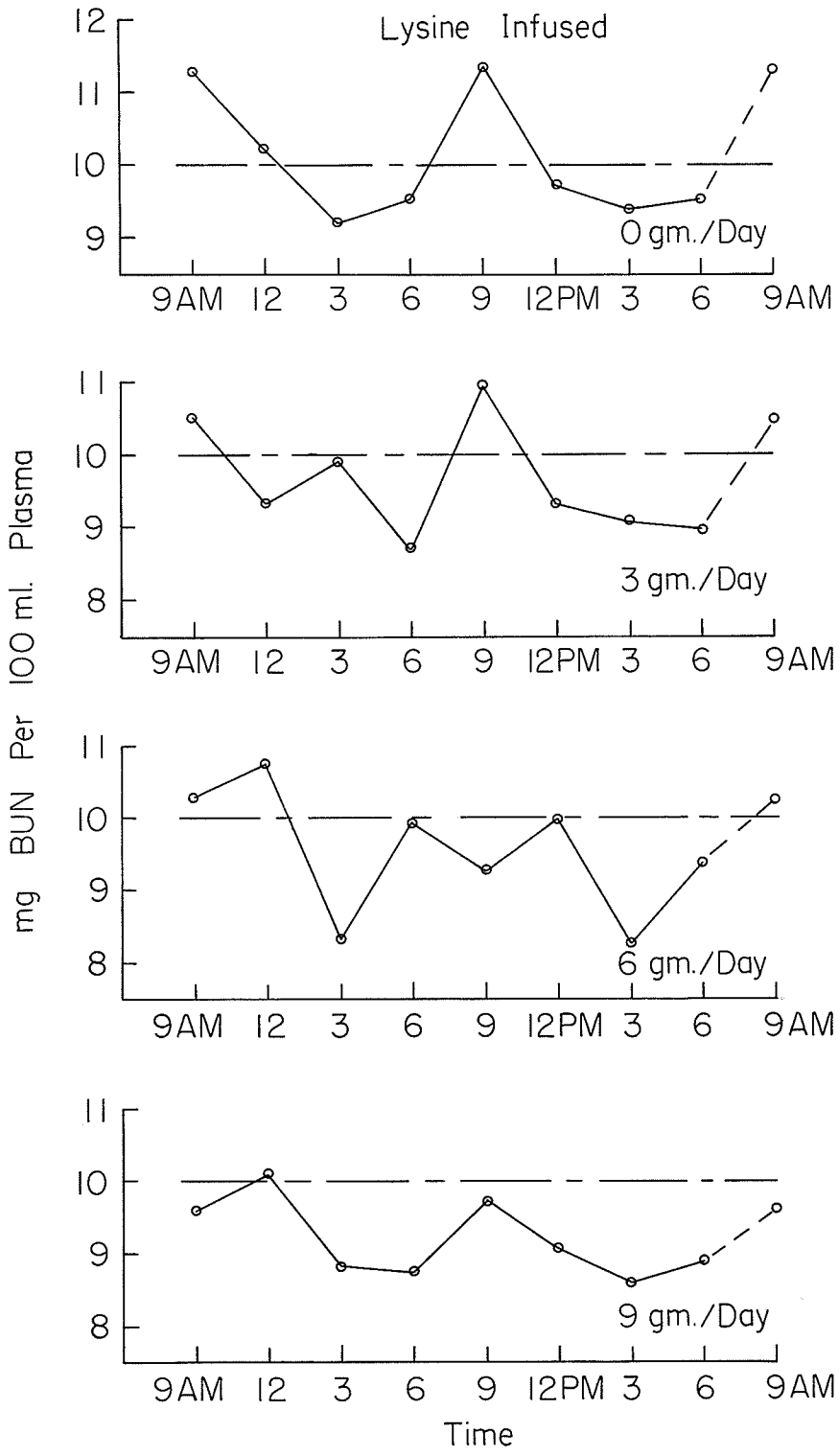
Analysis of BUN over 24 hours indicated quite definite patterns for all treatments (Figure 2). The zero gram level of lysine infusion yielded the most biphasic pattern in relation to times of feeding (7 AM and 7 PM). With each subsequent increase in lysine infusion, the pattern became less marked. Each successive addition of lysine per infusion (Figure 2) resulted in average values over the 24 hour period which were significantly lower ($P<0.01$) for BUN (Table 19) when analyzed as a set of eight 4 x 4 Latin Squares, each time period being an individual Latin Square. The 9 gram treatment (9.19 mg. BUN per 100 ml. plasma) was significantly lower ($P<0.01$) than the zero gram treatment (10.07 mg. BUN per 100 ml. plasma), while neither was significantly different from the 3 and 6 gram treatments (9.62 and 9.53 mg. BUN per 100 ml. plasma, respectively).

Analysis of each individual time period for BUN data was not significantly different. When analyzed as a set of eight 4 x 4 Latin Squares a significant ($P<0.01$) treatment was observed. Treatment averages for BUN are presented in Figure 2 and Figure 3b. Results in Figure 2 were extrapolated from 6 AM to 9 AM in order to indicate a total 24 hour effect. Only one sample was analyzed at the 9 AM time period, the one result having been used twice on Figure 2 at each level of lysine infusion.

Table 19 Part B. Blood urea nitrogen (mg./100 ml. plasma) over a 24 hour collection period.

Time	Grams lysine infused per day				Standard Error
	0	3	6	9	
6 AM	9.57	8.97	9.40	8.85	0.97
9 AM	11.30	10.55	10.25	9.60	0.97
12 NOON	10.17	9.35	10.75	10.10	0.57
3 PM	9.20	9.95	8.35	8.87	0.41
6 PM	9.50	8.70	9.92	8.72	0.70
9 PM	11.42	10.97	9.27	9.70	0.65
12 PM	9.72	9.30	10.00	9.05	0.98
3 AM	9.37	9.07	8.30	8.60	0.85
OVERALL	10.07^{A}	9.62^{AB}	9.53^{AB}	9.19^{B}	0.19

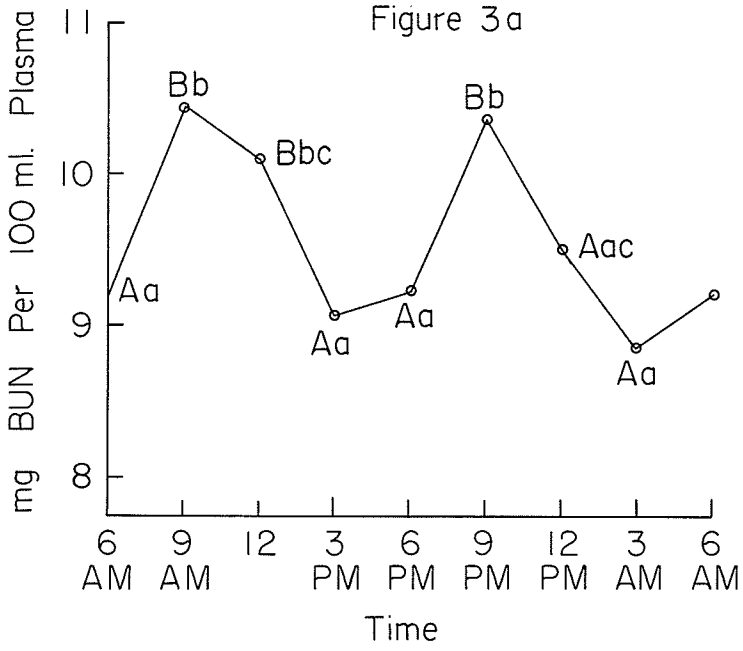
A, B - Treatment means not showing the same superscripts were significantly different ($P<0.01$).

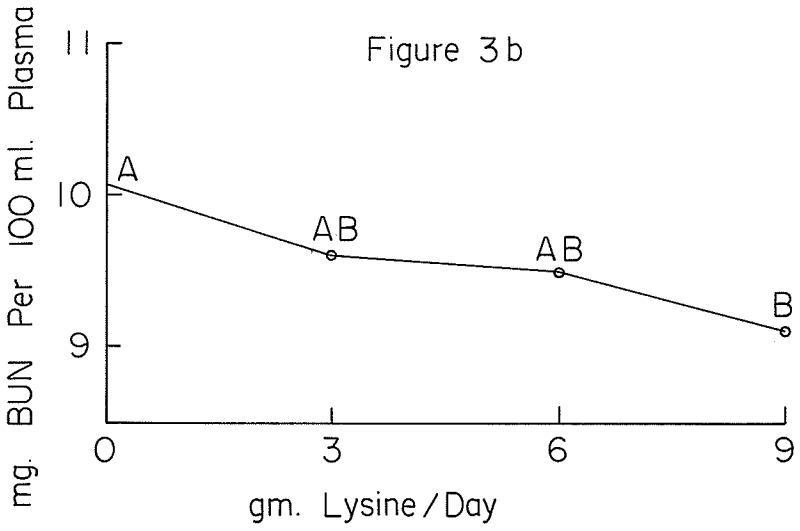


—— - —— Indicates 10 mg BUN/100 ml. Plasma

– – – – 6 AM to 9 AM Extrapolated to a 24 Hour Day

Figure 2. Blood Urea Nitrogen (BUN)





a, b, c Significantly Different ($P<0.05$)

A, B Significantly Different ($P<0.01$)

Figure 3 Blood Urea Nitrogen (BUN)

Abomasal Factors

Rumen organic matter digestibility, total rumen crude fiber + ether extract + nitrogen free extract digestibility and nitrogen reaching the abomasum were determined using chromic oxide as a marker and are reported individually.

Rumen Organic Matter Digestibility (Table 20)

Organic matter (OM) included protein (nitrogen x 6.25), crude fiber, ether extract and nitrogen free extract but excluded the ash portion which was included in dry matter calculations. Abomasal samples analyzed, indicated that at 9 AM the rumen OM digestibility was significantly lower ($P<0.05$) for the 9 gram treatment (21.53%) than the 0, 3 and 6 gram treatments (56.08%, 52.13% and 41.53%, respectively). Increasing the level of lysine infusion appeared to lower rumen OM digestibility. The rumen OM digestibility at 6 grams lysine infusion was slightly lower relative to the zero and 3 gram levels of lysine infusion. The above data referred to the 9 AM collection time only.

