AMINO ACID REQUIREMENTS AND EFFECT OF EXCESS DIETARY CRUDE PROTEIN ON VOLUNTARY FEED INTAKE AND NITROGEN METABOLISM OF GROWING STEERS

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ABSTRACT

AMINO ACID REQUIREMENTS AND
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By

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This dissertation is concerned with two studies which were conducted to determine the essential amino acid requirements and the effect of excess dietary protein on feed intake and nitrogen metabolism of growing steers.

In the first study, 7 growing Holstein steers (274 kg body weight) were fitted with soft plastic abomasal cannulae and fed a 9.5% crude protein ration. Amino acids were infused into the abomasum at incremental levels of 3.5, 7, 11 and 15 g per day for lysine (lys), methionine (met) and threonine (thr), and 1.7, 3.5, 7 and 11 g per day for tryptophan (try). Changes in plasma amino acid concentrations were used to evaluate amino acid requirements. Nitrogen balances were conducted as a check of the plasma amino acid method. Daily passage of nitrogen and amino acids to the abomasum was: nitrogen 14.9, lys 4.5, cystine 1.5, met 1.6, total sulfur amino acids (TSAA) 3.1, thr 3.7 and try 0.6 g per kg of feed consumed or 105.3, 32.8, 10.7,

11.2, 21.9, 21.6 and 4.7 g per day, respectively. Plasma met was constant until 7 g per day of met were infused and thereafter increased with each higher infusion level. Hence 7 g per day of infused met apparently met the requirements. Plasma lys, thr and try increased at all infusion levels indicating that the requirements for these amino acids were met by the digesta from the rumen. Assuming a digestibility of 70% for abomasal digesta, daily absorption of met, cystine, TSAA, lys, thr and try reaching the small intestine were calculated at 7.9, 7.4, 15.3, 22.5, 15.1 and 3.3g respectively. Daily amino acid requirements of the slow growing 274 kg steers were calculated to be: met, 14.9; TSAA, 22.3; lys, $\leq$ 22.5; thr, $\leq$ 15.1; try, $\leq$ 3.3g. The above data were adjusted to fit the requirement pattern of similar sized steers fed 9.5 or 12% crude protein ration . By setting the determined TSAA requirements (22.3g per day) at unity and assuming that cystine can fulfill 56% of the need, and by comparing the 1973 NRC pattern of swine amino acid requirements with the above data, the daily requirements of the growing 274 kg steer fed a 9.5% crude protein ration were estimated to be TSAA 22.3, met 9.8, lys 31.2, phenylalanine plus tyrosine 22.3, valine 22.3, isoleucine -2.3, leucine 26.8, thr 20.1, try 5.8, histidine 8.0, arginine 8.9 and total essential amino acids 177.5g per day. The requirements for the respective amino acids of the steer fed a 12% crude protein ration were estimated at 25.4,

11.2, 35.1, 25.4, 25.4, 25.4, 30.4, 22.8, 6.6, 9.8, 10.2 and 202.3g per day.

In the second study, Holstein steers (average body weight, 300 kg) were allotted to a 4 x 4 Latin Square design with 11 days for adjustment to a low protein ration followed by a 14 day experimental period. Rations were pelletted and composed of ground corn, oats, soybean meal, isolated soy protein, minerals and vitamins. Crude protein contents were 10.7, 20.2, 32.5 and 40.0% for rations 1, 2, 3 and 4 respectively. Steers were fed at 12 hour intervals and total daily feed intakes were recorded. Blood samples were taken before feeding (T_0) and rumen samples taken at T_0 and 3 hours after feeding (T_3) on days 1, 2, 3, 5, 7, 10 and 14 of the experimental period. Blood samples were analysed for plasma urea nitrogen and free amino acid levels. Rumen samples were fractionated and analysed for total nitrogen, soluble nitrogen, insoluble nitrogen, ammonia nitrogen, tungstic acid precipitable nitrogen, peptide and amino acid nitrogen and non protein nitrogen concentrations. Daily feed intake (kg) declined markedly as the dietary crude protein increased (9.0, 8.4, 7.1 and 5.0, for rations 1, 2, 3 and 4 respectively) whereas daily protein intake increased (1.0, 1.7, 2.3, 2.0 kg for the respective rations). For rations 3 and 4 feed intake was high on the first day, decreased drastically on the second and third day and then increased over the remainder of the 14-day period.

The mechanism of feed intake depression was not determined. Steers fed rations 3 and 4 were nibblers while steers fed rations 1 and 2 were meal eaters with no consistent pattern. Rumen ammonia (mg/100 ml) increased with increased dietary crude protein level (11.6, 39.8, 69.0, 81.9 for the respective rations. Plasma urea nitrogen increased with dietary protein but total plasma essential and nonessential amino acids were not affected by treatment.

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INTRODUCTION

It is now well recognized that the rumen and its symbiotic microbiota allow the ruminant animal to uniquely utilize cellulose and non protein nitrogen. Adequate dietary nitrogen in one form or another is therefore essential for the optimal growth. Many feeding systems have been useful in increasing nitrogen utilization in the ruminant. However, quantitative protein and amino acid requirements of the growing ruminant have not been determined.

Ruminants, like all other classes of animals, have a definite tissue requirement for essential amino acids. Since extensive ruminal degradation and synthesis of protein dictate the quantity and pattern of amino acids that reach the duodenum, it is impossible to determine these requirements by merely manipulating dietary levels of individual amino acids as in the case of non ruminants. It is therefore necessary to develop a technique in which interferences by the rumen are completely eliminated. The first experiment of the present study was designed to quantitate the essential amino acid needs of growing steers. The second experiment was to investigate the effects of very high levels of dietary crude protein on feed intake, plasma

amino acid levels, plasma urea and the various ruminal nitrogen fractions in growing steers. It is hoped that the information obtained from these investigations will be beneficial in the effort to further exploit the productive capacities of ruminants.

LITERATURE REVIEW

A. Protein Metabolism in the Ruminant

1. Protein Degradation and Synthesis in the Rumen

Protein metabolism in the ruminant has been a subject of considerable investigation over the years. The use of non protein nitrogen for protein synthesis by rumen microorganisms and the subsequent conversion of such protein to tissue protein by the host have made the ruminant unique.

McDonald (1948) and Mangan (1972) have demonstrated that proteins entering the rumen are first degraded by rumen microorganisms to peptides, amino acids, and ammonia. Quantitatively, ammonia forms the largest fraction. The extent of protein degradation depends on its solubility in rumen fluid (el-Shazly, 1958; Blackburn and Hobson, 1960; Hendrickx and Martin, 1963). Ingested non protein nitrogen as well as urea entering the rumen from saliva (McDonald, 1948; Somers, 1961) or diffusion across the rumen wall from blood (Houpt, 1959; Juhasz, 1965; Decker et al. 1960) is converted to ammonia by microbial urease. The ammonia is then incorporated into microbial protein, but the ability of the rumen microorganisms to utilize the ammonia is dependent on the type and amount of carbohydrate present (Lewis 1960). Thus, the nitrogenous nutrients absorbed by

the host are not merely those of the diet, as in the case of the nonruminant, but a mixture of dietary constituents, products of microbial metabolism in the rumen and amino acids from the microorganisms themselves (McDonald, 1954).

Ammonia that is not reincorporated into microbial protein will diffuse into the rumen vein, then to the portal vein and finally to the liver which converts it to urea (McDonald, 1948; Cocimano and Leng, 1966). However, very large excesses of ruminal ammonia will diffuse across the rumen wall into the peripheral circulatory system (Chalmers et al., 1972). Diffusion of ammonia across the rumen wall into the circulatory system is recognized as movement along a concentration gradient (Houpt, 1959). Thus, ammonia toxicity is caused primarily by diffusion of ammonia to the peripheral circulatory system per se, so that overloading of the liver is not a necessary condition for toxicity (Chalmers et al. 1972). Chalmers et al. (1972) observed that there were no visible signs of ammonia toxicity after administering 20 g of urea or 10 g of ammonia to the abomasum of sheep. However, when these workers administered such treatments to the cecum, ammonia toxicity occurred within minutes. Payne and Morris (1969) observed that there was no increase in plasma ammonia in sheep which had been adapted to high dietary protein when they were dosed with 0.5 g of urea per kilogram of body weight. However, when sheep maintained on low dietary crude

protein were subjected to the same treatment they exhibited very high plasma ammonia and died. Since these workers found increased levels of urea cycle enzymes without any change in alkaline phosphatase activity in the liver of the sheep maintained on the high dietary crude protein they concluded that tolerance of ammonia toxicity in sheep is at least partly a function of urea cycle enzymes in the liver.

Significant positive correlations between dietary nitrogen, rumen ammonia and circulating blood urea have been reported (Lewis, 1957; Tagari et al., 1964; Preston et al., 1965; Cocimano and Leng, 1966; McIntyre, 1970). According to Lewis (1957), the major factor controlling blood urea level is the concentration of rumen ammonia, in that a change in rumen ammonia concentration is usually accompanied by a change in blood urea concentration. Cocimano and Leng (1966) working with sheep, and Robins et al. (1974) working with white tailed deer found that the plasma urea pool size and urinary urea excretion rate increased as dietary nitrogen intake increased.

Ruminal ammonia is the most important source of nitrogen for rumen bacteria but is less important for protozoa (Allison, 1969). Abe (1968) and Allison (1969) observed that rumen ammonia is first incorporated into bacterial cells and then into protozoal cells following the ingestion of the bacteria by protozoa. Thus, the lower

concentration of rumen ammonia observed in defaunated lambs than faunated lambs by Klopfenstein, Purser and Tyznik (1966) and Males and Purser (1970) may be a direct result of a greater concentration of bacteria in the rumen of the defaunated animals.

The type of diet will quantitatively influence the microbial protein reaching the small intestine (Bergen, Purser and Cline, 1968). According to Bergen et al. (1968), microfauna represent a higher percentage of the total microbial protein when the ruminant is fed a natural diet, than on a semipurified diet where microflora make up most of the microbial protein.

The extent to which rumen ammonia is incorporated into microbial protein depends mainly on the available energy, the caloric source and the level of sulfur present in the rumen fluid (Lewis, 1960; Hungate, 1966; Hume, 1970). Hume (1970a) reported that the addition of a mixture of higher volatile fatty acids to a purified diet containing non protein nitrogen as the sole source of nitrogen significantly increased microbial protein production from 71 g per day to 81 g per day in sheep. Houpt (1959) observed a significant increase in the utilization of rumen ammonia in sheep fed increasing levels of a highly digestible carbohydrate supplement.

The level of sulfur in rumen fluid will affect the number of bacteria and protozoa in the rumen and not the

percentage of sulfur within these organisms. Thus, the increase in rumen protein production from 82 to 94 g per day, resulting from increasing the dietary sulfur from .61 to 1.95 g per day, was due to an increase in the rumen microbial population (Hume and Bird, 1970). Walker and Nader (1968) determined the nitrogen to sulfur ratio in bacteria to be 11:1. Bird (1973) found that the nitrogen to sulfur ratio in bacteria was 20.2 to 21.8. He argued that the high sulfur value found by Walker and Nader was due to faulty technique in sulfur isolation from bacteria. Bird (1972) found that the ratio of the increment of nitrogen:sulfur stored by sheep was 13.5:1 when sulfate was added to sulfur deficient diet. He therefore concluded that in order to maximize dietary nitrogen utilization in sheep the nitrogen to sulfur ratio should not be greater than 13.5:1. Moir, Somers and Bray (1967) found maximum nitrogen utilization in sheep when the nitrogen:sulfur ratio was 10:1. Thus, according to Hume and Bird (1970) there is a potential 69 g of microbial protein produced from every gram of sulfur entering the rumen.

Several workers (Hungate, 1966; Hume, 1970b; Ørskov, Fraser and McDonald 1972; Walker and Nader, 1970; Bucholtz and Bergen 1973) used a variety of techniques to quantitate the amount of microbial protein that is synthesized in the rumen. Hungate (1966) estimated that a maximum of 10 g microbial protein can be synthesized from 100 g of digestible

organic matter (DOM). On the same basis, Walker and Nader (1970) calculated a yield of 14.4 g of microbial crude protein while Hume (1970) with passage studies obtained a mean yield of 13 g and suggested that maximum yield may exceed 20 g. Ørskov et al. (1972) analyzed diaminopimelic acid in abomasal digesta and estimated a rumen bacterial protein synthesis of 15.6 g, and Bucholtz and Bergen (1973) measured rumen microbial phospholipid synthesis and calculated synthesis of true rumen microbial protein to be 16 g.