Analysis of rumen OM digestibility as a set of eight 4 x 4 Latin Squares was significantly ($P<0.10$) different for overall treatment means (Table 20, Figure 4b). Duncan's Multiple Range test ($P<0.05$) on overall rumen OM digestibility yielded a similar response to the 9 AM

Table 20 Part B. Percent organic matter digestibility in the rumen (excluded ash, included protein).

	Grams lysine infused per day				Standard Error
	0	3	6	9	
6 AM	37.45	43.91	36.22	33.78	7.65
9 AM *	56.08[a]	52.13[a]	41.53[a]	21.53[b]	4.77
12 NOON	48.71	56.38	22.53	31.20	12.84
3 PM	40.27	34.17	20.60	29.01	13.29
6 PM	33.01	38.52	39.15	30.42	13.87
9 PM	44.93	52.87	49.95	47.13	5.45
12 PM	33.42	20.01	32.60	31.43	10.40
3 AM	38.09	42.70	49.09	33.49	7.64
OVERALL**	41.49[a]	42.57[a]	36.47[ab]	32.25[b]	3.09

* Analysis of Variance was significant ($P<0.01$), Duncan's multiple Range test was significant ($P<0.05$).

** Treatment means were significantly different ($P<0.10$) Duncan's Multiple Range test was significant ($P<0.05$).

Figure 4a

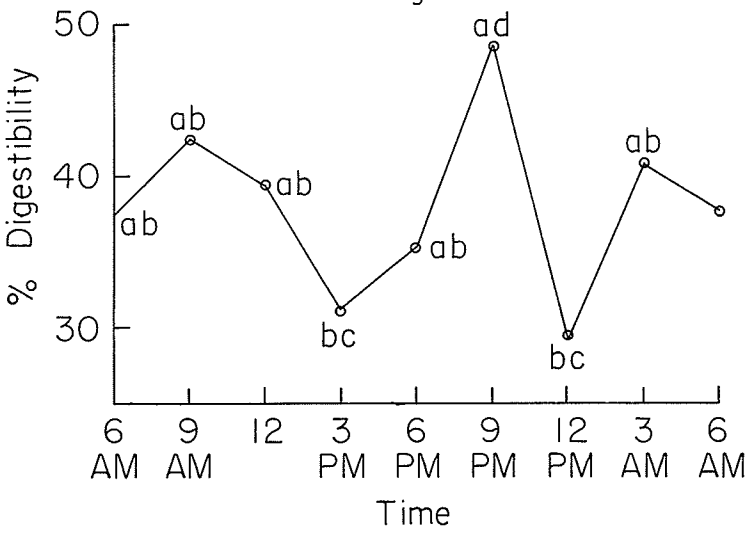
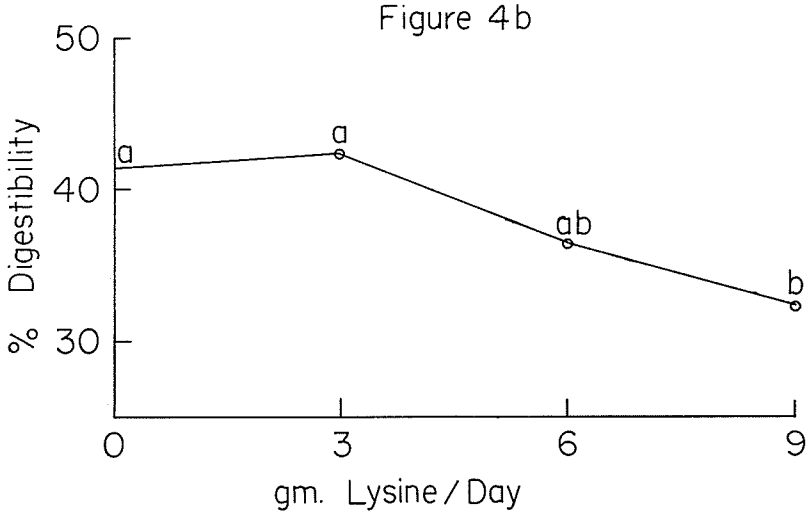


Figure 4b



a,b,c,d Significantly Different ($P<0.05$)

Figure 4 Organic Matter Digestibility

collection time. Rumen OM digestibility for the 9 gram treatment (32.25%) was significantly lower ($P<0.05$) than the zero (41.49%) and 3 (42.57%) gram treatments. Rumen OM digestibility for the 6 gram treatment (36.47%) was intermediate and not significantly different from the other treatments.

Total Crude Fiber (CF), Ether Extract (EE) and Nitrogen Free Extract (NFE) Digestibility in the Rumen (Table 21)

Total CF+EE+NFE was calculated as the non nitrogenous component of total organic matter. The results of CF+EE+NFE digestibility in the rumen demonstrated a more consistent response with respect to the utilization of the carbohydrate portion of the diet in the rumen. Rumen CF+EE+NFE digestibility calculation provided a means of accounting for the highly variable output of nitrogen from the rumen as indicated by the levels of nitrogen reaching the abomasum.

There was a significant ($P<0.05$) treatment effect for CF+EE+NFE digestibility in the rumen at the 9 AM collection time. Increasing levels of lysine infusion decreased ($P<0.05$) rumen CF+EE+NFE digestibility to 34.71% at the 9 gram treatment level, as compared to the 0, 3 and 6 gram treatments which were 62.53%, 62.18 and 50.92% respectively for CF+EE+NFE digestibility in the rumen.

Analysis of overall treatment means for rumen CF+EE+NFE digestibility as a set of eight 4 x 4 Latin Squares was not related to the values observed at the 9 AM

Table 21 Part B. Percent digestibility of crude fiber, ether extract, and nitrogen free extract in the rumen (excluded ash and protein).

	Grams lysine infused per day				Standard Error
	0	3	6	9	
6 AM	47.40	52.82	45.20	43.06	7.52
9 AM *	63.53[a]	62.18[a]	50.92[a]	34.71[b]	4.66
12 NOON	56.64	72.34	32.43	39.35	13.17
3 PM	47.67	46.02	29.51	36.27	14.89
6 PM	43.76	49.59	47.89	39.14	13.33
9 PM	54.81	62.40	58.27	55.93	4.44
12 PM	45.55	34.19	42.74	43.51	9.44
3 AM	45.62	54.22	58.89	45.62	6.91
OVERALL**	50.65[ab]	54.22[a]	45.73[ab]	42.20[b]	3.08

* Treatment means were significantly different ($P<0.05$).

** Treatment means were significantly different ($P<0.10$). Duncan's Multiple Range Test was significant ($P<0.05$).

Figure 5a

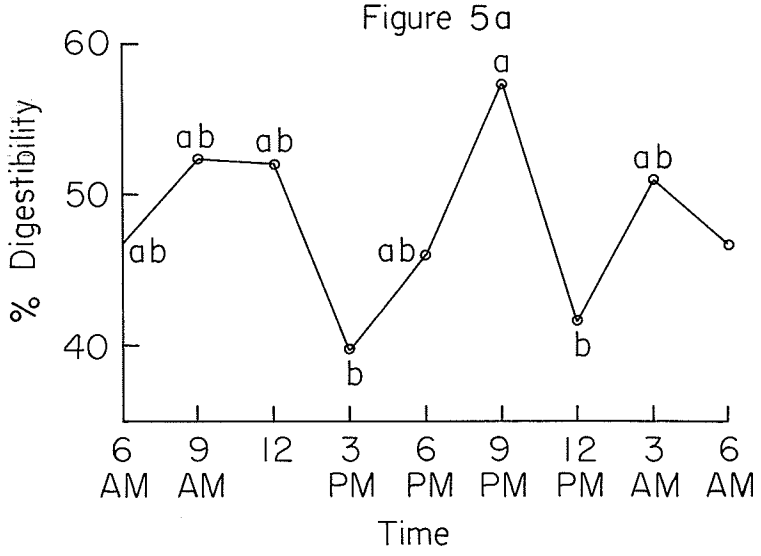
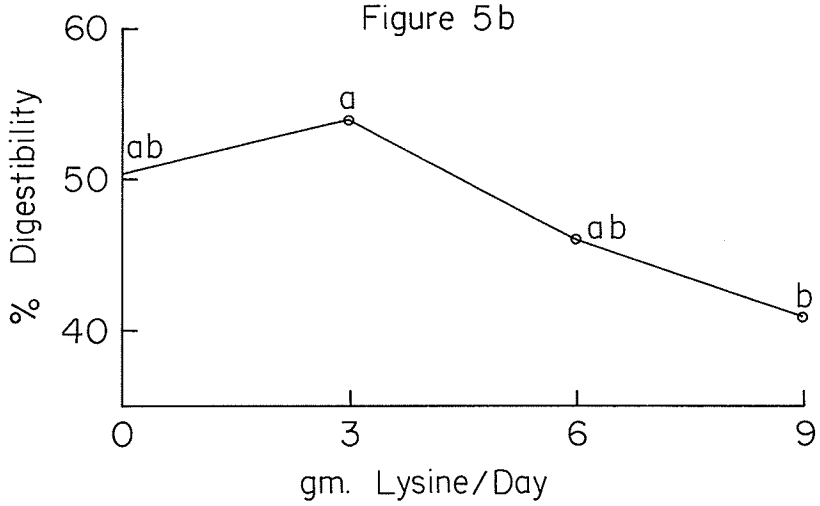


Figure 5b



a, b Significantly Different ($P<0.05$)

Figure 5 Total Crude Fiber, Ether Extract and Nitrogen Free Extract Digestibility in The Rumen.

collection time. Overall treatment means for rumen CF+EE+NFE digestibility were significant ($P<0.10$) according to the analysis of variance and Duncan's Multiple Range test ($P<0.05$). Rumen CF+EE+NFE digestibility at the 3 gram lysine treatment (54.22%) was significantly higher ($P<0.05$) than the 9 gram treatment (42.20%), while neither result was significantly different from intermediate values for the zero (50.65%) and the 6 (45.73%) gram treatments. Overall treatment means for rumen CF+EE+NFE digestibility yielded a similar pattern of response to that of rumen ammonia levels (Table 18) with the 3 gram lysine treatment having the highest level for rumen ammonia and rumen CF+EE+NFE digestibility (16.2 mg. ammonia per 100 ml. rumen fluid and 54.20% rumen CF+EE+NFE digestibility, respectively). A higher rumen ammonia level could have been more conducive to microbial use of CF+EE+NFE, mainly as carbohydrates (CF+NFE), as an energy source for metabolic processes.

Abomasal Nitrogen (Table 22)

Nitrogen reaching the abomasum expressed as a percent of intake nitrogen was highly variable within treatments for each collection time. There was a significant ($P<0.05$) treatment response for nitrogen reaching the abomasum at the 9 AM collection time. The 9 gram lysine infusion (170.71% an average of 247.85%, 177.20%, 128.78% and 129.01% for Periods I, II, III and IV,

Table 22 Part B. Nitrogen reaching the abomasum as a percent of intake nitrogen.

	Grams lysine infused per day				Standard Error
	0	3	6	9	
6 AM	132.14	108.04	125.95	131.15	10.09
9 AM *	96.04[a]	104.53[a]	123.00[ab]	170.71[b]	14.27
12 NOON	106.70	141.86	146.06	125.83	14.71
3 PM	110.99	136.96	140.48	121.80	18.38
6 PM	142.20	121.31	146.47	130.59	27.66
9 PM	124.18	101.22	107.59	114.48	10.57
12 PM	151.48	156.68	137.48	153.07	19.64
3 AM	116.28	125.07	118.67	151.44	15.61
OVERALL	122.50	124.44	130.72	137.39	6.53

* Treatment means were significantly different ($P<0.05$).

Figure 6a

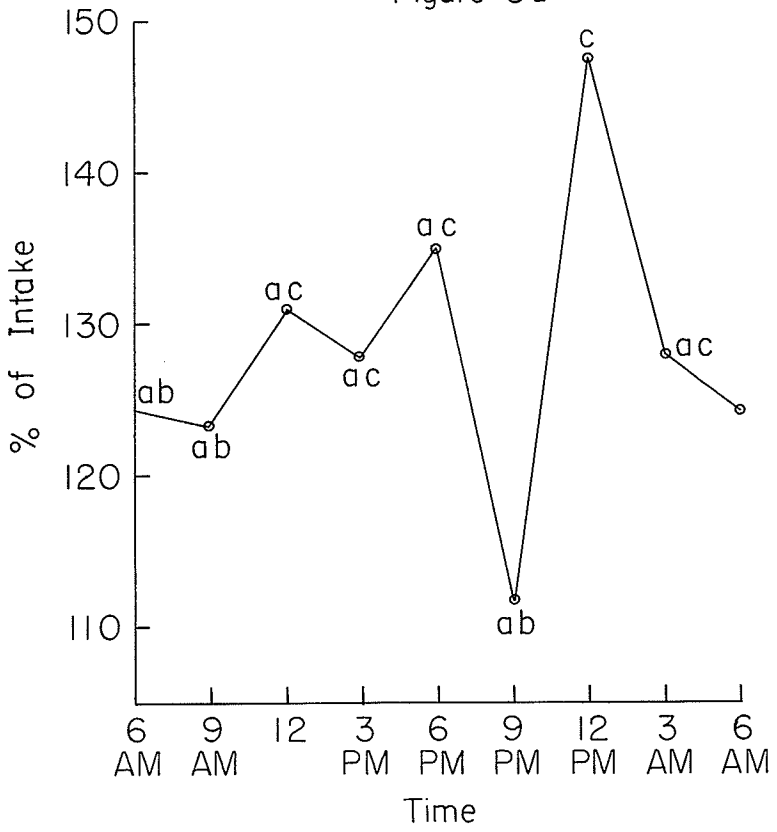
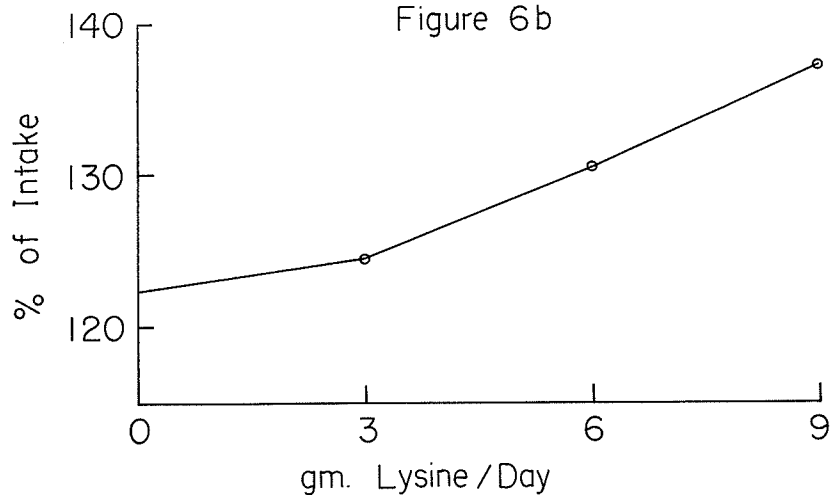


Figure 6b



a, b Significantly Different ($P<0.05$)

Figure 6 Abomasal Nitrogen (Percent of Nitrogen Intake)

respectively) was highest for level of nitrogen reaching the abomasum, and was significantly different ($P<0.05$) from the zero (96.04%) and 3 (104.53%) gram lysine infusions. The 6 gram treatment (123.00%) yielded an abomasal level of nitrogen which was not significantly different from other treatments. Peaks for abomasal nitrogen within treatments exhibited some measure of variation, although the peaks appeared to coincide (Figure 6a).

Increasing the level of lysine infusion increased the level of nitrogen reaching the abomasum when expressed as a percent of intake nitrogen (122.50%, 122.44%, 130.72% and 137.39% for treatments 0, 3, 6 and 9 grams lysine per day respectively). Within treatments, values varied considerably. Overall treatment means for nitrogen reaching the abomasum analyzed as a set of eight 4 x 4 Latin Squares were not significantly different.

Collection Time Period Variation

The term collection time periods refers to the individual collection times at three hour intervals over a 24 hour day. Results for time period analysis were highly variable for the four parameters studied over a 24 hour time period (organic matter and CF+EE+NFE digestibility in the rumen, nitrogen reaching the abomasum and blood urea nitrogen). Values at each time period averaged across treatments gave an indication of the diurnal variation

Table 23 Part B. Twenty-four hour analysis factors - Organic matter (OM) digestibility in the rumen, Total Crude Fiber (CF) + Ether Extract (EE) + Nitrogen Free Extract (NFE) digestibility in the rumen, Nitrogen reaching the abomasum as a percent of intake nitrogen and blood urea nitrogen

Collection time	OM Digestibility	CF+EE+NFE Digestibility	Abomasal Nitrogen	BUN
6 AM *	37.84^{ab}	47.12^{ab}	124.32^{ab}	9.20^{Aa}
9 AM	42.83^{ab}	52.83^{ab}	123.57^{ab}	10.43^{Bb}
12 NOON	39.72^{ab}	50.19^{ab}	130.11^{ac}	10.09^{Bbc}
3 PM	31.01^{cb}	39.87^{b}	127.51^{ac}	9.09^{Aa}
6 PM *	35.27^{ab}	45.09^{ab}	135.14^{ac}	9.21^{Aa}
9 PM	48.72^{ad}	57.85^{a}	111.87^{ab}	10.34^{Bb}
12 PM	29.36^{bc}	41.50^{b}	149.68^{c}	9.52^{Aa}
3 AM	40.84^{ab}	51.15^{ab}	127.87^{ac}	8.84^{Aa}
Standard Error	4.37	4.35	7.80	0.27

a, b - Collection time means within a column not showing the same superscript were significantly different ($P<0.05$).

A. B - Collection time means within a column not showing the same superscript were significantly different ($P<0.01$).

* Animals were fed 7 AM and 7 PM.

encountered with an animal fed twice daily (Figures 3a, 4a, 5a and 6a). Blood urea nitrogen (Figure 3a) with steers fed twice a day was the most uniform and peaked following feed intake,(7 AM, 7 PM). For the other three parameters, rumen organic matter digestibility and CF+EE+NFE digestibility in the rumen and nitrogen reaching the abomasum, results were not consistent between the two twelve hour periods, 6 AM to 6 PM and 6 PM to 6 AM. The interval 6 AM to 6 PM had a pattern of maxima and minima as was expected following the 7 AM feeding time. Observations made following the 7 PM feeding fluctuated considerably more with regard to the same parameters. Nitrogen in the abomasum as a percent of intake nitrogen was most variable within both twelve hour periods. Data on collection time period variation is presented in Table 23 and Figures 3a, 4a, 5a and 6a.

DISCUSSION

Part A. Continuous Feeding

Pure corn protein (zein) as used by Ely *et al.* (1967) and McDonald (1954) contained an insufficient level of lysine and tryptophan for growth of monogastric animals. The lysine content of the diet used in the present experiment was relatively low in lysine at 0.281 (100% Dry Matter) as a percent of the total diet (2.23% of the total amino acids designated as dietary sources of α-amino nitrogen). Although tryptophan was not analyzed, it was also considered to be low in the diet. Leucine was present at the highest level (1.508% of the diet, 100% Dry Matter) and could have influenced nitrogen retention of steers through competition at absorption sites in the small intestine.

The expected dietary deficiency of lysine would have been supplemented by the lysine infusion to the abomasum and the molar percent of lysine would have been increased in the abomasum. Lysine as a molar percent of total amino acids in the abomasum increased the most in relation to the dietary level, as indicated by feed analysis (Table 5). In relation to total abomasal lysine, the infused lysine was not critical in affecting the total lysine in the abomasum although lysine did form a greater molar percent of total amino acids in the abomasum at the higher levels of lysine infusion. The highest level of

infusion of lysine (9 grams per day) resulted in a decreased nitrogen retention as compared to the other treatments. Nimrick et al. (1970a) infusing lysine with a urea diet observed a decreased nitrogen retention in sheep unless methionine was infused along with lysine.