2. Protein and Amino Acid Reaching the Small Intestine

The capacity of the rumen and the metabolic activities of its microorganisms affect the flow and composition of digesta passing to the small intestine of the ruminant (Kay, 1969). If the bulk of the ingested nitrogen is from a non protein or highly soluble protein source the protein reaching the abomasum will be mainly of microbial origin (Ørskov, Fraser and McDonald, 1971). If, on the other hand, the ingested protein is resistant to rumen degradation, only a small percentage of the total protein reaching the abomasum will be of microbial origin (McDonald, 1954; Ørskov et al., 1971). Under normal dietary regimens, a high proportion of the dietary nitrogen is converted to microbial protein (McDonald, 1954). After feeding a diet of wheaten hay to sheep, Pilgrim and Weller (1953) found that over a

16 hour post prandial period, 50% of the rumen nitrogen was of microbial origin. Amos et al. (1972) fed fish meal and corn gluten meal to wethers and found that 55 to 60% of the nitrogen in the rumen was associated with the particulate portion and 40 to 55% in the liquid phase. Since fish meal and corn gluten meal are fairly resistant to rumen degradation, a large fraction of the particulate portion was thought to be of dietary origin. Amos et al. (1971) found that the abomasal fluid of steers fed corn gluten meal contained significantly higher levels of total nitrogen, protein, nonessential amino acids and essential amino acids than that of steers fed distillers dried solubles or soybean meal. Soybean meal and distillers dried solubles are more extensively degraded in the rumen, resulting in a substantial loss of rumen ammonia; whereas, most of the protein from the corn gluten meal reached the abomasum.

The amino acid composition of rumen bacteria or protozoa is not influenced by crude protein intake (Bergen et al. 1968; Purser and Buechler, 1966; Fenderson and Bergen, 1972; Burris, Bradley and Boling, 1974). Bergen et al. (1968) reported that there was no significant difference in the true digestibility, biological value or net protein utilization of microbial proteins isolated from the rumens of sheep fed different rations.

Hogan and Weston (1967) investigated the passage of nitrogen to the abomasum of sheep and found that the

quantity of non ammonia nitrogen passing to the abomasum was similar whether the diet contained 7.8 or 19.8% crude protein. This can be interpreted to mean that due to insufficient energy a high percentage of the ammonia from the high protein diet was not utilized by rumen microorganisms. This conclusion was substantiated by Ørskov et al. (1971) who observed that in the presence of adequate dietary energy the amount of non ammonia nitrogen reaching the small intestine increased with increased dietary crude protein intake up to 19.9%.

Burris et al. (1974) used an in vitro pepsin-pancreatin digestion procedure and found that the release of threonine, valine, methionine, phenylalanine and lysine from isolated microbial preparation varied significantly with the source of ingested protein. These workers also found that lysine had a greater rate of release from bacteria obtained from the rumen of steers fed soybean meal than steers fed fish meal. They concluded that the availability of certain amino acids to ruminants can vary with the source of dietary crude protein. However, since there are numerous interrelated factors affecting post ruminal proteolysis such conclusions may not be valid under in vivo conditions.

According to Bergen (1969) the release pattern of amino acids may affect the digestibility of a particular amino acid and influence absorption rates from the ruminant's

alimentary tract. In his review on amino acid nutrition for the ruminant, Hatfield (1970) suggested that the preponderance of data in the literature indicates that the qualitative pattern of essential amino acids at the absorption sites is one of the rate limiting factors in the growth of ruminants. Johns and Bergen (1973) and Hume, Jacobson and Mitchell (1972) found significant competition for transport binding sites among essential amino acids of the same transport class. However, it is unlikely that under normal in vivo conditions competitive inhibition among amino acids would be a limiting factor in amino acid absorption and utilization since Johns and Bergen (1973) further suggested an unlimited absorptive capacity throughout the length of the digestive tract.

According to Hume (1970) and Chalupa (1974) the maintenance of optimum rumen function along with the maximization of rumen bypass of good quality dietary protein or amino acids would be a feasible approach to meet the amino acid requirements of ruminants. Thus, improved protein utilization is possible by protecting dietary protein from rumen microbial proteolysis. Feeding naturally resistant protein or increasing the resistance of soluble protein and amino acid by chemical treatments are suggested techniques. Mowat and Deelstra (1972) found that addition of 0.45% encapsulated methionine to the diet of lambs increased weight gains by 11% and feed efficiency by 9% when the basal

diet was supplemented with corn-blood and feather meal. Weight gains and feed efficiency were increased by 12% and 10%, respectively, with urea in the basal diet. At 0.6%, encapsulated methionine the above diets severely depressed gains and feed efficiency. Faichney (1971) found that lambs fed the formaldehyde treated casein had a faster growth rate and improved feed efficiency than those fed the untreated casein. Macrae et al. (1972) observed a significant increase in daily amounts of non ammonia nitrogen and individual amino acids reaching the intestine of sheep fed formaldehyde treated casein. Ferguson, Hemsley and Reis (1967) fed formaldehyde treated casein as a supplement to roughage-fed sheep and obtained substantial increases in wool growth. These workers claimed that formaldehyde forms acid reversible cross linkages with amino and amide groups. Thus, the protein is insoluble at rumen pH and soluble at abomasal pH. Faichney and Weston (1971) observed significant decreases in organic matter digestion, VFA and ammonia production in the rumen and significant increases in non ammonia nitrogen and starch reaching the abomasum of sheep fed formaldehyde treated casein.

Corn gluten meal and fish meal are protein sources which are resistant to microbial degradation. Thus, when these proteins bypass rumen degradation their amino acids are reflected in plasma and tissue pools (Bergen, Henneman and Magee, 1973). Amos et al. (1971) observed a significantly

higher total nitrogen, isoleucine, leucine, methionine and phenylalanine in abomasal fluid of steers fed corn gluten meal than steers fed soybean meal or distillers dried solubles.

B. Plasma Amino Acids and What They Represent

The investigation of plasma amino acids over the years has yielded invaluable information on their role in mammalian protein metabolism. According to Munro (1970) the plasma free amino acid pool comprises a very small proportion of the total free amino acid pools of the body. Plasma free amino acids are rapidly renewed since the daily amino acid intake is large in comparison to the plasma pool. Changes in plasma free amino acids therefore may not reflect changes in body free amino acids as a whole (Munro , 1970). However, there is an interchange of free amino acids between plasma and tissue pools. Munro (1970) suggested that the body free amino acid pools have 3 metabolic outlets: a) protein synthesis, b) synthesis of low molecular weight compounds, and c) degradation through amino acid catabolism.

It has been shown by several workers (Munro et al., 1962; Crofford, Felts and Lacy, 1964; Potter, Purser and Cline, 1968; Bergen and Purser, 1968; Halfpenny, Rook and Smith, 1969; Knipfel et al., 1969) that the administration of energy to a fasted animal causes a significant depression of plasma free essential amino acids. Such depressions in plasma free essential amino acids have been interpreted to

be a direct result of tissue uptake of these amino acids. Such interpretation is substantiated by the data of Halfpenny et al. (1969) which showed that an increase in energy to lactating cows caused a significant decrease in plasma essential amino acids and a substantial increase in milk protein. Call et al. (1972) showed that the injection of insulin into sheep caused a severe depression in plasma levels of glucose, acetate and both essential and non-essential amino acids. Hence, it appears that infusion of energy does not directly depress plasma free amino acids, but induces the release of insulin which directly stimulates the uptake of amino acids (Munro, 1964; Potter et al., 1968; Preston et al., 1973). Such a conclusion can be substantiated by the fact that fat as an energy source does not stimulate amino acid uptake, mainly because it does not influence insulin secretion (Munro, 1964).

Plasma amino acids will reflect protein intake only in the sense that an increase in dietary protein will be accompanied by an increase in plasma amino acid level during the absorptive phase. An exception is when one amino acid is severely limiting the utilization of the other amino acids present in large quantities (Zimmerman and Scott, 1965; Dean and Scott, 1965). Purser (1970) in his review on amino acid requirements of ruminants, pointed out that plasma concentrations of a specific amino acid do not always reflect the nutritional status of an animal unless that

amino acid is substantially limiting. McLaughlan (1963) reported that the plasma amino acid concentrations of rats increased after a meal of good quality protein, but the duration of the increase was dependent on the amount and composition of the fed protein. According to Cecyre, Jones and Gandreau (1973) plasma amino acid level decrease gradually with time after feeding. Hogan, Weston and Lindsay (1968) observed that the total plasma essential amino acids of sheep increased with each successive level of casein infused into the abomasum, but as a proportion of the total essential amino acids only valine, leucine and phenylalanine increased. The sulfur amino acids remained unchanged and histidine and arginine decreased. In an effort to explain plasma amino acid response to dietary protein intake in ruminants, Bergen et al. (1973) pointed out that the changes observed in plasma amino acid parameters in response to various rations in ruminants are related primarily to the quantity of protein reaching the small intestine and not to dietary protein per se. With the exception of methionine and histidine, plasma amino acids, for the first 4 days of infusion, did not reflect the amino acid pattern of protein sources infused into the duodenum of sheep (Potter, Purser and Bergen, 1972).

The metabolic state of the animal will influence the plasma amino acid level independently of dietary protein (Munro, 1964). During starvation or fasting, plasma level

of the essential amino acids increased significantly (Leibholz and Cook, 1967; Zimmerman and Scott, 1967; Hogan et al., 1968; Leibholz, 1970; Bloxam, 1971). Leibholz and Cook (1967) attributed this large increase in plasma free amino acids to tissue breakdown and amino acid release during starvation. According to Bloxam (1971) there is a general flow of most amino acids from extra hepatic tissues to the liver, whereas lysine and the branched chain amino acids flow from the liver to extra hepatic tissues during starvation. This out flow of branched chain amino acids from the liver to extra hepatic tissue may be due in part to the inability of the liver to deaminate branched chain amino acids (Leibholz, 1970; Neale, 1972). During starvation Leibholz (1970) observed an increase in the essential to nonessential amino acid ratio from 0.35 to 0.56. This may be explained by the degradation of tissue protein during starvation and the ultimate deamination of the circulating amino acids, other than the branched chain amino acids, by the liver. Thus, the increased level of circulating branched chain amino acids were the major contributor to the increased plasma amino acid ratio. However, this may only be partially true during extended fast since Brown et al. (1961) measured plasma amino acid levels of cattle during an 88 hour fast and found approximately a two-fold increase in glycine, a significant increase in the aromatic and branched chain amino acids, lysine and

threonine. There was a decrease in serine and alanine and no significant changes in glutamic acid, cystine, histidine and arginine. Zimmerman and Scott (1967) observed that plasma lysine, methionine, leucine, isoleucine, tyrosine, phenylalanine and histidine increased with each extension of fasting in chickens, whereas plasma cystine was decreased and proline, glutamic acid and arginine were unaffected.

Interrelationships between the different plasma amino acids in several species have been well documented. Several workers (Snyderman and Holt, 1970; Clark, Umezawa and Swendseid, 1973; Keiichiro, Takeuchi and Sakurae, 1973; McLaughlan, Karsrud and Knipfel, 1973) observed a reciprocal relationship between the different branched chain amino acids in plasma. Snyderman and Holt (1970) reported a significant decrease in plasma valine, isoleucine tyrosine and threonine after loading the animal with leucine. Clark et al. (1973) observed very high plasma levels of isoleucine and valine after feeding a diet devoid of leucine to rats. Keiichiro et al. (1973) and McLaughlan et al. (1973) observed that an increase in plasma lysine above requirement caused a significant decrease in plasma threonine. Zimmerman and Scott (1965) reported that severe deficiencies of either lysine or arginine and large excesses of lysine or valine caused a substantial increase in plasma threonine; also, plasma lysine accumulated when dietary arginine was deficient. Zimmerman and Scott (1965) concluded that a

decrease in plasma threonine should not be interpreted that threonine is limiting.