Calculated amounts of amino acids reaching the abomasum in relation to daily intake levels (Table 24) indicated a substantial increase in lysine in the abomasum, as well as minor increases for some other amino acids. These levels were calculated using dietary levels of nitrogen (averaged for each treatment), amino acid nitrogen as a percent of dietary nitrogen (89.5%), abomasal nitrogen as a percent of dietary nitrogen (Table 11C) and percent amino acid nitrogen in abomasal nitrogen (Appendix Table 5). The decrease in proline level was attributed to the Stickland reaction (Lewis, 1955; Clarke et al., 1966). The steers on the 3 gram level of infusion exhibited virtually no increase in amino acids in the abomasum when compared to intake levels of amino acids. The higher level of ammonia in the rumen probably resulted in an ammonia nitrogen loss through absorption across the rumen wall. A lower level of nitrogen (104% of intake nitrogen) with the 3 gram infusion entered the abomasum when compared to the other treatment levels (0, 6 and 9 grams lysine per day which averaged 125% of intake nitrogen).

Table 24 Part A. Calculated Levels of Amino Acids Reaching the Abomasum expressed As Grams per day

	Intake *		Abomasum			
	0,3,6	9	0	3	6	9
Lysine	17.72	18.29	53.73	44.12	60.49	68.88
Histidine	11.53	11.90	15.42	13.53	14.78	17.06
Arginine	24.54	25.33	33.21	27.76	30.45	34.12
Threonine	25.17	25.98	43.43	33.28	37.44	43.15
Valine	28.33	29.24	47.12	35.56	39.76	46.90
Methionine	13.01	13.43	22.46	17.19	19.76	21.35
Isoleucine	25.87	26.71	41.75	32.73	36.63	42.42
Leucine	95.06	98.12	93.73	73.46	95.68	97.62
Phenylalanine	49.92	51.53	45.45	34.73	38.96	46.35
Aspartic Acid	53.01	54.72	93.02	68.42	75.43	90.14
Serine	33.68	34.76	42.81	33.83	37.60	44.43
Glutamic Acid	130.14	141.60	146.84	114.27	127.89	152.64
Proline	60.25	62.20	55.23	44.95	48.76	54.83
Glycine	26.57	27.43	39.55	30.93	43.70	40.96
Alanine	54.98	56.75	62.98	50.95	55.99	66.33
Cystine	-	-	3.70	3.04	4.74	4.38
Tyrosine	27.35	28.23	40.61	31.90	35.43	40.78

* Intake of Nitrogen varied:
0,3,6 - 128.25 grams per day
9 - 136.52 grams per day

Ammonia levels were significantly higher ($P<0.05$) in the rumen fluid at the 3 gram level of lysine infusion. This could be partly explainable if lysine nitrogen was recycling to the rumen as urea. Infused lysine was equivalent to 0.45 grams nitrogen, 1.00 grams of urea (45% nitrogen) or 1800 milligrams ammonia (82% nitrogen). This amount of ammonia equaled 2.25 milligrams of ammonia per 100 ml of rumen fluid (80 litres or 20 gallons of fluid in the rumen). This would account for 20% of the increase in level of ammonia observed (15 mg. per 100 ml. rumen fluid at zero grams lysine infusion to 25.5 mg. per 100 ml. at 3 grams lysine infusion). Recycling of the nitrogen as urea was feasible as 8.2 mg. blood urea nitrogen per 100 ml. plasma was equivalent to 18.2 mg. urea per 100 ml. at the point of maximum urea transport across the rumen wall for cattle (Vercoe, 1969). The higher ammonia level in the rumen increased rumen organic matter digestibility and occurred mainly as a disappearance of total crude fiber, ether extract and nitrogen free extract. A more active fermentation process might have been indicated, which did not affect nitrogen retention. The main contributing factor to the lower abomasal level of nitrogen at the 3 gram lysine infusion was apparently the high ammonia level in the rumen.

Infusion of lysine increased the level of lysine in plasma (Table 4) from an average of 7.6 u moles per 100 ml.

plasma for the 0, 3 and 6 gram treatments to 11.58 u moles per 100 ml. plasma for the 9 gram treatment. This appeared to be a definite response to lysine infusion, both expressed as absolute values (Table 6) and as a molar percent of total plasma amino acids (Table 7). The plasma level of lysine was believed to be contributory to the reduced nitrogen retention of steers at the 9 gram lysine infusion. Absolute values for other amino acids did not yield any differences (Table 6). The molar percent of leucine and isoleucine in plasma were significantly different. The leucine effect could have been a result of the high level of leucine in corn, while its relationship to isoleucine at sites of absorption in the small intestine could have affected isoleucine levels. Valine, however, did not seem to be affected. Valine, isoleucine and leucine have been shown to share a common absorption site in the small intestine (Hagihira et al., 1960).

The rumen fermentation process could have been expected to remain relatively constant over the twenty-four hour day with continuous feeding (Ibrahim et al., 1969). Treatment variations could therefore have been expected to remain constant. The pattern of treatment effects within the two collection times (Table 11) were in close agreement for rumen organic matter digestibility, rumen digestibility of the non nitrogenous dietary component, and abomasal nitrogen as a percent of intake nitrogen. Both protozoa

and bacteria were expected to contribute a steady level of microbial protein as microbial population levels were stabilized by a higher frequency of feeding (Nottle, 1956; Moir and Somers, 1957).

Amino acid metabolism, particularly synthesis of the essential amino acids, was favoured in the rumen. Except for leucine, which was at a higher level in the diet than the abomasum, total essential amino acids increased from 37% of total amino acids in the diet to 44% of total amino acids in the abomasum (molar percent basis), using the zero gram lysine treatment as a baseline. A further concentrating of essential amino acids was evident by an increase in molar percent of total essential amino acids from the abomasum at 44% of total amino acids to plasma at 50% of total amino acids (Table 8). The rumen environment, endogenous nitrogen, as well as selective absorption of amino acids in the small intestine upgraded the dietary levels of amino acids to quite an extensive degree, enough to cover up dietary deficiencies, and notably lysine in the case of a high corn diet.

Part B. Twice a day feeding

Feeding steers twice a day at 12 hour intervals resulted in diurnal variations in relation to rumen fermentation. The products of fermentation and levels of bacteria and protozoa, as well as the digesta flow characteristics along the lower gastrointestinal tract were also influenced by diurnal variation. Many of the parameters in Part B. (twice a day feeding) did not form as clear a relationship as was observed in Part A. (continuous feeding). Levels of significance ($0.05<P<0.015$) only served to indicate a response that was most likely affected by the diurnal rhythmicity encountered in an animal fed twice a day. Apart from the diurnal rhythm in rumen fermentation as exemplified by ammonia (Lewis, 1958) and volatile fatty acids in the rumen (Wright and Grainger, 1969), and protozoal and bacterial population variation (Warner, 1965), plasma amino acid levels (Theurer et al., 1966) and blood urea nitrogen (Lewis, 1958) could also have been under the influence of diurnal regulation with respect to times of feeding.

Balance data (Table 12) indicated a reduction in nitrogen retention with the 6 gram level of lysine infusion. A significantly lower ($P<0.05$) nitrogen digestibility (higher fecal nitrogen output), plus a slightly increased level of urinary nitrogen excretion, resulted in a slightly decreased although non significant nitrogen

retention. The main contributing factor appeared to be the greater amount of fecal nitrogen output either as a result of a high metabolic fecal nitrogen output or a lower absorption of amino acids along the small intestine. Plasma amino acid levels, both absolute (Table 14) and as a molar percent of total plasma amino acids (Table 16) were affected although not consistently with the 6 gram lysine infusion.

Amino acids in the abomasum, in relation to intake of amino acids expressed as grams per day are presented in Table 25. Individual amino acids as a molar percent of total abomasal amino acids did not vary among treatments. However, the abomasal levels of amino acids increased as total nitrogen levels increased in the abomasum from zero to 9 grams lysine infusion. Abomasal proline levels in Part B. at zero grams lysine infusion were lower relative to proline intake as compared to abomasal versus dietary proline levels in Part A. Proline has been implicated in the Stickland reaction (Lewis, 1955; Clarke et al., 1966) as a hydrogen acceptor in bacterial amino acid metabolism. A higher proline activity in the rumen in Part B. than Part A. with respect to utilization of dietary proline in the Stickland reaction could possibly have indicated that a greater turnover of microbial protein appeared to have occurred in twice a day fed animals.

Table 25 Part B. Calculated Levels of Amino Acids Reaching the Abomasum Expressed As Grams Per Day

Amino Acid	Intake Amino Acid	Abomasum grams lysine per day			
		0	3	6	9
Lysine	20.57	54.19	52.65	51.98	66.82
Histidine	13.39	18.62	14.44	16.47	23.08
Arginine	28.50	31.69	32.75	33.67	35.94
Threonine	29.23	39.95	44.55	43.56	47.48
Valine	32.90	43.82	45.25	45.66	51.13
Methionine	15.10	20.39	23.42	24.07	25.92
Isoleucine	30.05	39.60	41.64	42.37	46.67
Leucine	110.39	90.76	89.36	101.12	111.57
Phenylalanine	57.97	43.74	45.69	47.86	53.15
Aspartic Acid	61.56	79.89	86.28	93.43	97.90
Serine	39.11	41.29	42.00	44.75	49.51
Glutamic Acid	159.30	145.12	150.29	153.82	172.22
Proline	69.97	48.12	57.40	61.22	62.57
Glycine	30.86	40.54	42.35	42.00	43.74
Alanine	63.85	63.37	67.18	68.36	73.71
Cystine	-	5.31	2.82	3.66	5.16
Tyrosine	31.76	37.33	42.35	41.18	46.57

Amino acids as a molar percent of total amino acids in the abomasum in Part B. were nearly identical to results obtained in Part A. There was no difference among treatments in lysine as a molar percent of total amino acids in Part B. However, the increase in level of lysine in the abomasum relative to intake levels of lysine was of the same order as the increase observed in Part A.