Reis, Tunks and Sharry (1973) found that infusion of 4.9 to 10 g of methionine per day into the abomasum of sheep significantly increased plasma methionine, cystine, taurine and cystathionine and significantly decreased plasma levels of the branched chain amino acids. However, the infusion of equimolar cystine significantly increased plasma cystine, taurine and cystathionine, significantly decreased plasma branched chain amino acid levels but had no effect on plasma methionine level. The results of these experiments agree with the 1973 NRC pig requirement report that methionine can fulfill the total sulfur amino acid need whereas cystine can only fulfill a part of such need. Byington and Howe (1972) fed a 70:30 ratio of methionine to cystine to rats and found high levels of plasma methionine, alanine, isoleucine, valine and α-aminobutyric acid and low levels of threonine and taurine. However, reversing the ratio resulted in high plasma levels of threonine and taurine. Shannon, Howe and Clark (1972) observed that rats fed 4.8 or 3.6 millimoles of sulfur amino acids consumed comparable amounts of food and had similar weight gains regardless of whether 100% methionine was fed or 25 or 50% of the methionine was replaced by cystine. However, replacement of 75% of the methionine by cystine significantly depressed feed intake and decreased weight gains to 25% of normal.

The effects of insulin, protein intake and metabolic state of the animal on plasma amino acids along with the constant turning over and interrelationships of plasma amino acids, imply that an authentic overall interpretation of plasma amino acid is difficult unless these interrelated factors are given due consideration.

C. The Amino Acid Requirement of Growing Animals

The amino acid requirements of man, pigs, chickens, sheep and rats have been directly determined with a variety of techniques. Such techniques include plasma amino acid response curves (Zimmerman and Scott, 1965; Young et al., 1971; Tontisirin et al., 1972; Young et al., 1972; McLaughlan et al., 1973), nitrogen retention (Nimrick et al., 1970a; Nimrick et al., 1970b; Boila and Devlin, 1972), weight gain (Stockland et al., 1970; Boomgaardt and Baker, 1973a; Boomgaardt and Baker, 1973b), oxidation rates of amino acids (Brooks, Owens and Garrigus, 1972) and plasma urea concentration (Puchall et al., 1962; Christensen et al., 1972; Mercer and Miller, 1973; Brown and Cline, 1974). Regardless of the method used the fundamental criterion for any amino acid requirement determination is that the amino acid under investigation be limiting. The limiting amino acid may be defined as that amino acid which, by quantity, is least able to satisfy the animal's requirement. The concept of a limiting amino acid implies that the

other amino acids (which are in excess relative to that which is limiting) will be utilized in quantity as dictated by the most limiting amino acid (Velu, Baker and Scott, 1971).

The approach of feeding incremental levels of an essential amino acid and quantitatively measuring its appearance in plasma has been proven to be quite satisfactory in non ruminants (Zimmerman and Scott, 1965; Velu et al., 1971; Young et al., 1971; Young et al., 1972). Working with chickens, Zimmerman and Scott (1965) found that the first limiting amino acid in the diet remained at a low and constant level in plasma irrespective of the severity of the deficiency. The break point at which the amino acid began to accumulate was regarded as the requirement level. These workers concluded that the plasma technique can be used to determine the amino acid requirement. However, such technique cannot be utilized in the ruminants because of amino acid degradation by ruminal microorganisms. Thus, the determination of the essential amino acid requirement of the ruminant animal can be achieved only if the amino acid under investigation is infused directly into the abomasum of animals where the quantity of that amino acid passing from the rumen is limiting (Wakeling, Annison and Lewis, 1970). The response curve of plasma amino acids versus the quantity of amino acid passing the duodenum should show a rapid increase in plasma level beyond the

requirement level. Thus, the point of inflection on the plasma amino acid response curve denotes the requirement level (Wakeling et al., 1970). McLaughlan and Illman (1967) defined the amino acid requirement as that dietary level at which the corresponding plasma amino acid is equal to normal fasting level. Tontisirin et al. (1974) explained that the amino acid requirement should be considered as the intake of that amino acid which is capable of just maintaining higher blood plasma amino acid plateau level. Working with young men, Young et al. (1971) regarded the level of dietary tryptophan above which a linear increase in plasma tryptophan occurred as the tryptophan requirement. Tontisirin et al. (1972) found that both pre and post prandial plasma free tryptophan levels in children became constant at a tryptophan intake of 4 mg per kg body weight and below, but they increased linearly between 4 and 7 mg per kg body weight. These workers concluded that the tryptophan requirement of children 7 to 13 years old is 4 mg per kg body weight per day. Young et al. (1972) observed that free valine in plasma of young men remained constant below valine intakes of 14-16 mg per kg body weight per day and increased with intakes above this level. The lysine requirement of rats was determined by McLaughlan et al. (1973) who found that plasma lysine remained low until supplemental lysine exceeded .12% of the diet then increased rapidly beyond this point of intake. These workers regarded

the point of lysine intake at which the plasma lysine curve deflected upward as the lysine requirement. Tontisirin et al. (1973) observed that after fasting or 3 1/2 hour post prandial, plasma tryptophan levels of elderly people decreased as daily tryptophan intake was lowered to 2 mg per kg body weight, below that level plasma tryptophan remained constant. Thus they concluded that the tryptophan requirement of elderly people (65 years and over) was 2 mg per kg body weight per day. After compiling their data on the tryptophan requirement of children, young men and elderly people (4, 3, 2 mg/kg body weight/day) these workers concluded that the minimum tryptophan requirement per unit of body weight in humans decreases with increasing age.

Several workers (Nimrick et al., 1970a; Nimrick et al., 1970b; Nimrick and Kaminiski, 1970; Boila and Devlin, 1972; Schelling, Chandler and Scott, 1973) have used nitrogen retention as an indicator of amino acid requirements. The level of amino acid intake or intake plus infusion which coincided with the point of inflection on the nitrogen retention curve was regarded as the requirement of that amino acid. Nitrogen retention was maximized in lambs at 0.40, 0.10, 0.16 and 0.10% of the diet for glutamic acid, methionine, lysine-HCl and threonine, respectively (Nimrick et al., 1970a). These workers further found a relationship between nitrogen retention, plasma free methionine and methionine supplementation. Plasma methionine

remained low and constant as nitrogen retention increased with increased methionine supplementation and then increased rapidly after nitrogen retention was maximized. Schelling et al., (1973) carried out a series of abomasal methionine infusions in sheep and found that 2 to 3 g of methionine per sheep per day were adequate for optimal nitrogen retention. Nimrick and Kaminiski (1970) infused combinations of lysine-HCl, methionine, threonine and urea into the abomasum of sheep at 0.25, 0.18, 0.11 and 0.12% of feed intake and found the mean daily nitrogen retentions to be 2.76, 0.52, 0.89, 0.94 and 1.76 g for lysine-HCl plus methionine, lysine-HCl plus threonine, methionine plus threonine, lysine-HCl and methionine respectively. Scrimshaw et al. (1973) reported that lysine supplementation of 2.25% of the total protein intake significantly improved nitrogen balance in young men.

Maximum weight gain has been used as an indicator in amino acid requirement in growing animals (Stockland et al., 1970; Boomgaardt and Baker, 1973a; Boomgaardt and Baker, 1973b). The level of amino acid intake or infusion which corresponds to the point of inflection on the weight gain curve is the requirement of that amino acid. Thus Stockland et al. (1970) found the lysine requirement for maximum weight gain in rats to be 0.6% of a diet containing 10% dietary crude protein. Boomgaardt and Baker (1973a) reported that the lysine requirement for maximum weight gain in chickens fed 14, 18.5 and 23% dietary protein was

4.73, 4.72 and 4.62% of the protein or 0.66, 0.88 and 1.05% of the diet, respectively. Boomgaardt and Baker (1973b) found the lysine requirement for maximum growth rate in chickens to be a constant 4.62% of the dietary protein with increasing age.

The use of blood urea concentration as an indicator of amino acid requirement in growing animals is promising although not extensively used. The rationale underlying this technique is that plasma urea level will decrease to a minimum when maximum utilization of the amino acid in question is achieved. Working with pigs, Christensen et al., (1972) observed that serum urea concentration decreased to a minimum as dietary methionine supplementation increased from 0.25 to 0.45% of the diet. These workers used plasma amino acid and weight gain techniques to test their results and obtained identical values for the methionine requirement of .46 and .45% of the diet respectively. Puchall et al. (1962) found that plasma urea concentration was proportional to feed per gain ratio and inversely proportional to gain when pigs were fed proteins of different quality. Mercer and Miller (1973) investigated the validity of using plasma urea concentration as an indicator of amino acid requirement and found that the methionine requirement of lambs growing at a mean rate of 154 g per day was 2.63g per day. Using urinary ^{35}S as an indicator to confirm their results these workers obtained a value of 2.35g

of methionine per day.

The oxidation rate of an amino acid as an indicator of its requirement was investigated in rats by Brooks, Owens and Garrigus (1972). These workers used radioactive CO_2 as an index for the amount of lysine oxidized and found that the oxidation rate of lysine did not increase markedly until the dietary lysine intake was increased above that level at which the average daily gain and gain per feed ratio were maximal. They concluded that the oxidation of amino acid technique is a very good method for determining the dietary amino acid requirement of a growing rat.

MATERIALS AND METHODS

A. Experiment One

1. General Design of Experiment

Seven Holstein steers with an average body weight of 274 Kg were fitted with soft plastic abomasal cannulae. These steers were fed a 9.5% crude protein ration (Table 1) at 12 hour intervals at 3% of their body weight. Steers were housed individually in 91 cm x 244 cm metal metabolism collection stalls and given free access to water.

Amino acids (dissolved in 500 ml of water and pH adjusted to 2.5) were infused into the abomasum with a Harvard peristaltic pump at the rate of 0.42 ml per minute. Methionine, lysine and threonine were infused at incremental levels of 3.5, 7, 11 or 15 g per day and tryptophan at 1.7, 3.5, 7.0 or 11 g per day. Each level of amino acid was infused for seven days during which time a nitrogen balance study was conducted (Figure 1). Nitrogen balance was expressed as total nitrogen intake (dietary and infused) minus the total nitrogen excreted (urine and feces).

Abomasal samples were collected prior to each amino acid infusion period to determine the quantity of each amino acid (per Kg of feed consumed per day) reaching the abomasum. Chromic oxide and lignin were used as insoluble markers to

TABLE 1

Ration Used in Experiment One

Ingredients	%
Oats, grain (4) 4-03-309	10.00
Wheat, bran, (4) 4-05-191	5.00
Corn, dent yellow grain, gr 2 US mn wt 54, (4) 4-02-931	51.55
Soybean, seeds, sov-ext. grnd, mx 7% fiber, (5) 5-04-604	3.75
Corn, cobs, grnd, (1) 1-02-782	20.00
Sugarcane, molasses, mn 48% invert sugar mn 79.5 degrees br/x, (4) 4-04-696	5.00
Urea (45% N)	0.25
Limestone, grnd, mn 33% calcium, (6) 6-02-632[a]	1.45
Trace mineral salt [bc]	2.00
Chromic oxide-flour	1.00
Crude protein	9.50
Vitamin A[de]	2,000,000
Vitamin D[ef]	250,000
Vitamin E[eg]	55,000

[a]Calcium Carbonate Co., Quincy Illinois.

[b]Contained in %: Zn, mn 0.35; Mn, mn 0.2; Fe, mn 0.2; Mg, mn 0.15; Cu, mn 0.03; Co, mn 0.05; I_2, mn 0.007; NaCl, mx 98.5.

Table 1 (continued . . .)

[c]International Mineral Co.

[d]Vitamin A Palmitate (Pfizer Co., Terre Haute, Indiana).

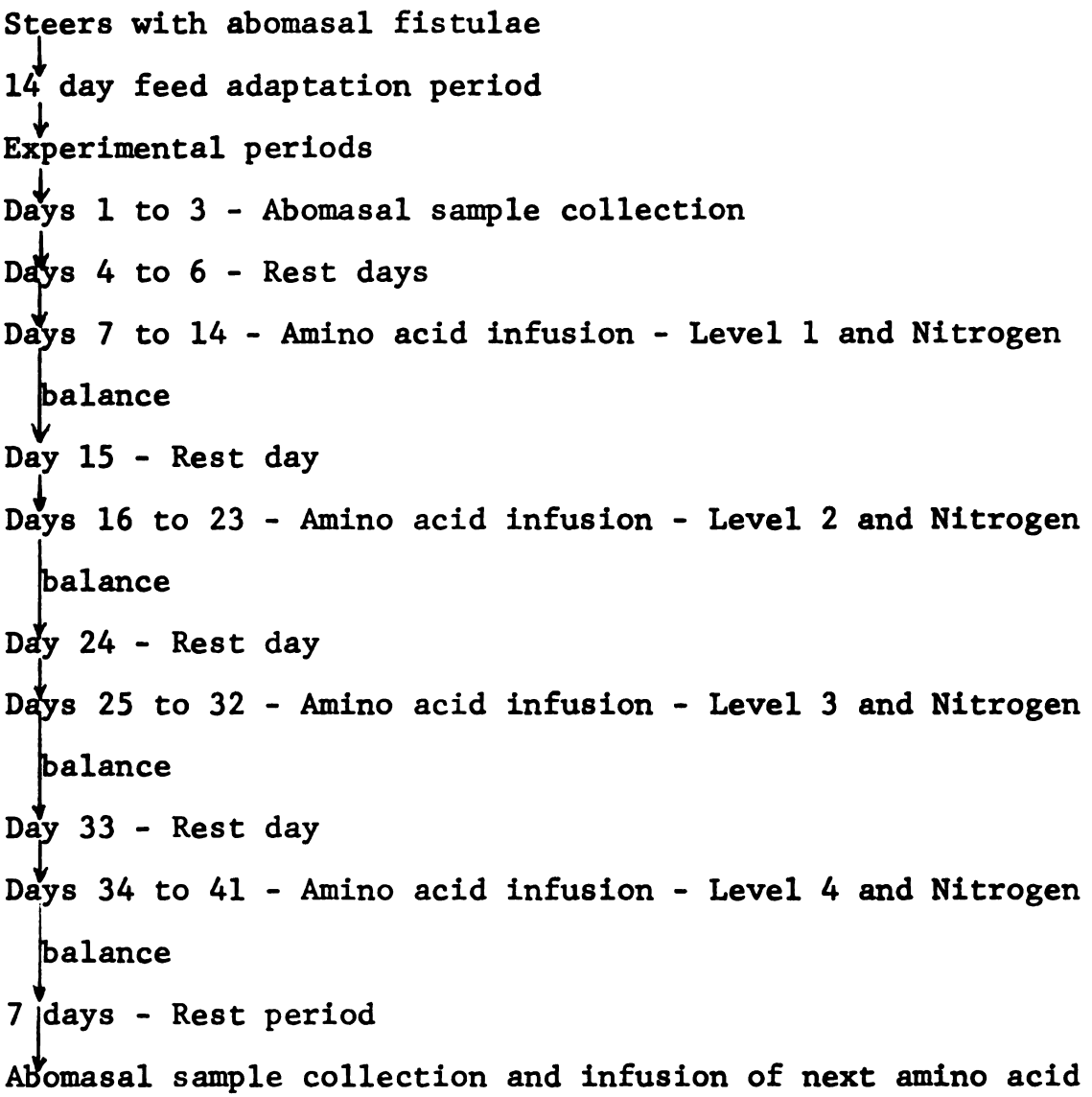
[e]International Units

[f]Ergocalciferol (Fleichman Irradiated Dried Yeast).

[g]Alpha tocopherol acetate (Eastman Kodak, Rochester, N.Y.).

FIGURE 1

Flow Chart of Experiment One



quantitate the total nitrogen passage to the abomasum. To this end, chromic oxide was mixed with wheat flour (ratio of 1:4) into a paste with water and baked in an oven at 100°C. The baked mixture was ground in a Wiley mill and added to the ration (1%) Ørskov et al. (1971).

Amino acids were obtained from General Biochemicals (Laboratory Park, Chagrin Falls, Ohio). They were of the L-isomer and NRC grade containing less than 0.3% moisture. Lysine was lysine hydrochloride.

2. Cannula Design

Cannulae were made from clear plastisol (obtained from Norton's Plastic and Synthetics Division, Akron, Ohio) in a brass mould obtained from Dr. L.D. Satter of the University of Wisconsin. The plastisol was placed under strong vacuum for one hour to remove air bubbles, after which it was poured into the preheated mould and allowed to be baked in an oven at 190°C for approximately 20 minutes. After cooking the mold was cooled by immersing it in cold water for a few minutes. Properly prepared cannulae were pliable,transparent, amber in color and free of air bubbles.

3. Sample Collection and Preparation

a. Abomasal samples

Abomasal samples were collected twice daily at 6 hour intervals over a 3 day period. The abomasal contents were agitated with air from a small air pump and approximately

200 ml of the digesta were collected in a beaker. A preliminary study revealed that a representative sample could be obtained by agitating the abomasal contents during sampling. Samples from each steer were pooled and freeze-dried. Dry matter, nitrogen, acid lignin, chromium and amino acids were then determined.

b. Blood samples

Blood samples were collected immediately before the morning feeding on days 4 and 8 of each infusion period. A preliminary study of sampling on days 1, 2, 3, 4, 5, 6, 7 and 8 revealed that days 4 and 8 were the best. At each sampling time 10 ml of blood was drawn from the right jugular vein of each steer into a heparinized syringe and placed into a bucket of ice until all steers were sampled. Plasma was prepared for amino acid analysis according to Bergen et al. (1973) and stored at -70°C.

c. Urine samples

Total urine from each steer was collected in a plastic carboy containing 200 ml of 18 N sulfuric acid. The sulfuric acid was added to prevent liberation of ammonia or other nitrogen compounds from the urine. The carboy was emptied once per day and the urine volume measured, diluted to 10 liters with water and a 1 liter aliquot stored in the cooler. The remaining dilute urine was discarded. At the end of each 7 day collection period samples from each steer were pooled into a composite sample and properly mixed. A 10%

subsample was then secured for nitrogen determination.

d. Fecal samples

Total feces were allowed to pass through a wide spaced steel grid in the floor immediately behind each steer and were collected in large plastic containers in the pit below the collection stalls. Feces were removed once daily and the total output for each steer weighed, properly mixed and a representative sample (10% of the total weight) secured by taking several small subsamples from different points in the mixture. At the end of each 7 day collection period, samples from each steer were properly mixed and into a composite sample and a 10% subsample retained for nitrogen analysis.

4. Nitrogen Determinations

Feed, abomasal contents, fecal and urinary nitrogens were determined with a standard semi-micro Kjeldahl method (copper catalyst) using Aminco system and Sargent Spectro-Electro Titrator.

5. Plasma Amino Acid Determinations

a. Plasma lysine, methionine and threonine

Plasma lysine, methionine and threonine were determined from the protein free filtrate by ion exchange chromatography (Technicon Amino Acid Analyser) as detailed by Bergen and Potter (1971) and Bergen et al. (1973).

b. Plasma tryptophan

Plasma tryptophan was assayed using the simplified

spectrofluorometric micromethod of Wapnir and Stevenson (1969) as modified by Fenderson and Bergen (1972). The detailed procedure was outlined by Fenderson (1972).

c. Abomasal lysine, methionine and threonine

Abomasal lysine, methionine and threonine were determined from acid hydrolysates of the freeze-dried abomasal samples on the amino acid autoanalyzer. The crude protein content of the sample was first determined by the micro Kjeldahl procedure. A sample containing approximately 10 mg of protein was weighed into a 35 ml screw capped test tube and 1 ml of 1 mM norleucine, 1 ml 12 N HCl and 8 ml of 6N HCl were added. The tube was flushed with nitrogen (to exclude air) capped and autoclaved for 16 hours at 121°C. The contents of the tube were filtered through Whatman No.2 filter into an evaporating flask. The filtrate was evaporated to dryness under vacuum (at 60°C) washed twice with approximately 10 ml of deionized distilled water, evaporated to dryness and finally resuspended in 4 ml of pH 2 (.3 lithium .05 citrate) buffer. The buffered filtrate was then analyzed on the amino acid analyzer as previously outlined in section 5a.

d. Abomasal tryptophan

Tryptophan determination of the freeze-dried abomasal samples was done by a modified $Ba(OH)_2$ protein hydrolysis procedure as outlined by Fenderson (1972).

6. Chromium Analysis

Chromium content of feed and abomasal samples was determined by nitric-perchloric acid digestion followed by atomic emission spectrophotometry using the I.L. 453 Atomic Absorption/Emission Spectrophotometer.

Between 200 and 500 mg of the finely ground sample were digested in 60 ml of concentrated nitric acid in a 250 ml Phillips beaker on a hot plate until the volume was 1-2 ml. The flask was then cooled to room temperature and the digest was oxidized by adding 7 ml of 72% perchloric acid and heating it on the hot plate until 1-2 ml of the solution was left in the flask. The flask was again cooled and the content diluted to 100 ml with deionized distilled water. The oxidized diluted samples were then read on the atomic emission spectrophotometer at a wave length of 425.4 nm, scale of 2.5, slit width of 80, photomultiplier voltage at 700 volts, acetylene flame, hollow cathode chromium lamp and nitricoxide solid burner. A chromium standard curve was obtained by subjecting ammonium chromate solutions of 1, 2, 5, 10 and 15 ppm chromium to the same treatments as the samples.

7. Lignin Determination

Acid lignin content of feed and abomasal freeze-dried samples was determined by the standard Van Soest procedure (Van Soest, 1963; Van Soest and Wine, 1965).

B. Experiment Two

1. General Design of Experiment

Four growing, rumen fistulated, Holstein steers with an average body weight of 328 Kg were randomly allotted to a 4 x 4 Latin Square experiment (Table 2). In this experiment four rations containing different levels of crude protein (Table 3) were fed to the steers in four 14 day periods. Steers were housed indoors (temperature between 55 and 70^{o}F) in 91 cm x 244 cm metal stalls, given free access to water and fed twice daily at ad libitum. Unconsumed feed was weighed back to quantitate daily feed intake.

In each experimental period, blood samples were collected immediately before the morning feeding (T_o) on days 1, 2, 3, 5, 7, 10 and 14 and rumen samples were taken immediately before the morning feeding and 3 hours after feeding (T_3) on days 1, 2, 3, 5, 7, 10 and 14. Each treatment period was preceded by an 11 day adjustment period during which the steers were fed ration 1 (low protein control diet).

2. Sample Collection and Preparation

a. Blood samples

Blood samples were collected from the right jugular vein of each steer and prepared for amino acid analysis as previously outlined in section 3b of experiment 1. Three ml

TABLE 2

Experimental Design for Experiment Two

	Steer Number			
Period	1	2	3	4
1	A	B	C	D
2	B	C	D	A
3	D	A	B	C
4	C	D	A	B

Treatments: A = RATION 1

B = RATION 2

C = RATION 3

D = RATION 4

TABLE 3

TABLE 3

Rations Used in Experiment Two

Ingredients	1 %	2 %	3 %	4 %
Oats, grain (4) 4-03-309	10.0	10.0	7.5	7.5
Sugarcane molasses, (5) 5-04-604	5.0	5.0	5.0	5.0
Corn, dent, yellow, grain, gr 2 US mn wt 54 (4) 4-02-931	75.0	50.0	30.0	20.0
Soybean, seeds, solv-extd, grnd, mx 7% fiber (5) 5-04-604	5.0	30.0	32.5	22.5
Isolated soybean protein (70%)[a]	-	-	20.0	30.0
Isolated soybean protein (90%)[a]	-	-	-	10.0
Limestone, grnd, mn 33% calcium, (6) 6-02-632[b]	1.0	1.0	1.0	1.0
Corn, cobs, grnd, (1) 1-02-782	2.0	2.0	2.0	2.0
Trace mineral salt [cd]	2.0	2.0	2.0	2.0
Crude protein	10.7	20.2	32.5	40.0
Vitamin A[ef]	1,000,000	1,000,000	1,000,000	1,000,000
Vitamin D[fg]	125,000	125,000	125,000	125,000
Vitamin E[fh]	22,500	22,500	22,500	22,500

Table 3 (Continued . . .)

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[a]Central Soya, Decatur, Indiana.

[b]Calcium Carbonate Co., Quincy, Illinois.

[c]Contained in %: Zn, mn 0.35; Mn, mn 0.2; Fe, mn 0.2; Mg, mn 0.15; Cu, mn 0.03; Co, mn 0.05; I_2, mn 0.007; NaCl, mx 98.5.

[d]International Mineral Co., Libertyville, Illinois.