Several plasma amino acids were significant ($P<0.15$) for absolute values (Table 15) or as a molar percent of total plasma amino acids (Table 16). The amino acids arginine, phenylalanine, tyrosine, methionine, and leucine peaked at the 6 gram lysine infusion in plasma. Plasma cystine as a molar percent of total plasma amino acids was lowest at the 3 gram lysine infusion and highest at the 9 gram lysine infusion. This did not coincide with peaks for other plasma amino acids which occurred at the 6 gram lysine infusion. In relating the various amino acids several relationships could be involved. Lysine and arginine could be related as they have been previously studied with regard to an apparent antagonism (Jones, 1964); phenylalanine and tyrosine are both phenyl derivatives of alanine and methionine and cystine are both sulphur containing amino acids. The latter two amino acid

relationships are as a result of interconversions and the sparing action of one amino acid upon the other (cystine on methionine, tyrosine on phenylalanine). The difference for the molar percent of leucine in plasma could have been a result of the high dietary level having been maintained in the abomasum.

The highest level for plasma tyrosine observed at the 6 gram lysine infusion was also the treatment with the reduced nitrogen balance. Tyrosine, although not considered a true essential amino acid, could have been termed essential according to Downes (1961), as tyrosine like other essential amino acids did not exhibit radioactive carbon as a result of amino acid synthesis in sheep body tissue. Plasma phenylalanine paralleled plasma tyrosine both for absolute values (Table 15) and as a molar percent of total plasma amino acids (Table 16). It is to be noted that in Part A. (continuous feeding) both abomasal and plasma responses for tyrosine as a molar percent of total amino acids were similar, while the abomasal and plasma tyrosine responses were different in Part B. (twice a day feeding). The main interaction was probably at absorption sites, although the effect of diurnal rhythmicity in tyrosine plasma levels (Wurtman 1970) could also have been important. Care must be taken, however, to differentiate the true effect of tyrosine on phenylalanine, the latter having been regarded as an essential dietary

amino acid, the former acting as an interconversion source, and supplementary in a phenylalanine deficiency.

The cystine effect encountered in plasma which was different from other plasma levels of amino acids, could not be explained in relation to the interaction with other amino acids.with the 6 gram lysine infusion. Methionine and cystine combined as a molar percent of total plasma amino acids increased as the level of lysine infusion increased (1.70, 1.77, 2.08 and 2.41 for 0, 3, 6 and 9 grams lysine infused per day respectively). This could be interpreted as a mobilization of sulphur containing amino acids from body tissue proteins. Other work with sheep (Hogan, Weston and Lindsay, 1968) has supported the fact that methionine and cystine responses in plasma should be considered together.

Essential amino acids having been implicated according to plasma levels in Part B. were tyrosine and phenylalanine, the sulphur containing amino acids, methionine and cystine, followed by arginine and leucine. A lysine-arginine antagonism as shown by a higher plasma lysine level and a lower plasma arginine level in response to an increased intake of lysine in monogastrics (Jones, Petersburg and Burnett, 1967) did not occur in the present study. Plasma arginine levels appeared to parallel plasma lysine increases to some extent in both Part A. and Part B. A true antagonism as has been shown

to exist in rats (Jones, Wolters and Burnett, 1966) and chickens (Jones et al.,1967) did not appear to occur in steers, specifically with respect to plasma levels of arginine and lysine. A leucine antagonism could also have been implicated through high dietary levels of leucine, which were maintained in the abomasal contents. Leucine, isoleucine and valine have been shown to share a common absorption site in the small intestine (Hagihira et al., 1960).

Rumen organic matter (OM) digestibility and crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE) digestibility in the rumen plus nitrogen reaching the abomasum and blood urea nitrogen (BUN) yielded very definite patterns of diurnal rhythmicity (Table 20). The 9 AM period demonstrated a significant influence among all collection times for rumen OM digestibility (significantly lower ($P<0.05$) at 9 grams lysine per day), total CF+EE+NFE digestibility in the rumen (significantly lower ($P<0.05$) at 9 grams lysine per day) and nitrogen in the abomasum as a percent of intake nitrogen (significantly higher ($P<0.05$) at 9 grams lysine per day). Abomasal nitrogen was the most variable between collection times. There was no similar response over the two twelve hour periods (6 AM to 6 PM, 6 PM to 6 AM animals having been fed at 7 AM and 7 PM). Animals appeared to have consumed the ration more quickly at the 7 AM feeding than

the 7 PM feeding. Consequently a greater availability of substrate in the rumen for microbial use during the early morning would have increased rumen fermentation activity in relation to times of feeding. The data appeared to support this to some extent. The possibility that a circadian rhythm influenced the abomasal nitrogen response must be considered. Rumination time periods, or microbial responses in the rumen to daylight (Gordon and McAllister, 1970) might have contributed to the rumen fermentation characteristics. However, due to confounding by times of feeding, as well as lack of support data in this experiment, the reason for the differences between the two twelve hour periods could not be substantiated. Sampling error for abomasal samples over the day from 6 PM to 6 AM, particularly as it affected the animals during the night period of rest, when samples were obtained every three hours could have affected flow patterns to some extent.

Infusion of lysine continuously, while in conjunction with feeding twice a day, could have resulted in a variable addition of nitrogen as lysine in the abomasum in relation to the diurnal variation of nitrogen passing out of the rumen. During peaks of nitrogen flow lysine would have contributed less to total abomasal nitrogen, while having contributed to a greater extent during reductions in flow rate. If a normal biphasic diurnal

rhythmicity, with identical twelve hour intervals with respect to times of feed intake had occurred, one would have expected the animal to be in a state of lysine excess at absorption sites in the small intestine for one half the total 24 hours. Lysine could possibly have adversely affected absorption of other amino acids with respect to their availability for use by body tissues.

Ammonia levels in the rumen (Table 18), sampled at 5 PM, did not indicate a response to lysine infusion. If one were to rely on calculations of Part A., the recycling of lysine nitrogen as urea could have accounted for the ammonia increase in the rumen at the 3 gram level of lysine infusion. At 6 grams lysine infusion, one would have expected less of a recycling response as a greater portion of the dietary nitrogen was excreted both in feces and urine, and most markedly in the feces. Recycling of infused lysine nitrogen as urea would have increased the rumen pool of total nitrogen available for use by the rumen microbial population. Consequently a higher output of nitrogen to the abomasum and less total organic matter could possibly have been a result of a more active rumen fermentation due to a more available source of nitrogen as ammonia in the rumen. The 9 AM collection time yielded a significant effect both for rumen organic matter digestibility and rumen nitrogen

output to the abomasum. This response was expected as intake of feed has been shown to cause the fermentation rate of the rumen to increase in response to the influx of new substrate for the rumen microbial population.

Blood urea nitrogen (BUN) in ruminants can be derived either from conversion of ammonia absorbed from the rumen to urea or from amino acid degradation to urea. These amino acids would be derived either from body protein or absorbed from the small intestine and deaminated in the liver. With respect to times of feeding, BUN alone appeared to yield a near perfect biphasic pattern of maxima and minima for the 24 hour period (Figure 3a). Lower BUN values (24 hour averages) for the lysine infusion treatments were associated with a higher rumen output of nitrogen to the abomasum. Increased infusions of lysine appeared to have resulted in an increased output of nitrogen from the rumen to the abomasum, most likely by way of utilization of recycled BUN as ammonia by the rumen microbial population.

As with Part A. (continuous feeding) the rumen contribution as microbial protein, endogenous secretions as well as selective amino acid absorption had increased the molar percent of essential amino acids from diet (37%) to abomasum (44%) to plasma (55%) as compared to non essential amino acids. However, the plasma levels of amino acids were different between Part A. (continuous

feeding) and Part B. (twice a day feeding). A dietary deficiency of lysine in a corn diet appeared to have been corrected in the rumen.

General Discussion

The responses of steers in Part A. (continuous feeding) and Part B. (twice a day feeding) were very different. The method of feeding appeared to have affected the rumen fermentation to the greatest extent, while possible differences in nitrogen intake could also have been implicated.

Nitrogen intakes varied between Part A. at 128 grams per day and Part B. at 149 grams per day. The highest level of lysine infusion (9 grams lysine per day) resulted in a significantly reduced nitrogen retention in Part A., while in Part B., the 6 gram lysine infusion gave some indication of a similar reduction in nitrogen retention. The Part B. response may have been a result of interaction between amino acids other than lysine.

In Part A., it was expected that a continuous feeding mechanism would have maintained a steady level of fermentation in the rumen over 24 hours (Ibrahim et al., 1969). There would have been a constant high level of a protozoal population (Moir and Somers, 1957) and a bacterial population (Nottle, 1956) in response to a more frequent feeding schedule. Consequently the type of digesta and the availability of the various amino acids would have varied according to the type of population in the rumen in relation to dietary components. For Part B. the situation was very much different. Bacteria and

protozoa exhibited a diurnal rhythmicity in relation to the availability of fermentation products and time of introduction of dietary components into the rumen system (Warner, 1965). Therefore at any time during the 24 hour day, the type of digesta entering the abomasum as α-amino nitrogen would have varied according to the various fluxes in microbial population. Hence the response of the animal would have varied accordingly.

The abomasal levels of amino acids in Parts A. and B. were similar while plasma levels differed. The plasma level differences could have been associated with different availabilities of amino acids between Parts A. and B. Although all animals ate the same diet, rumen fermentation differed as a result of the method of feeding. The microbial contribution to the digesta could have yielded amino acids which were not all similar in availability for absorption.