[e]Vitamin A Palmitate (Pfizer Co., Terre Haute, Indiana).

[f]International Units

[g]Ergocalciferol (Fleichman Irradiated Dried Yeast).

[h]Alpha tocopherol acetate (Eastman Kodak, Rochester, New York).

of plasma from each sample were frozen (-70°C) for plasma urea nitrogen determinations.

b. Rumen samples

Rumen samples were collected at specified times by inserting the full length of the arm through the cannula into the rumen and mixing the contents thoroughly. A 200 cc sample of rumen contents was then removed. The rumen samples were then immediately frozen and stored at -70°C for further processing at a later date. At the time of processing, 25 g of thawed, well mixed sample (Figure 2) were strained through 2 layers of cheese cloth to obtain rumen liquor. Four ml of the rumen liquor were used for the ammonia determination and another 5 ml portion was treated with 1 ml of 25% metaphosphoric acid to precipitate the soluble proteins. The metaphosphate-treated rumen liquor was then centrifuged at 12,100 x g for 15 minutes and the supernatant was saved for volatile fatty acid (VFA) determination.

Twenty five g of the original rumen sample (Figure 2) were fractionated into total ruminal nitrogen, soluble nitrogen, insoluble nitrogen, nonprotein nitrogen, soluble tungstic acid precipitable nitrogen and peptide nitrogen as follows. One hundred ml of distilled water were added to the 25 g of original rumen contents and the mixture was homogenized for 2 minutes in an Omnimixer placed in an ice bucket. Two 2 ml aliquots of the homogenate were used for

the total rumen content nitrogen determination. Forty five ml of the remaining homogenate were centrifuged at 18,000 x g for 15 minutes and two 1 ml portions of the supernatant were kept for soluble rumen nitrogen determination. Twenty ml of the supernatant were mixed with 5 ml of 1.07 N sulfuric acid and 5 ml of 10% sodium tungstate (w/r) (Winter et al., 1964) and left to precipitate the protein over night in the cold room. The mixture was then centrifuged at 13,000 x g for 10 minutes and two 2 ml portions of the supernatant were used for non-protein nitrogen analysis.

Insoluble nitrogen, soluble precipitable nitrogen and peptide nitrogen were calculated as the difference between total and soluble nitrogens, soluble and non-protein nitrogens and non-protein nitrogen and ammonia nitrogen respectively.

3. Nitrogen Determination

Total, soluble and non-protein nitrogens in rumen samples were determined as outlined in section 4 of experiment 1.

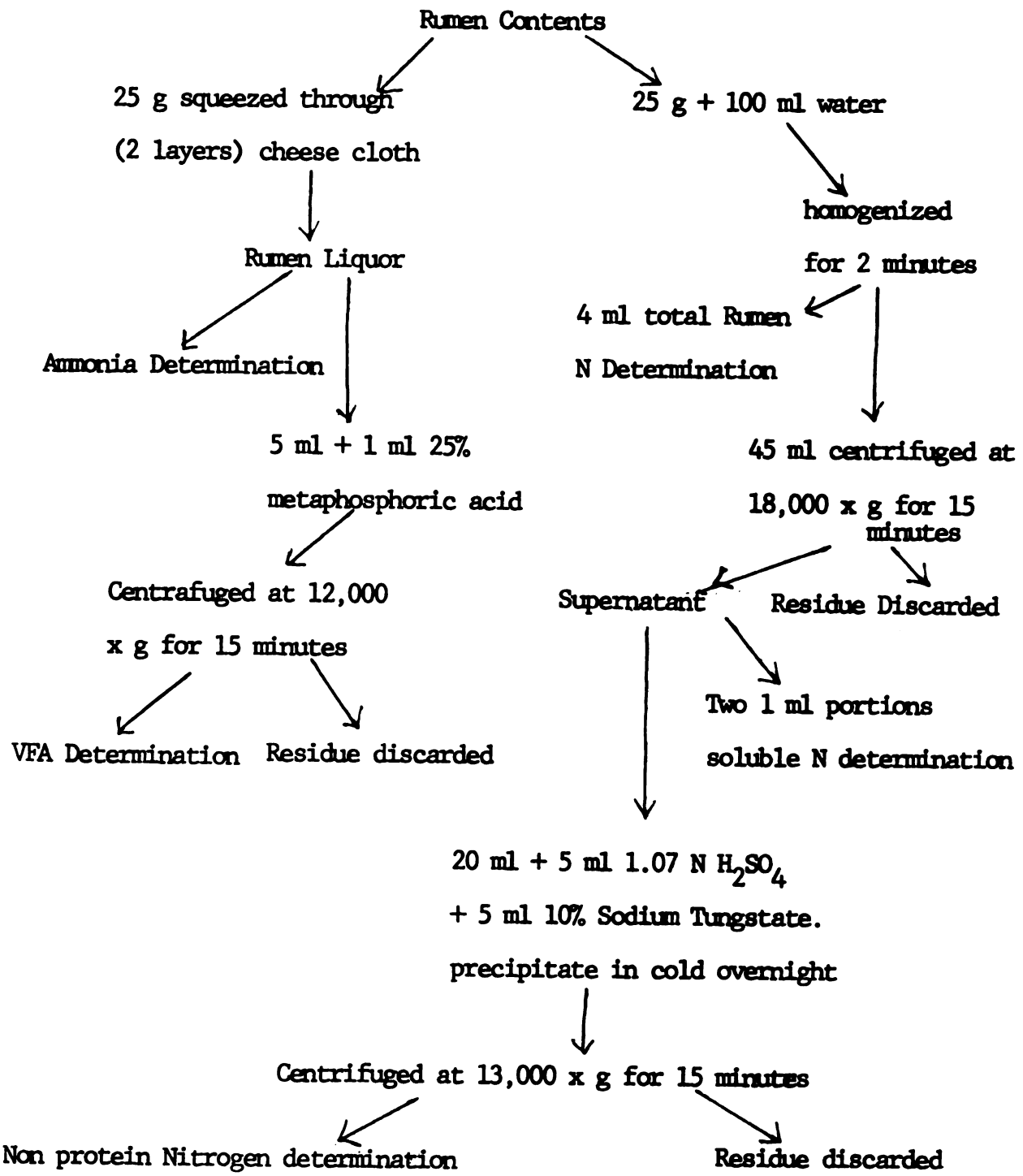
4. Rumen Ammonia and Blood Urea Nitrogen Determination

Rumen ammonia and blood urea nitrogens were determined by the microdiffusion method of Conway (1960) as outlined by Fenderson (1972).

FIGURE 2

Flow Chart of Fractionation of Rumen Contents,

Experiment Two



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5. Volatile Fatty Acids Determination

Rumen VFA concentrations were determined by gas liquid chromatography using a Packard G.L.C. (Model 840). The column (183 cm long and 2 mm internal diameter, teflon tubing) was packed with Chromosorb 101 (Anspec Co., Inc., Ann Arbor, Michigan), the flow rate of the carrier gas (Nitrogen) was 40 ml/minute and the column temperature was maintained at 185°C (Ponto and Bergen, 1974). Volatile fatty acids in rumen fluid samples were identified quantitatively by comparison with a standard solution of volatile fatty acids.

6. Statistical Analysis

Data were statistically analyzed by the usual analysis of variance technique on a CDC 6500 computer at Michigan State University Computer Laboratory. Difference between means were determined by the "Duncan's New Multiple Range Test" on the CDC 6500 computer.

RESULTS

Experiment One

1. Plasma Amino Acid Concentration Changes As An Indicator of Amino Acid Requirements.

In the present study, plasma amino acid changes in response to incremental abomasally infused amino acid levels were used as a response criterion to determine the essential amino acid requirements of growing steers. Plasma level of the infused amino acid was expected to remain constant until the animal's requirement for that amino acid was satisfied and then increase thereafter with higher infusion levels. The infused level, at which the plasma concentration began to deflect upward, along with the quantity entering the small intestine was regarded as the requirement.

The effects of incremental abomasal methionine, lysine, threonine and tryptophan infusions on their respective plasma concentrations are presented in Table 4 and average daily feed intake in Table 5.

Plasma methionine concentration, on both day 4 and day 8 did not change until 7 g of methionine were infused. Thereafter a large linear increase was observed with each successive increment of infused methionine. A graphical

TABLE 4

Plasma Amino Acid Levels[a] in Relation to The Level of Amino Acid Infused, Experiment One

Amino Acid	Sampling day	Infusion Level[b] 0.0	1.7	3.5	7.0	11.0	15.0
Methionine[c]	4	2.65±0.3	-	2.51±0.1	2.82±0.2	4.65±0.3	6.56±0.5
	8	3.06±0.8	-	3.70±0.1	3.92±0.5	5.46±0.5	6.60±0.0
Lysine[d]	4	3.90±0.2	-	5.20±0.4	7.22±1.1	8.77±0.6	10.44±1.8
	8	3.90±0.2	-	6.12±1.7	8.48±0.8	8.50±1.5	8.37±1.5
Lysine in presence of Methionine[c]	4	5.95±0.6	-	7.71±0.7	9.51±0.5	11.12±0.9	12.20±1.1
Threonine[d]	4	4.75±1.1	-	8.26±0.7	11.53±1.2	12.56±0.3	17.62±3.2
	8	4.75±1.1	-	7.08±1.7	8.57±0.8	10.79±1.1	17.83±1.9
Tryptophan[df]	4	0.70±0.1	0.90±.1	1.20±0.3	1.44±0.2	1.70±0.0	-
	8	0.70±0.1	0.92±.1	1.20±0.2	1.27±0.1	1.72±0.0	-

[a]Micromoles of amino acid per 100 ml plasma

[b]Grams of amino acid per animal per day

[c]Mean and standard error of 5 steers

[d]Mean and standard error of 2 steers.

[e]Mean and standard error of 3 steers.

[f]Milligrams of tryptophan per 100 ml plasma.

TABLE 5

Average Daily Feed Intake of Steers During Infusion Periods, Experiment One

Infusion Period	No. of Steers	Avg. Daily Feed Intake Kg/100 Kg B.W.
Methionine	7	2.6
Lysine	7	2.6
Lys + met	7	2.6
Threonine	2	2.3
Tryp	2	2.7

representation of plasma methionine levels in relation to the level of infused methionine are presented in Figure 3. The point of inflection on days 4 and 8 of plasma methionine occurred when 7 g of methionine were infused.

Plasma lysine increased linearly with each successive infusion increment of lysine indicating that the animal's requirement for lysine was satisfied by the quantity leaving the rumen. A graphical representation of these results is presented in Figure 4. Incremental levels of lysine along with 11 grams of methionine were infused into the abomasum of 3 larger steers (311 kg) to determine if lysine was the second limiting amino acid. Under these conditions, plasma lysine increased immediately with the first infusion level (3.5g of lysine) and then continued to

increase rapidly with each successive increment. This indicated that lysine was not limiting in the presence of adequate methionine and that lysine passing to the small intestine from the rumen-reticulum met the requirement of this amino acid.

Plasma threonine level at both sampling times increased with the first infusion level of 3.5g of threonine and with each successive increase in infused threonine. These results indicated that the animal's threonine requirement was met by the level of threonine in the ingesta passing to the small intestine. A graphical representation of these results is presented in Figure 5.

Plasma tryptophan at both sampling times increased immediately upon tryptophan infusion and at each successive increment of infused tryptophan into the abomasum. This also indicated that the animal's tryptophan requirement was satisfied by the tryptophan leaving the rumen. A graphical representation of these results is presented in Figure 6.

2. Nitrogen Balance As An Indicator of Animo Acid Requirement

Results of nitrogen balance studies carried on two steers during the infusion of each amino acid are presented in Table 6 with graphical representations accompanying the specific amino acid in Figures 3, 4, 5 and 6.