Amino acids affected by lysine infusion were lysine, phenylalanine, tyrosine, methionine, cystine, arginine and leucine. The infused lysine could have interacted with arginine, and neither would have been limiting as such in the rumen digesta as presented to the host animal. Sulphur-containing amino acids cystine and methionine, and specifically methionine, have at times been indicated to be limiting in the diets of sheep (Nimrick et al., 1970a; Reis and Schinckel, 1963,

1964). Care must be exercised in interpreting these results with respect to cattle, as sheep show a requirement for sulphur containing amino acids for wool growth. The plasma leucine response could have been an interaction with isoleucine mainly, and valine to a lesser extent, at absorption sites in the small intestine. An unexpected response occurred with phenylalanine and tyrosine in plasma of twice a day fed animals. A high level of tyrosine in plasma relative to the abomasum in Part B., but not Part A., indicated an interaction at absorption sites in the small intestine, or at metabolic sites in the body. In order to determine a phenylalanine requirement by body tissues, both phenylalanine and tyrosine must be evaluated together. In reevaluating the phenylalanine and tyrosine requirements for growing rats Stockland, Lai, Meade, Sowers and Oestermer (1971) concluded that for a dietary phenylalanine deficiency in rats, plasma tyrosine levels were a better gauge of the dietary phenylalanine deficiency than plasma phenylalanine levels. Plasma tyrosine yielded a more accurate picture relative to a dietary phenylalanine or a dietary phenylalanine plus tyrosine deficiency or excess. Such appeared to be the case in the present study with steers.

The contribution of infused lysine to the total lysine in the abomasum was masked in the abomasum by the rumen output of lysine. Lysine as a molar percent of total amino acids remained constant over all treatments. However, with each successive addition of infused lysine, a higher level of total nitrogen reached the abomasum. Consequently infused lysine did not increase in total to a basal level of lysine entering the abomasum. Therefore, a linear increase of lysine in the abomasum (i.e. from 3 to 6 to 9 grams lysine infusion) was not indicated when compared to the basal level observed at zero grams lysine infusion.

SUMMARY AND CONCLUSIONS

Lysine was infused at 0, 3, 6, and 9 grams daily per abomasum with steers fed continuously or twice a day. A corn based, corn gluten meal supplemented diet (11.2% crude protein, 90% dry matter) was fed.

Part A. Continuous Feeding

(1) Infusion of 9 grams lysine daily per abomasum resulted in a reduced nitrogen retention (percent nitrogen absorbed retained) in steers fed 16 lb. daily. An increased excretion of urinary nitrogen as well as an increased level of plasma lysine were believed to be partly responsible for the reduced nitrogen retention indicated at 9 grams lysine infused per day. Approximately 20% of the increased nitrogen excretion was attributable to the lysine infusion.

(2) Rumen ammonia was significantly higher at 3 grams lysine infusion per day as compared to the other treatments. A higher digestibility of non nitrogenous dietary components in the rumen was observed, as well as a reduction of rumen output of nitrogen to the abomasum. A loss of ammonia from the rumen was indicated, but nitrogen balance data did not demonstrate a loss of nitrogen at 3 grams lysine infused per day.

(3) Continuous feeding has been shown to stabilize rumen fermentation as exemplified by rumen ammonia and

volatile fatty acid levels, rumen pH and microbial population variations in the rumen. Since rumen population variation was reduced, a constant homogeneous level of amino acids from dietary as well as microbial sources would have reached the abomasum during a twenty-four hour period. Amino acid studies with ruminants continuously fed could possibly be an experimental tool in determining specific amino acid deficiencies in the digesta leaving the rumen. A minimum of sampling intervals, without reference to diurnal variation encountered on an animal fed once or twice a day, could be used to indicate amino acid interactions quite readily.

Part B. Twice a day feeding

(1) Lysine infusion did not appear to have a direct effect on nitrogen retention (expressed as percent absorbed nitrogen retained). However, a significant ($P<0.05$) reduction in nitrogen digestibility (higher fecal nitrogen output) at 6 grams lysine per day demonstrated a possibility of an amino acid interaction at absorption sites in the small intestine.

(2) The plasma amino acid levels of methionine, arginine, leucine, phenylalanine and tyrosine peaked at 6 grams lysine infusion per day. A possible higher activity of essential amino acids in plasma was indicated, the effect having been to mobilize body protein to meet a deficiency relative to amino acid availability in the digesta in the small intestine.

General Conclusions

(1) Lysine infused per abomasum of steers did not increase the level of total available lysine in the abomasum. Rumen microbial protein contributed to a total lysine level in the abomasum two to three times the dietary lysine level. Nitrogen retention reduction in Part B. (twice a day feeding) appeared to have been following the similar trend observed in Part A. (continuous feeding).

(2) For both Parts A. and B. total essential amino acids as a molar percent of total amino acids increased from diet (37.2%), to abomasum (averaging 44%), to plasma (averaging 50% in Part A., 55% in Part B.). The rumen contribution as microbial protein, endogenous nitrogen secretions as well as selective absorption of amino acids in the small intestine appeared to have increased the total essential amino acids available for use by ruminant tissue.

(3) A difference between plasma amino acid levels was noted between Part A. (continuous feeding) and Part B. (twice a day feeding). The amino acid availability of microbial protein within each feeding mode could have been different. The method of feeding (once or twice a day versus a more frequent feeding schedule) has been shown to affect the metabolism of the rumen microbial population. Most likely a different rumen population had

developed within each feeding mode. The amino acids subsequently made available through proteolytic activity in the small intestine could have been affected by the type of microbial population in the rumen, and the microbial protein contribution to total protein in the lower gastrointestinal tract.

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A P P E N D I X

APPENDIX Table 1 Division of experimental animals by breed, age, and weight, within and between squares.

	Animal Number	Breed *	Age to Aug 31/70 (days)	Weight Period I (pounds)
Part A. Continuous Feeding	10	H	211	740
	15	H(AxBSxH)	204	804
	17	H(AxBSxH)	193	750
	21	H	186	696
Part B. Twice A Day	8	H	220	631
	12	H	206	767
	13	H(AxBSxH)	203	670
	23	H(BSxH)	183	696
		Average		
		Continuous Feeding	199	645
		Twice A Day	203	696

* - H - Holstein
A - Ayrshire
BS - Brown Swiss

APPENDIX Table 2 Nitrogen Balance data expressed as grams nitrogen per day

Part A.

	Grams lysine per day				Standard Error
	0	3	6	9	
Total Intake	128.25	128.81	129.38	138.21	-
Retained	70.87	74.88	71.47	65.36	4.61
Urine *	24.35[a]	20.13[a]	22.23[a]	36.95[b]	2.50
Feces	33.02	33.74	35.57	36.31	1.01

Part B.

	Grams lysine per day				Standard Error
	0	3	6	9	
Total Intake	148.93	149.49	150.06	150.62	-
Retained	82.54	86.88	64.61	75.36	5.97
Urine	27.52	26.12	42.05	35.74	1.17
Feces *	38.88[a]	36.44[a]	43.30[b]	39.37[a]	1.04

* Treatment means were significantly different ($P<0.05$).

APPENDIX Table 3 Nitrogen intakes for Parts A. and B. over the total experiment (grams nitrogen per day) including lysine nitrogen added per day.

Part A.

Period	grams lysine infused per day 0	3	6	9	Period** Average
I	148.93 (17)*	149.49 (21)	150.06 (15)	150.62 (10)	149.77^{A}
II	132.38 (21)	99.84 (10)	133.51 (17)	134.07 (15)	124.95^{B}
III	132.28 (15)	132.94 (17)	100.41 (10)	134.07 (21)	124.95^{B}
IV	99.28 (10)	132.94 (15)	133.51 (21)	134.07 (17)	124.95^{B}
Treatment Average	128.25	128.81	129.38	138.21	

Standard Error = 4.14

Animal** Average	10	15	17	21
	112.53^{A}	137.36^{B}	137.36^{B}	137.36^{B}

* Animal Numbers are in brackets below each value (10, 15, 17, 21)

** Period and Animal Means are significantly different ($P<0.01$)

Part B.

	grams lysine infused per day 0	3	6	9
Treatment Means *	148.39	149.49	150.06	150.62

* All variation attributed to treatment differences.

APPENDIX Table 4 Lysine nitrogen infused as a percent of total nitrogen, including lysine nitrogen

Part A.

Period	grams lysine infused per day 0	3	6	9
I	0 (17)*	0.37(21)	0.75(15)	1.12(10)
II	0 (21)	0.56(10)	0.85(17)	1.26(15)
III	0 (15)	0.42(17)	1.13(10)	1.26(21)
IV	0 (10)	0.42(15)	0.85(21)	1.26(17)
Treatment Means	0 ^{A}	0.44^{B}	0.90^{C}	1.23^{D}
				Standard Error 0.05

* Animal numbers are in brackets (10,15,17,21).

Part B.

	grams lysine infused per day 0	3	6	9
Treatment Means	0.00	0.37	0.75	1.12

A,B,C - Treatment means were significantly different ($P<0.01$).

APPENDIX Table 5 Amino acid nitrogen in the abomasum as a percent of total nitrogen (microkjeldahl)

	grams lysine infused per day					
	0	3	6	9	Standard Error	Range
Part A.*	86.03	82.45	82.14	83.52	2.24	72.26→97.29
Part B.*	82.58	84.93	84.03	88.46	2.41	68.20→96.90

Overall Averages **

	Mean	Standard Deviation	Standard Error
Part A.	83.53	6.50	1.62
Part B.	85.00	6.80	1.70
Combined	84.27	6.58	1.16

* Data is calculated by way of analysis of variance

** Data is calculated by way of:

$$\text{Mean} = \frac{\Sigma x}{n}$$

$$\text{Standard Deviation} = \frac{\Sigma (X-\bar{x})^2}{n-1}$$

$$\text{Standard Error} = \frac{\text{Standard Deviation}}{\sqrt{n}}$$

APPENDIX Table 6 Total Amino Acids in the Feed and Abomasum (grams per day)

Part A.	grams lysine per day			
	0	3	6	9
Intake Amino Acids	717.35	717.35	717.35	740.50
Abomasal Amino Acids	880.89	690.45	803.33	912.36
Abomasal As % Intake	122.80	96.25	111.99	123.21

Part B.	grams lysine per day			
	0	3	6	9
Intake Amino Acids	833.06	833.06	833.06	833.06
Abomasal Amino Acids	842.73	880.43	915.07	1012.46
Abomasal As % Intake	101.17	105.69	109.84	121.54

APPENDIX Table 7 Intake of phosphorus per day

Intake per day (lb)	Dry matter intake (gm)	Phosphorus * grams per day
18	7318.93	20.49
16	4879.28	13.66
12	6505.71	18.22

* For optimal RNA-DNA formation in the microbial population, a requirement of 11.2 gm/day was shown to exist (Barnard, 1969).