The quantity of nitrogen retained in one steer infused with methionine increased linearly until after 7 g

■ Methionine D_4. $Y_1 = .03x + 2.78$. ($r = .55$)
$Y_2 = .47x + (-.46)$. ($r = -.99$)

● Methionine D_8. $Y_3 = .12x + 3.13$. ($r = .96$)
$Y_4 = .36x + (-1.61)$. ($r = -.99$)

▲ Nitrogen balance

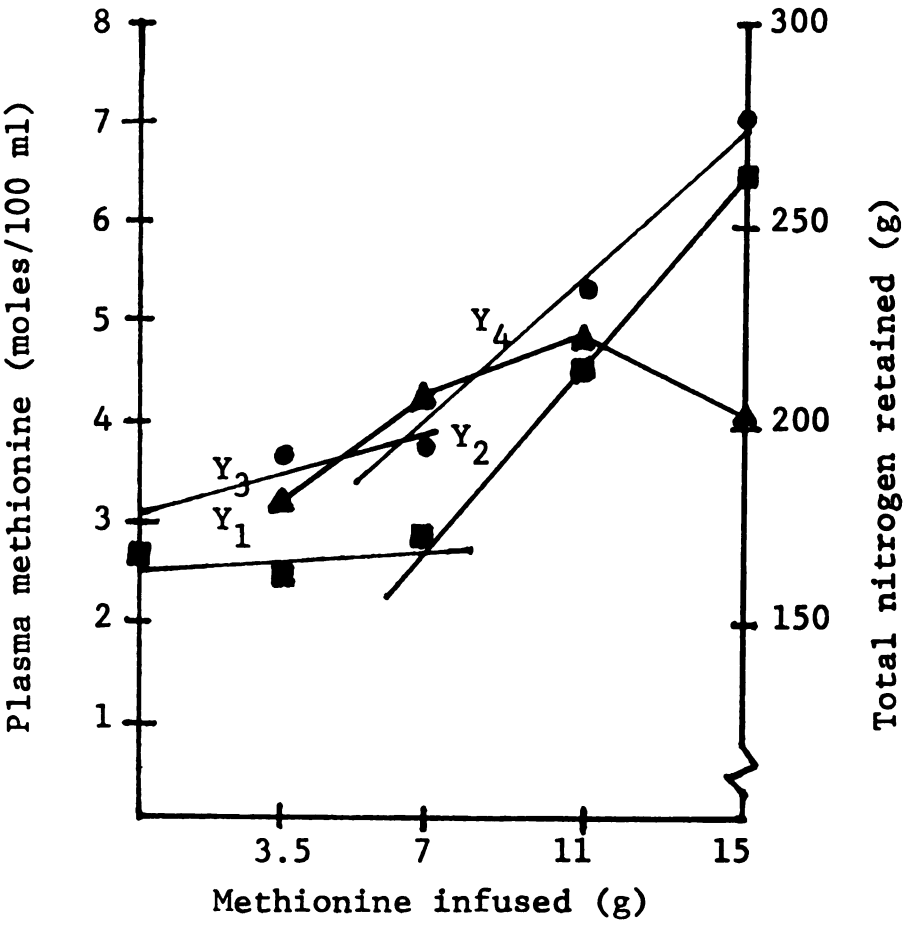

FIGURE 3. The effect of abomasal methionine infusions on plasma methionine and nitrogen balance.

■ Lysine D_4. Y_1 = .44x + 6.2. (r = .99)

● Lysine D_8.Y_2 = .30x + 4.91. (r = .85)

✕ Lysine + methionine. Y_3 = .42x + 6.2. (r = .99)

▲ Nitrogen balance. Y_4 = -3.14x + 285. (r = -.75)

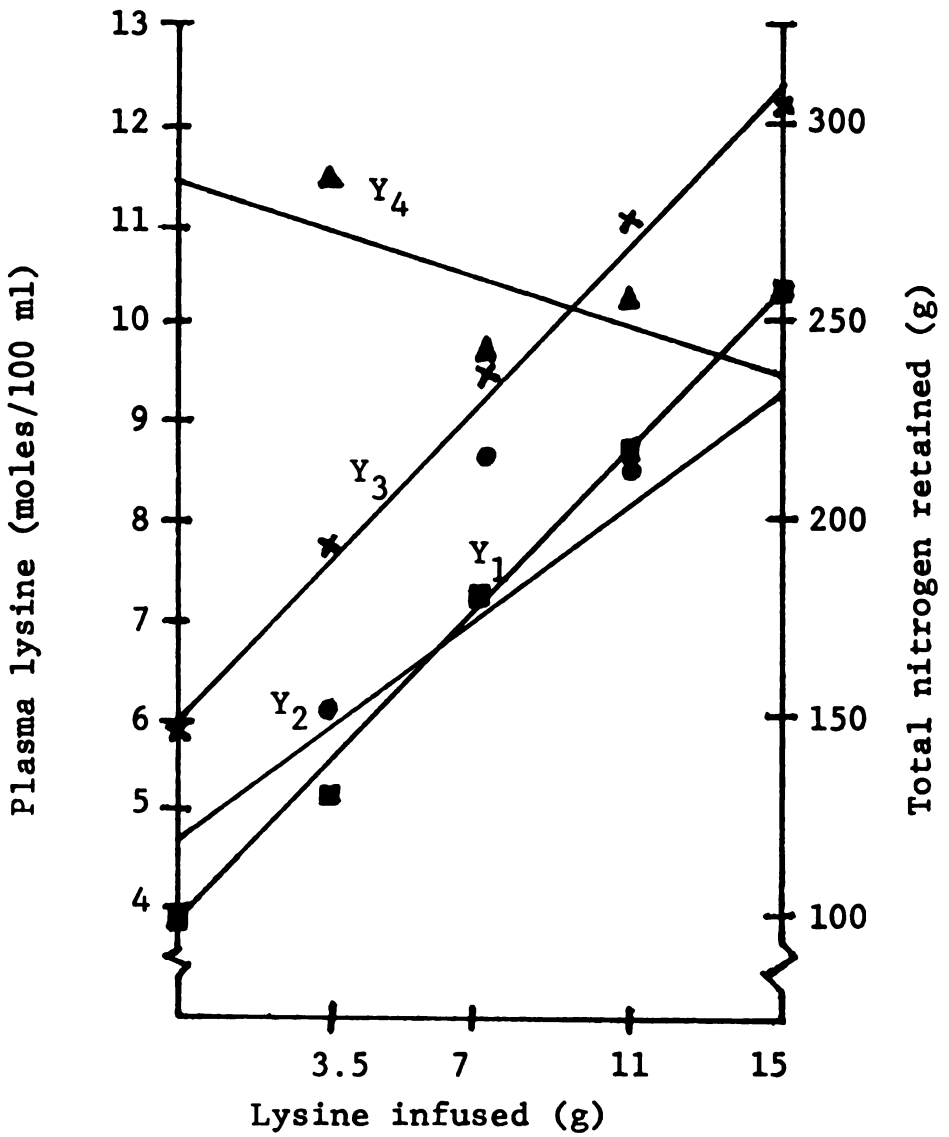

FIGURE 4. The effect of abomasal lysine infusions on plasma lysine and nitrogen balance.

■ Threonine D_4. Y_1 = .80x + 5.1. (r = .98)

● Threonine D_8. Y_2 = .80x + 3.96. (r = .96)

▲ Nitrogen balance. Y_3 = 6.18x + (-212). (r= -.99)

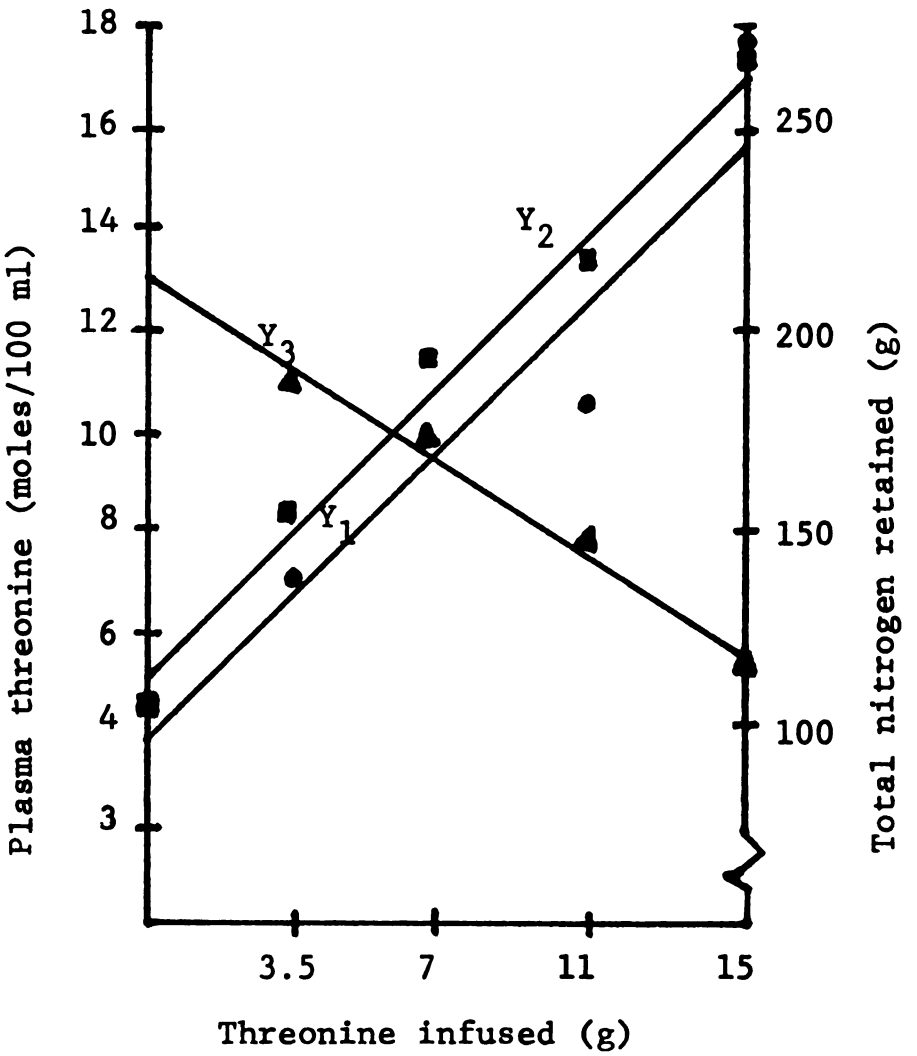

FIGURE 5. The effect of abomasal threonine infusions on plasma threonine and nitrogen balance.

■ Tryptophan D_4. $Y_1 = .09x + .78$. ($r = .98$)

● Tryptophan D_8. $Y_2 = .08x + .77$. ($r = .97$)

▲ Nitrogen balance. $Y = -6.05x + 222$. ($r = -.97$)

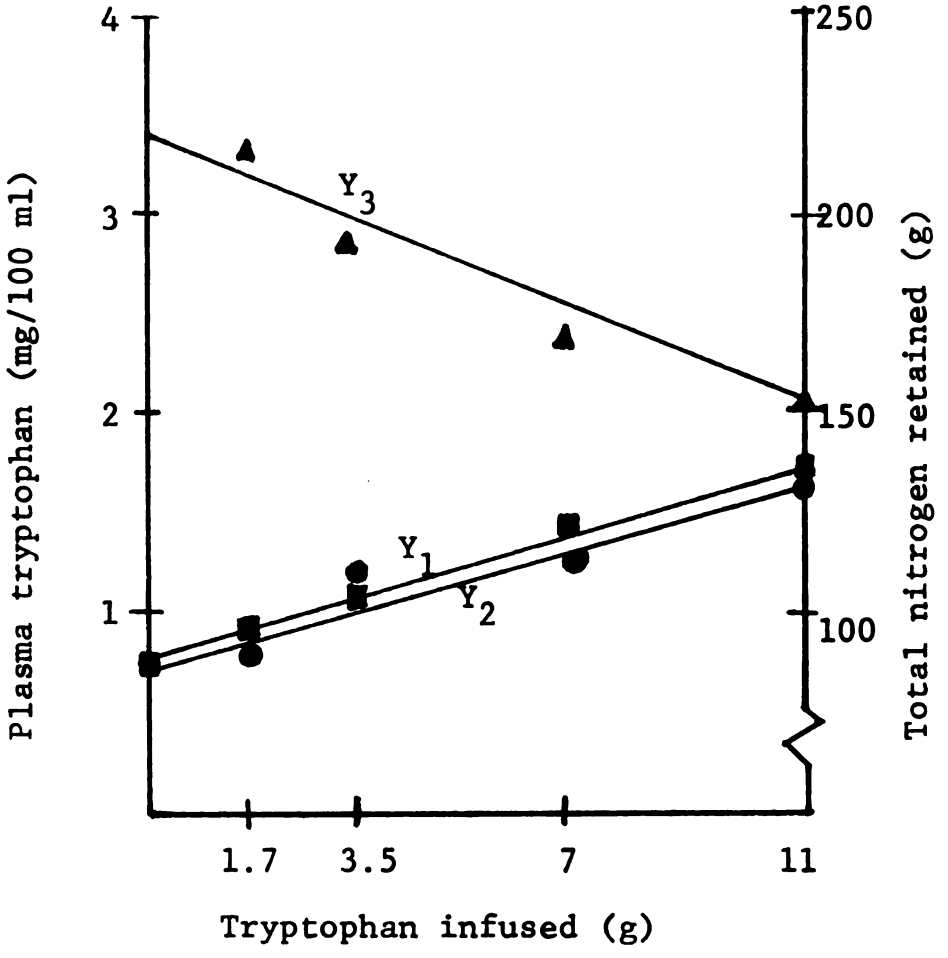

FIGURE 6. The effect of abomasal tryptophan infusions on plasma tryptophan and nitrogen balance.

of methionine were infused and then plateaued. However, that of the second steer infused with methionine decreased with each successive level of infusion. This is not surprising, since this steer had diarrhea throughout the infusion period. However, plasma responses to the methionine infusion were apparently not affected.

The quantity of nitrogen retained during the lysine, threonine and tryptophan infusion periods decreased linearly as their respective plasma concentrations increased with each increment of infusion. These results support those of the plasma studies and show that the animal's requirements for lysine, threonine and tryptophan were met by the quantity of each amino acid reaching the small intestine from the rumen.

3. Nitrogen and Amino Acid Passage to the Abomasum

Acid lignin/nitrogen and chromium/nitrogen ratios were used to quantitate the nitrogen passage to the abomasum (Table 7). The percentages of lignin, chromium and nitrogen in the experimental ration were 2.61, 0.13 and 1.49, respectively. The lignin,chromium and nitrogen contents in the abomasal ingesta of steers fed the experimental ration was 3.79, 0.16 and 2.12 percent respectively. The lignin to nitrogen ratios in the ration and abomasal ingesta (1.75 and 1.79 respectively) indicated that there was no significant net ruminal nitrogen loss (2%) under these

TABLE 6

TABLE 6

Nitrogen Retention in Steers Abomasally Infused With Different Levels of Amino Acids, Experiment One

Parameters	Infusion Levels[a]				
	1.7	3.5	7.0	11.0	15.0
Lysine[b]					
Total administered nitrogen g/7 days	-	721± 2	738± 0	742± 0	752± 0
Fecal nitrogen g/7 days	-	246± 2	260±16	262±19	282± 3
Urinary nitrogen g/7 days	-	189± 7	235± 4	217±21	231±28
Nitrogen retained g/7 days	-	286± 8	241±12	285± 3	240±25
Nitrogen retained %	-	40	33	38	32
Methionine[c]					
Total administered nitrogen g/7 days	-	655	651	600	700
Fecal nitrogen g/7 days	-	249	226	207	234
Urinary nitrogen g/7 days	-	224	217	168	231
Nitrogen retained g/7 days	-	182	209	225	204
Nitrogen retained %	-	28	32	38	29
Threonine[b]					
Total administered nitrogen g/7 days	-	614± 3	627±53	630± 3	613±25
Fecal nitrogen g/7 days	-	205±20	232±32	225± 8	216±11
Urinary nitrogen g/7 days	-	221± 4	224± 7	258± 6	280±14
Nitrogen retained g/7 days	-	190±26	170±28	148±11	117±28
Nitrogen retained %	-	31	27	23	19

Table 6 (Continued . . .)

Table 6 (Continued . . .)

Parameters	Infusion Levels[a]				
	1.7	3.5	7.0	11.0	15.0
Tryptophan[b]					
Total administered nitrogen g/7 days	816± 5	799±39	759±82	843± 0	-
Fecal nitrogen g/7 days	305±24	292±23	256±19	273± 0	-
Urinary nitrogen g/7 days	294±42	312±32	336±17	371± 0	-
Nitrogen retained g/7 days	217±12	194±31	167±46	160±40	-
Nitrogen retained %	27	24	22	19	

[a]Grams of amino acid/steer/day.

[b]Average of 2 steers

[c]Data from 1 steer

TABLE 7

TABLE 7

Ratios of Markers in Ration and Abomasal Ingesta, Experiment One

Sample	Acid-Lignin	Chromium	Nitrogen	Lignin/ Nitrogen	Lignin/ Chromium	Chromium/ Nitrogen
	%					
Ration	2.61	.13	1.49	1.75	20.08	.09
Abomasum[a]	3.79±.16	.16±.01	2.12±.08	1.79	23.69	.08

[a]Mean of 14 composite samples from 7 steers.

particular circumstances. The chromium/nitrogen ratio in the ration, 0.09 and in the abomasal ingesta 0.08, and lignin/chromium ratio in the ration 20.08 and in the abomasal ingesta 23.69 tend to indicate that chromium was reaching the abomasum at a slightly faster rate than lignin and nitrogen.

The quantities of nitrogen and each amino acid reaching the abomasum are presented in Table 8. The average quantities of nitrogen, lysine, cystine, methionine, total sulfur amino acids, threonine and tryptophan reaching the abomasum per kg of feed consumed were determined by the use of nitrogen to marker ratios. The quantity (g) of each amino acid per kg of feed consumed (lysine 4.5, cystine 1.5, methionine 1.6, total sulfur amino acid 3.1, threonine 3.7 and tryptophan 0.6) was obtained by multiplying the quantity of each amino acid per g of abomasal nitrogen by g of nitrogen passing to the abomasum for each Kg of feed. The average daily quantity of each amino acid reaching the abomasum was determined by multiplying the average quantity of each amino acid reaching the abomasum per Kg of feed consumed by the average daily feed intake. These values are 103.3, 32.3, 10.7, 11.2, 21.9, 21.6 and 4.7 g per day for nitrogen, lysine, cystine, methionine, total sulfur amino acids, threonine and tryptophan, respectively.

TABLE 8

Nitrogen and Amino Acid Passage to the Abomasum in Steers Fed a 9.5% Crude Protein Ration[a], Experiment One

Parameter	g/Kg Feed	g/day
Nitrogen[bc]	14.9	105.3±6.2
Lysine [bc]	4.5	32.1±0.9
Cystine[bc]	1.5	10.7±1.1
Methionine[bc]	1.6	11.2±0.8
Total sulfur amino acid[bc]	3.1	21.9
Threonine[de]	3.7	21.6±0.5
Tryptophan[df]	.6	4.7±0.9

[a]Fed at 3% of body weight

[b]Mean and standard error of 7 steers

[c]Steers consumed 7.07±.4 Kg/feed/day

[d]Mean and standard error of 2 steers

[e]Steers consumed 5.88 Kg feed/day

[f]Steers consumed 7.55 Kg feed/day

4. The Quantitative Aspects of Amino Acid Requirement

The quantitative aspects of lysine, cystine, methionine, total sulfur amino acids, threonine and tryptophan are presented in Table 9. According to Hogan (1973) postruminal protein has an average digestibility coefficient of approximately 0.70. Thus, the quantity of each absorbable amino acid reaching the small intestine was obtained by multiplying the quantity of each amino acid that was reaching the abomasum by the coefficient of digestibility. These values were for methionine, 7.9; cystine, 7.4; total sulfur amino acid, 15.3; lysine, 22.5; threonine, 15.1; and tryptophan, 3.3.

The daily requirement for each amino acid was obtained as the sum of the absorbable amino acid and the determined infusion requirement level. These values were for methionine, 14.9 and the total sulfur amino acids, 22.3g per day. Since the animal's lysine, threonine and tryptophan requirements were satisfied by the quantity of these amino acids in the digesta reaching the small intestine, their daily requirements were regarded as equal to or less than the absorbable amount.

During the infusion periods the steers were growing very slowly or in spurts and were fed a dietary protein level that would not support maximal growth. These data were used to derive an essential amino acid requirement pattern for the same size steers growing continuously and

TABLE 9

Quantitative Aspects of Methionine, Cystine, Total Sulfur Amino Acid, Lysine, Threonine and Tryptophan Requirements, Experiment One

Amino Acid	Passage to Abomasum (g/day)	Dig. Coef. (%)	Absorbable AA (g)	AA Infused to Infl.pt. (g)	Requirement (g/day)
Methionine	11.3±.5[a]	70	7.9	7.0[c]	14.9
Cystine	10.6[a]	70	7.4	-	-
Total Sulfur Amino Acid	21.9	70	15.3	-	22.3
Lysine	32.1±.9[a]	70	22.5	-	≤22.5
Threonine	21.6±.5[b]	70	15.1	-	≤15.1
Tryptophan	4.7±.9[b]	70	3.3	-	≤ 3.3

[a]Mean and standard error of 7 steers

[b]Mean and standard error of 2 steers

[c]Mean of 5 steers

fed a 9.5 or 12% crude protein ration at approximately 2.5% of body weight. Results of these tabulations are given in Table 10.

In order to calculate these amino acid requirement patterns, the experimentally determined total sulfur amino acid requirement (22.3 g per day) was taken as unity and was then multiplied by the amino acid ratios of the 1973 NRC pig requirement pattern (using the pig's total sulfur amino acid requirement as unity). On this basis cystine can fulfill 56% of the total sulfur amino acid need (NRC pig requirement, 1973) and the methionine requirement in the presence of adequate cystine for the 274 kg growing steer fed 9.5% crude protein was calculated to be 9.8 g per day. The other essential amino acid requirements were calculated for lysine 31.2, phenylalanine plus tyrosine 22.3, valine 22.3, isoleucine 22.3, leucine 26.8, threonine 20.1, tryptophan 5.8, histidine 8.0, arginine 8.9 g per day.

The total sulfur amino acid requirement for the 274 Kg steer fed the 12% ration was calculated in relation to the determined total sulfur amino acid requirement of the 9.5% ration, with the assumption that only 90% of the nitrogen from the 12% ration is reaching the abomasum (see discussion). The individual essential amino acids were then calculated in a manner similar to that of the 9.5% ration. These values for the respective amino acids were

TABLE 10

Estimated Daily Essential Amino Acid Requirement of Growing Steers, Experiment One

Amino Acid	Present study 9.5% C.P.	274 Kg steer 12% C.P.	Chalupa (1973) (300 Kg steer)
	g		
TSAA[a]	22.3	25.4	-
Met.	9.8[b]	11.2[b]	12.0
Lysine	31.2[b]	35.1[b]	36.5
Phe & Tyr	22.3[b]	25.4[b]	21.0
Valine	22.3[b]	25.4[b]	25.0
Isoleucine	22.3[b]	25.4[b]	25.0
Leucine	26.8[b]	30.4[b]	39.0
Thr.	20.1[b]	22.8[b]	20.5
Try.	5.8[b]	6.6[b]	3.0
His.	8.0[b]	9.8[b]	13.5
Arginine	8.9[b]	10.2[b]	30.0
TEAA[c]	177.5[b]	202.3[b]	225.5

[a]Total sulfur amino acids

[b]Calculations based on pig requirement (NRC 1973) pattern

[c]Total essential amino acids

25.4, 11.2, 35.1, 25.4, 25.4, 25.4, 30.4, 22.8, 6.6, 9.8, 10.2 and 202.3g per day. With the exception of arginine and leucine these values compare favorably with those of Chalupa (1974) who based his calculations on estimation of the total absorbable protein that was bypassing ruminal degradation, total ruminal protein synthesis and post ruminal infusion data.