* Proximate Analysis - Feed
Dry matter 89.64% on air dry basis
Phosphorus 0.28% on dry matter basis.

APPENDIX Table 8 Part A. Analysis of Variance for Nitrogen Balance as Percent Retention of Absorbed Nitrogen and Urinany and Fecal Nitrogen as a Percent of Intake Nitrogen.

		Mean Squares		
	df	Nitrogen Balance	Urinary Nitrogen	Fecal Nitrogen
Total	15			
Treatment	3	156.06 **	94.36 *	2.88
Period	3	37.19	20.48	9.73
Animal	3	42.07	37.82	10.88
Error	6	32.31	15.27	2.03

* Significant ($P<0.05$)

APPENDIX Table 9 Part A. Analysis of Variance for Lysine and Histidine as a Percent of Total Amino Acids in the Abomasum

		Mean Squares	
	df	Lysine	Histidine
Total	15		
Treatment	3	2.19 *	0.032 *
Period	3	1.16	0.175 **
Animal	3	2.25 *	0.005
Error	6	0.25	0.007

* $P<0.05$
** $P<0.01$

APPENDIX Table 10 Part A. Analysis of Variance for Plasma Lysine, Absolute Value and Percent of Total Plasma Amino Acids.

	df	Mean Squares	
		Absolute Value	Percent of Total
Total	15		
Treatment	3	1635.62 *	5.64 *
Period	3	249.37	0.36
Animal	3	103.40	1.12
Error	6	195.56	0.40

* ($P<0.05$)

APPENDIX Table 11 Part A. Analysis of Variance for Plasma Leucine and Isoleucine as a Percent of Total Plasma Amino Acids.

	df	Leucine	Isoleucine
Total	15		
Treatment	3	2.58 *	0.39 *
Period	3	2.52	0.17
Animal	3	0.20	0.05
Error	6	0.27	0.06

* ($P<0.05$)

APPENDIX Table 12 Part A. Analysis of Variance for Rumen Ammonia

	df	Mean Squares
Total	15	
Treatment	3	80.06 **
Period	3	33.68
Animal	3	32.10
Error	6	8.04

** ($P<0.01$)

APPENDIX Table 13 Part B. Analysis of Variance for Nitrogen Balance as Percent Retention of Absorbed Nitrogen and Urinany and Fecal Nitrogen as a Percent of Intake Nitrogen

	df	Mean Squares		
		Nitrogen Balance	Urinary Nitrogen	Fecal Nitrogen
Total	15			
Treatment	3	227.40	96.55	13.80 *
Period	3	969.29 *	490.38 *	4.55
Animal	3	7.40	4.77	3.13
Error	6	141.80	69.91	1.97

* ($P<0.05$)

APPENDIX Table 14 Part B. Analysis of Variance for Rumen Ammonia

	df	Mean Squares
Total	15	
Treatment	3	14.61 *
Period	3	34.61 **
Animal	3	22.50
Error	6	3.84

* ($P<0.10$)
** ($P<0.05$)

APPENDIX Table 15 Part B. Analysis of Variance for Plasma Arginine (Arg), Methionine (Meth), Leucine (Leuc), Phenylalanine (Phe) and Tyrosine (Tyr) (Absolute Values)

	df	Mean Squares Arg	Meth	Leuc	Phe	Tyr
Total	15					
Treatment	3	356.11 *	69.62 *	3472.08*	304.20 *	398.41 *
Period	3	224.93	30.54	439.89	26.76	59.70
Animal	3	526.06 **	4.94	1495.17	124.61	127.62
Error	6	109.15	22.01	1211.60	99.69	125.19

* (P<0.15)
** (P<0.05)

APPENDIX Table 16 Part B. Analysis of Variance for Plasma Methionine (Meth), Leucine (Leuc), Tyrosine (Tyr) and Cystine (Cys) as a percent of total Amino Acids.

	df	Mean Squares Meth	Leuc	Tyr	Cys
Total	15				
Treatment	3	0.13 +	4.02 *	0.64***	0.41 **
Period	3	0.14	0.81	0.07	1.17
Animal	3	0.08	2.11	0.69	0.31
Error	6	0.05	1.21	0.11	0.13

\+ (P<0.20)
* (P<0.15)
** (P<0.10)
*** (P<0.01)

APPENDIX Table 17 Part B. Analysis of Variance for Organic Matter (OM) Digestibility in the Rumen Digestibility of Total Crude Fiber, Ether Extract and Nitrogen Free Extract in the Rumen (CF+EE+NFE), Blood Urea Nitrogen (BUN) and Nitrogen reaching the abomasum as a percent of intake nitrogen.

	df	Mean Squares OM digestibility	CF+EE+NFE digestibility
Total	127		
Squares	7	639.69 **	577.00 **
Treatment	3	731.08	900.03 ***
Period	24	420.94 *	395.38
Animal	24	530.54 ***	681.33 ****
Error	69	304.49	303.45

	df	Mean Squares BUN	Nitrogen in Abomasum
Total	127		
Squares	7	6.01 ****	1861.88 **
Treatment	3	12.50 ****	1451.60
Period	24	1.03	1431.64 *
Animal	24	5.19 ****	1428.83 *
Error	69	1.14	971.65

* ($P<0.15$)
** ($P<0.10$)
*** ($P<0.05$)
**** ($P<0.01$)

APPENDIX Table 18 Part B. 9 AM Collection time Analysis of Variance for Organic Matter (OM) digestibility, Total Crude Fiber, Ether Extract and Nitrogen Free Extract digestibility in the Rumen and Nitrogen reaching the abomasum as a percent of nitrogen intake.

	df	Mean Squares OM digestibility	Mean Squares CF+EE+NFE digestibility	Mean Squares Nitrogen in Abomasum
Total	15			
Treatment	3	956.70 **	711.82 *	4457.48 *
Period	3	169.87	103.77	1239.81
Animal	3	142.82	262.43	922.03
Error	6	90.90	86.83	814.17

* ($P<0.05$)
** ($P<0.01$)

APPENDIX Table 19 Part A. Nitrogen balance Data - Nitrogen intake, Fecal Nitrogen, Retained Nitrogen, Percent Absorbed Nitrogen Retained (grams per day)

Treatment*	Period	Animal Number	Intake Nitrogen	Fecal Nitrogen	Urinary Nitrogen	Retained Nitrogen	Percent Absorbed Nitrogen Retained
0	I	17	148.93	43.97	25.91	79.05	75.31
	II	21	132.38	34.53	25.81	72.05	76.80
	III	15	132.38	29.09	27.15	76.13	73.72
	IV	10	99.28	24.51	18.53	56.24	81.40
3	I	21	149.49	40.69	19.98	88.76	81.55
	II	10	99.84	30.18	17.89	51.72	74.17
	III	17	132.94	33.87	23.85	75.17	67.92
	IV	15	132.94	30.23	18.78	83.88	81.48
6	I	15	150.06	41.29	25.23	83.45	76.71
	II	17	133.51	36.06	18.91	78.44	74.16
	III	10	100.41	28.96	18.56	52.79	72.23
	IV	21	133.51	35.98	26.23	71.20	73.08
9	I	10	150.62	43.90	28.30	78.27	73.35
	II	15	134.07	33.42	45.55	54.95	54.49
	III	21	134.07	33.13	30.94	69.84	69.29
	IV	17	134.07	34.77	42.96	56.19	56.67

* Grams lysine infused per day

APPENDIX Table 20 Part B. Nitrogen Balance Data - Nitrogen intake, Fecal Nitrogen, Retained Nitrogen, Percent Absorbed Nitrogen Retained (grams per day)

Treatment	* Intake Nitrogen	Period	Animal Number	Fecal Nitrogen	Urinary Nitrogen	Retained Nitrogen	Percent Absorbed Nitrogen Retained
0	148.93	I	23	37.53	17.46	93.95	84.33
		II	13	43.16	36.54	69.22	65.44
		III	12	36.05	25.41	87.47	77.49
		IV	8	38.77	30.65	79.52	72.18
3	149.49	I	8	35.61	16.43	97.39	85.54
		II	12	38.84	53.29	57.31	51.82
		III	23	36.21	11.45	101.79	89.89
		IV	13	35.12	23.31	91.01	79.61
6	150.06	I	13	46.50	44.30	59.15	57.12
		II	23	45.47	77.99	26.49	25.35
		III	8	39.95	24.91	85.10	77.36
		IV	12	41.27	21.01	87.68	80.67
9	150.62	I	12	41.57	34.77	74.13	68.02
		II	8	37.83	60.82	51.82	46.01
		III	13	40.19	17.60	92.68	84.03
		IV	23	37.90	29.75	82.82	73.58

* Grams lysine infused per day

APPENDIX Table 21 Part A. Rumen organic matter (OM) digestibility, Rumen Crude Fiber (CF) + Ether Extract (EE) + Nitrogen Free Extract (NFE) digestibility in the Rumen and Abomasal Nitrogen as a percent of intake nitrogen.

* Treatment	Period	Animal	OM Digestibility (%)		CF+EE+NFE Digestibility (%)		Abomasal Nitrogen	
			6 AM	6 PM	6 AM	6 PM	6 AM	6 PM
0	I	17	56.01	51.09	65.70	61.41	111.81	121.11
	II	21	45.85	33.31	58.94	46.18	141.50	152.56
	III	15	46.01	66.96	55.94	74.06	12o.24	80.41
	IV	10	56.12	10.50	64.29	25.33	101.03	193.27
	Avg.		51.00	40.47	61.22	51.75	118.65	136.84
3	I	21	62.49	66.44	69.79	73.13	88.54	80.36
	II	10	36.86	52.55	38.78	63.36	76.54	123.11
	III	17	54.99	47.55	62.45	59.69	94.74	133.44
	IV	15	64.11	52.80	74.55	65.74	105.55	133.56
	Avg.		54.61	54.84	61.39	65.48	91.34	117.62
6	I	15	52.69	67.37	60.46	74.65	101.70	83.58
	II	17	25.87	43.35	37.12	51.96	149.18	114.11
	III	10	54.45	30.89	62.81	43.19	104.06	155.17
	IV	21	47.21	39.15	58.48	51.06	127.97	140.32
	Avg.		45.06	45.19	54.72	55.22	120.73	123.30
9	I	10	16.04	42.33	29.65	53.38	179.20	135.00
	II	15	64.87	49.74	73.06	62.76	89.79	137.14
	III	21	44.16	60.59	57.26	67.47	143.26	85.27
	IV	17	32.45	49.76	47.94	59.57	170.87	115.67
	Avg.		39.38	50.61	51.98	60.80	145.78	118.27

* - Grams lysine infused per day

APPENDIX Table 22 Part B. Rumen Organic Matter Digestibility Twenty-four hour collection data (percent).

			Time							
Trt	Per	An	6 AM	9 AM	12NOON	3 PM	6 PM	9 PM	12 PM	3 AM
0	I	23	25.91	60.24	51.19	32.32	26.24	59.30	51.05	59.86
	II	13	46.76	44.41	33.21	16.38	32.53	46.40	-0.62	44.40
	III	12	38.18	61.63	57.11	54.41	30.07	34.93	40.18	11.06
	IV	8	38.95	58.04	53.34	57.97	43.19	39.08	43.05	37.04
	Avg.		37.45	56.08	48.71	40.27	33.01	44.93	33.42	38.09
3	I	8	59.85	45.75	42.48	48.95	24.57	48.51	6.58	43.77
	II	12	56.27	58.41	85.78	15.17	23.69	55.58	6.72	47.77
	III	23	40.72	52.32	60.77	55.24	51.91	43.36	53.18	46.25
	IV	13	22.78	52.05	36.49	17.30	53.89	63.72	13.54	33.02
	Avg.		43.91	52.13	56.38	34.17	38.52	52.87	20.01	42.70
6	I	13	50.01	23.68	12.94	18.69	47.62	53.37	44.19	47.42
	II	23	11.07	44.76	-5.76	-51.49	49.14	40.12	4.30	52.02
	III	8	44.20	55.92	48.39	63.10	50.68	55.54	48.46	56.49
	IV	12	39.58	42.02	34.55	52.09	9.14	50.75	33.43	39.93
	Avg.		36.22	41.53	22.53	20.60	39.15	49.95	32.60	49.09
9	I	12	23.57	6.49	24.02	-10.82	42.00	53.82	36.75	-1.62
	II	8	53.91	41.72	66.37	42.23	55.11	57.56	41.24	48.90
	III	13	63.68	22.92	-18.52	46.75	-23.21	40.62	5.23	48.70
	IV	23	-6.05	14.97	52.92	37.88	47.76	36.51	42.48	37.97
	Avg.		33.78	31.53	31.20	29.01	30.42	47.13	31.43	33.49

Trt - Treatment (grams lysine infused per day)
Per - Period
An - Animal Number

APPENDIX Table 23 Part B. Digestibility of a Total of Crude Fiber (CF) + Ether Extract (EE) + Nitrogen Free Extract (NFE) in the rumen Twenty-four hour collection data (percent).

			Time							
Trt*	Per	An	6 AM	9 AM	12NOON	3 PM	6 PM	9 PM	12 PM	3 AM
0	I	23	36.76	68.39	57.57	40.26	38.89	65.93	58.85	64.71
	II	13	53.62	52.47	42.56	21.13	44.06	55.54	16.21	51.67
	III	12	48.69	68.29	64.16	63.63	42.93	46.66	52.60	17.72
	IV	8	50.51	64.97	62.25	65.67	52.14	51.09	54.53	49.35
	Avg.		47.40	63.53	56.64	47.67	43.76	54.81	45.55	45.62
3	I	8	71.50	61.97	63.63	67.35	51.98	64.85	37.54	65.64
	II	12	63.84	67.53	114.29	32.59	27.18	64.98	11.71	58.53
	III	23	46.76	61.59	67.06	61.23	58.41	51.33	61.34	51.49
	IV	13	29.78	57.58	44.36	22.90	60.78	68.44	26.18	41.23
	Avg.		52.82	62.18	72.34	46.02	49.59	62.40	34.19	54.22
6	I	13	58.68	31.16	21.19	34.25	54.61	62.30	55.47	57.25
	II	23	18.37	53.99	2.03	-43.59	59.21	48.87	11.95	59.66
	III	8	52.96	64.03	59.28	71.21	60.92	64.83	59.86	66.59
	IV	12	50.80	54.48	47.22	56.17	16.82	57.09	43.69	52.04
	Avg.		45.20	50.92	32.43	29.51	47.89	58.27	42.74	58.89
9	I	12	37.54	28.54	36.87	-10.29	51.84	66.38	54.00	20.00
	II	8	65.47	58.71	76.68	55.74	65.55	67.90	55.45	61.98
	III	13	68.20	30.31	-14.54	51.98	-17.14	48.35	11.91	56.91
	IV	23	1.01	21.26	58.39	47.65	56.29	41.10	52.66	43.60
	Avg.		43.06	34.71	39.35	36.27	39.14	55.93	43.51	45.62

* - grams lysine infused per day

APPENDIX Table 24A Part B. Nitrogen Reaching the Abomasum as a percent of Intake nitrogen (6 AM, 9 AM 12 NOON, 3 PM).

Treatment*	Period	Animal Number	Time 6 AM	9 AM	12 NOON	3 PM
0	I	23	150.01	96.78	93.42	123.23
	II	13	101.21	111.96	132.19	116.81
	III	12	135.37	84.97	92.20	110.09
	IV	8	141.95	90.43	108.98	93.81
	Avg.		132.14	96.04	106.70	110.99
3	I	8	111.91	113.53	151.86	131.82
	II	12	96.67	105.42	213.79	206.71
	III	23	97.36	112.57	83.21	86.65
	IV	13	126.21	86.67	118.58	121.87
	Avg.		108.04	104.53	141.86	136.96
6	I	13	107.82	126.19	142.06	185.16
	II	23	140.02	119.79	160.25	206.72
	III	8	117.05	100.81	127.82	93.61
	IV	12	138.89	145.19	154.10	76.44
	Avg.		125.95	123.00	146.06	140.48
9	I	12	174.22	247.85	165.86	114.51
	II	8	126.98	177.20	105.76	152.34
	III	13	67.95	128.78	146.36	89.86
	IV	23	155.44	129.01	85.32	130.50
	Avg.		131.15	170.71	125.83	121.80

* - grams lysine infused per day

APPENDIX Table 24B Part B. Nitrogen Reaching the Abomasum as a percent of Intake nitrogen (6 PM, 9 PM, 12 PM, 3 AM).

Treatment*	Period	Animal Number	Time 6 PM	9 PM	12 PM	3 AM
0	I	23	162.26	87.10	103.53	74.06
	II	13	127.16	117.53	218.38	106.43
	III	12	159.94	147.15	146.70	135.54
	IV	8	119.43	144.95	137.31	149.08
	Avg.		142.20	124.18	151.48	116.28
3	I	8	196.59	113.03	219.72	157.98
	II	12	100.73	110.17	128.14	127.49
	III	23	93.59	112.36	103.94	90.42
	IV	13	94.34	69.30	174.92	124.40
	Avg.		121.31	101.22	156.68	125.07
6	I	13	98.98	106.23	131.05	114.31
	II	23	121.32	121.09	149.21	101.42
	III	8	120.97	109.48	131.34	114.16
	IV	12	244.61	93.57	138.33	144.79
	Avg.		146.47	107.59	137.48	118.67
9	I	12	126.83	134.08	183.93	252.91
	II	8	117.91	114.76	158.17	142.61
	III	13	165.68	113.46	141.46	108.82
	IV	23	111.95	95.61	128.72	101.43
	Avg.		130.59	114.48	153.07	151.44

* - Grams lysine infused per day

APPENDIX Table 25 Part B. Blood urea nitrogen (mg/100 ml. plasma) twenty-four hour collection data.

			Time							
Trt	Per	An	6 AM	9 AM	12NOON	3 PM	6 PM	9 PM	12 PM	3 AM
0	I	23	10.2	11.2	10.3	10.1	9.9	11.6	10.8	10.0
	II	13	10.0	10.0	10.7	8.3	9.2	8.7	10.6	8.9
	III	12	8.2	10.0	9.2	8.0	7.6	8.2	8.3	6.8
	IV	8	7.1	8.9	9.7	12.6	7.9	11.3	7.1	8.3
	Avg.		9.6	11.3	10.2	9.2	9.5	11.4	9.7	9.4
3	I	8	10.9	11.7	11.5	9.7	11.0	11.3	11.4	11.2
	II	12	6.8	9.6	7.1	7.7	7.2	9.8	7.4	7.8
	III	23	8.2	9.5	12.3	8.3	12.1	8.3	10.1	6.9
	IV	13	8.3	11.0	9.5	8.4	8.1	10.6	8.8	8.4
	Avg.		9.0	10.55	9.35	9.95	8.7	11.00	9.30	9.1
6	I	13	8.3	7.7	9.8	8.1	7.3	10.1	7.4	7.7
	II	23	11.9	13.4	9.7	10.7	9.5	13.2	11.9	11.3
	III	8	11.5	11.1	10.7	8.6	10.5	10.1	11.0	9.2
	IV	12	8.0	9.4	7.8	6.6	8.0	8.8	7.5	6.9
	Avg.		9.4	10.25	10.75	8.35	9.9	9.3	10.00	8.3
9	I	12	7.9	10.4	9.3	8.2	7.9	10.0	8.3	8.2
	II	8	11.8	13.6	13.1	11.7	12.0	13.4	11.8	12.2
	III	13	10.1	10.3	10.9	8.8	10.2	9.6	10.8	8.9
	IV	23	8.0	9.0	9.9	9.7	9.0	9.2	9.1	8.7
	Avg.		8.85	9.6	10.1	8.9	8.7	9.7	9.05	8.6

Trt - Treatment (grams lysine infused per day)
Per - Period
An - Animal Number