DISCUSSION

Amino Acid Requirements of Growing Steers, Experiment One

Numerous authors (Zimmerman and Scott, 1965; McLaughlan and Illman, 1967; Mitchell et al., 1968; Stockland et al., 1970; Brave et al., 1970; Wakeling et al., 1970; Young et al., 1971; Keith et al., 1971; Young et al., 1972; Tontisirin et al., 1972) have reported that changes in plasma amino acid levels in response to amino acid intake or infusion can be used as an indicator of essential amino acid requirements. However, such a technique will give valid results only if the amino acid under investigation is the first limiting amino acid in the ingesta reaching the small intestine (Zimmerman and Scott, 1965; Wakeling et al., 1970). Results of the present study shows that methionine was the first limiting amino acid in the ingesta reaching the small intestine of these steers. Similar results have been obtained in sheep by Wakeling et al. (1970). However, because of wool growth, the sulfur amino acid requirements of sheep are generally higher than those of steers. The observed changes in plasma methionine levels in response to methionine infusion substantiates the observations of Almquist (1954), Morrison et al. (1961), Zimmerman and

Scott (1965), Mitchell et al. (1968), Wakeling et al. (1970) and Keith et al. (1971) that plasma level of the limiting amino acid remains constant until the animal's requirement is met and increases rapidly as intake or infusion levels are increased. The immediate and linear increase in plasma lysine during the lysine infusion indicates that lysine was not limiting in the ingesta reaching the small intestine and neither was it made limiting when infused in the presence of adequate methionine. A similar result has been obtained in sheep by Wakeling et al. (1970). Also, threonine and tryptophan were not found limiting in the ingesta reaching the small intestine. This can be attributed to the slow growth rate of the steers due to the low protein diet and stresses arising from the confinement in the metabolism stalls. No attempt was made to determine if these amino acids would be limiting in the presence of adequate methionine.

Nitrogen balance has been successfully used as an indicator of amino acid requirements (Nimrick et al., 1970a; Nimrick et al., 1970b; Nimrick and Kaminiski, 1970; Schelling et al., 1973). However, little can be concluded from the nitrogen balance during the methionine infusion period since there was only one animal on this treatment. The observed decrease in nitrogen retention during the lysine, threonine, and tryptophan infusions was contrary to the expected plateau (Nimrick et al., 1970a).

Fecal nitrogen excretion remained constant throughout each nitrogen balance trial indicating that all the infused nitrogen was absorbed. However, the increase in urinary nitrogen was greater than the increase of nitrogen from infused amino acids. This suggests that an increased nitrogen load was placed on the animals either by excessive amino acids or other stresses such as extended confinement in the metabolism stalls or both. Similar results have been reported by Boila and Devlin (1972) who observed an 11% decrease in nitrogen retention after infusing 9g per day of lysine into the abomasum of steers. These workers claimed that the decrease in nitrogen retention was due to degradation of the very high level of plasma free lysine resulting from infusion. However, this is not an adequate explanation, because if the high level of plasma lysine balanced the amount of lysine infused, nitrogen retained should not have changed.

Since ruminal activities dictate the quantity and pattern of amino acids reaching the small intestine, it was imperative that the quantity of each amino acid in the abomasal ingesta be determined. The lignin to nitrogen ratios in feed and abomasal ingesta showed that there was no net ruminal nitrogen loss. This was expected, since the ration contained only 9.5% crude protein. Using lignin and chromic oxide as markers, similar results have been obtained by Weller, Pilgrim and Gray (1971) in sheep fed 8-10% crude

protein ration. Usually, when large amounts of soluble protein are fed, the nitrogen reaching the small intestine is less than dietary intake. Conversely, when a high energy, low protein ration is fed to the ruminant the nitrogen reaching the small intestine tends to be equal or higher than the dietary intake (Clarke et al., 1966). There is considerable controversy concerning the use of chromic oxide as an insoluble marker for the determination of nutrient digestibility. Drennan, Holmes and Garrett (1969) and Faichney (1972) after finding that chromic oxide behaved independently of the particulate matter in the digesta, concluded that chromic oxide is not a satisfactory indicator of flow of digesta through the gastrointestinal tract. However, Ørskov, Frazer and McDonald (1970), Offer, Axford and Evans (1971), Offer, Evans and Axford (1971a), Offer, Evans and Axford (1971b) and Weller et al. (1971) obtained satisfactory results with chromic oxide. Although fairly good results were obtained with chromic oxide in the present study, the slight differences between the lignin/chromium ratio of the ration and the lignin/chromium ratio of the abomasal ingesta indicates that chromic oxide was moving through the alimentary tract independent of lignin and nitrogen. However, regardless of the marker used the key to good passage studies is sampling technique (Weller et al., 1971; Axford et al., 1971).

In order to relate the determined sulfur amino acid requirement of steers in the present study to growing steers of the same body weight and consuming 12% protein at 2.5% of their body weight daily, it was necessary to make 3 basic assumptions: (1) That the tissue requirements for essential amino acids for pigs and cattle are similar (Black et al., 1957; Downes, 1961; Hutton and Annison, 1972). This assumption is based on a similar gain in carcass nitrogen expressed as a percentage of live weight gain (2.64) for pigs (Woodman and Evans, 1951) and steers (2.40) (Agricultural Research Council, 1965). Although these findings are from early data, no new data are available to challenge their authenticity. Another basis for the above assumption is that the amino acid composition of pig and beef muscles are similar (Black et al., 1957; Downes, 1961). After observing that isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and histidine were metabolically essential to growing and mature sheep, Downes (1961) concluded that tissue amino acid metabolism of sheep is similar to that of non ruminants. (2) That the requirement for each amino acid per 16g of nitrogen remains unchanged regardless of protein intake. This assumption is based on the findings of Boomgaardt and Baker (1973a) who observed that the lysine requirement for maximum weight gain in chickens remained a constant percentage of dietary protein whether the diet contained 14, 18.5 or 23% crude

protein. (3) That at least 90% of the nitrogen from a 12% protein ration would reach the abomasum. This assumption is well within reasonable limits. Weller et al. (1971) reported that when sheep were fed 8-10% crude protein, the amount of nitrogen reaching the abomasum was approximately 100% of the dietary intake. In the present study it was found that 98% of the dietary nitrogen reached the abomasum. Clarke et al. (1966) found that at low protein intakes the amount of nitrogen reaching the duodenum was equal to or greater than the dietary intake but as the nitrogen intake increased the amount reaching the duodenum decreased proportionally.

According to the 1973 NRC pig requirement report, methionine can fulfill the total sulfur amino need whereas cystine can meet only 56% of the total sulfur amino acid requirement. The results of the present study indicated that methionine fulfilled part of the cystine need. Hutton and Annison (1972) used the pig amino acid requirement pattern to calculate the essential amino acid requirement for a growing 200 Kg steer. However, these workers did not consider that cystine can supply at least 56% of the sulfur amino acid requirement, consequently most of their calculated requirement values were low when compared to those of the present study.

It can be concluded from this study that the method used to determine the essential amino acid requirements for growing steers was satisfactory. However, it

would be advisable to use a lower crude protein ration so that the requirement for several amino acids would not be satisfied by the quantity leaving the rumen. Also, since age and body weight influence protein and essential amino acid requirements (Young et al., 1971; Tontisirin et al., 1972; Tontisirin et al., 1973), future experiments should include different ages and body weights under more favorable growth conditions. Steers of different fatness should also be studied.

RESULTS

Experiment Two

This experiment was designed to study the effects of dietary crude protein content (10.7, 20.2, 32.5 and 40.0% for rations 1, 2, 3 and 4 respectively) on voluntary feed intake, protein intake, plasma amino acids, plasma urea nitrogen, rumen volatile fatty acids (VFA) and the various nitrogenous fractions in the rumen.

Intake Parameters

The results from the feed intake study are presented in Table 11. Feed intake decreased non significantly ($P>.05$) with each increment of dietary crude protein. The fourteen day patterns of feed intake of steers fed the four rations are presented in Table 12 and graphically in Figure 7. There were no significant changes in daily feed intake of steers fed ration 1 throughout the 14 day experimental period. Daily feed intake of steers fed ration 2 decreased on day 2 and then increased significantly ($P<.05$) for the remainder of the period. Daily feed intake of steers fed rations 3 and 4 started to decrease on day 2 and was significantly depressed ($P<.01$) by day 3. Thereafter, feed intake of steers fed these two rations increased throughout the rest of the period.

TABLE 11

Effects of Various Levels of Dietary Crude Protein on Feed Intake, Plasma Amino Acids and Urea Levels, Ruminal VFAs and Ruminal Nitrogenous Fractions, Experiment Two

Parameter	Rations				
	1	2	3	4	S.E.
Intake					
Daily feed[1]	9.0^{aA}	8.4^{abAB}	7.1^{bB}	5.0^{bAB}	1.1
Daily protein[1]	1.0^{a}	1.7^{a}	2.3^{a}	2.0^{a}	0.2
Kg (feed)/100 Kg B.W.	3.0^{a}	2.8^{a}	2.4^{a}	1.7^{a}	0.4
Rumen Nitrogen[2]					
Total	589.6^{aA}	747.6^{bAB}	741.6^{bcB}	666.3^{cB}	47.4
Soluble	120.3^{a}	156.9^{a}	187.7^{a}	199.0^{a}	9.3
Insoluble	469.3^{aA}	590.7^{bAB}	553.8^{bcB}	467.3^{cB}	46.6
Non Protein	56.0^{aA}	76.0^{bAB}	91.0^{cBC}	101.4^{cC}	5.1
Ammonia	11.6^{aA}	39.8^{bAB}	69.0^{cBC}	81.9^{cC}	5.9
Peptide & Amino Acid	44.4^{cB}	36.1^{bcA}	22.1^{abA}	19.5^{aA}	4.2
Precipitable	64.4^{a}	81.0^{a}	96.5^{b}	97.6^{b}	6.9
Rumen VFAs[3]					
Acetate	49.2^{bcAB}	65.9^{cB}	65.6^{bAB}	56.9^{aA}	7.3
Propionate	33.8^{a}	42.8^{b}	31.7^{a}	22.1^{a}	2.6
Butyrate	15.2^{a}	20.4^{ab}	15.5^{bc}	13.0^{c}	1.4
Isovalerate	3.2^{a}	4.0^{b}	4.5^{b}	3.9^{ab}	0.7
Valerate	3.2^{a}	4.3^{b}	5.6^{c}	6.4^{c}	0.3

Table 11 (Continued . . . .)

Parameter	Rations				
	1	2	3	4	S.E.
Plasma					
Urea Nitrogen[4]	7.0^{aA}	14.8^{bB}	20.1^{cB}	21.0^{cB}	1.2
Total EAA[5]	0.9^{a}	1.1^{a}	0.9^{a}	0.9^{a}	0.1
Total NEAA[5]	1.1^{a}	1.0^{a}	0.9^{a}	0.9^{a}	0.1
EAA/NEAA	0.9^{a}	1.1^{b}	1.0^{ab}	1.0^{ab}	0.1

[1]Kg per steer per day

[2]Mg nitrogen per 100 ml rumen content

[3]Micromoles of the respective VFA per ml rumen liquor

[4]Mg nitrogen per 100 ml blood plasma

[5]Micromoles per ml plasma

Values with different superscript in the same row are significantly different.

a,b,c $P<.05$; ABC $P<.01$.

TABLE 12

Daily Feed and Protein Intake, Experiment Two

Parameter	Days							S.E.
	1	2	3	5	7	10	14	
Feed Intake[1]								
Ration 1	8.5^{a}	8.6^{a}	8.8^{a}	8.7^{a}	9.3^{a}	9.2^{a}	9.4^{a}	0.3
Ration 2	7.5^{ab}	6.4^{a}	7.5^{ab}	8.7^{b}	9.5^{ab}	9.7^{ab}	9.6^{ab}	0.3
Ration 3	7.7^{bAB}	5.1^{aA}	5.0^{aA}	7.7^{bAB}	7.8^{bB}	8.2^{bB}	8.3^{bB}	0.3
Ration 4	5.7^{bcdBC}	3.3^{abAB}	2.3^{aA}	4.8^{bcABC}	5.3^{bcdBC}	6.1^{adc}	7.4^{dc}	0.3
Protein Intake[2]								
Ration 1	0.9^{a}	0.9^{a}	0.9^{a}	0.9^{a}	1.0^{a}	1.0^{a}	1.0^{a}	0.1
Ration 2	1.5^{abA}	1.3^{aA}	1.5^{abA}	1.8^{abA}	1.9^{abA}	2.0^{bA}	2.0^{bA}	0.1
Ration 3	2.5^{B}	1.7^{A}	1.6^{A}	2.5^{B}	2.5^{B}	2.7^{B}	2.9^{B}	0.1
Ration 4	2.3^{bCD}	1.3^{aAB}	0.9^{aA}	2.0^{bBC}	2.1^{bC}	2.5^{bcCD}	3.0^{cD}	0.1

[1]Average Kg. of feed per steer per day

[2]Average Kg. of protein per steer per day

Values with different superscripts in the same row are significantly different

a,b,c,d $P<.05$; ABCD $P<.01$

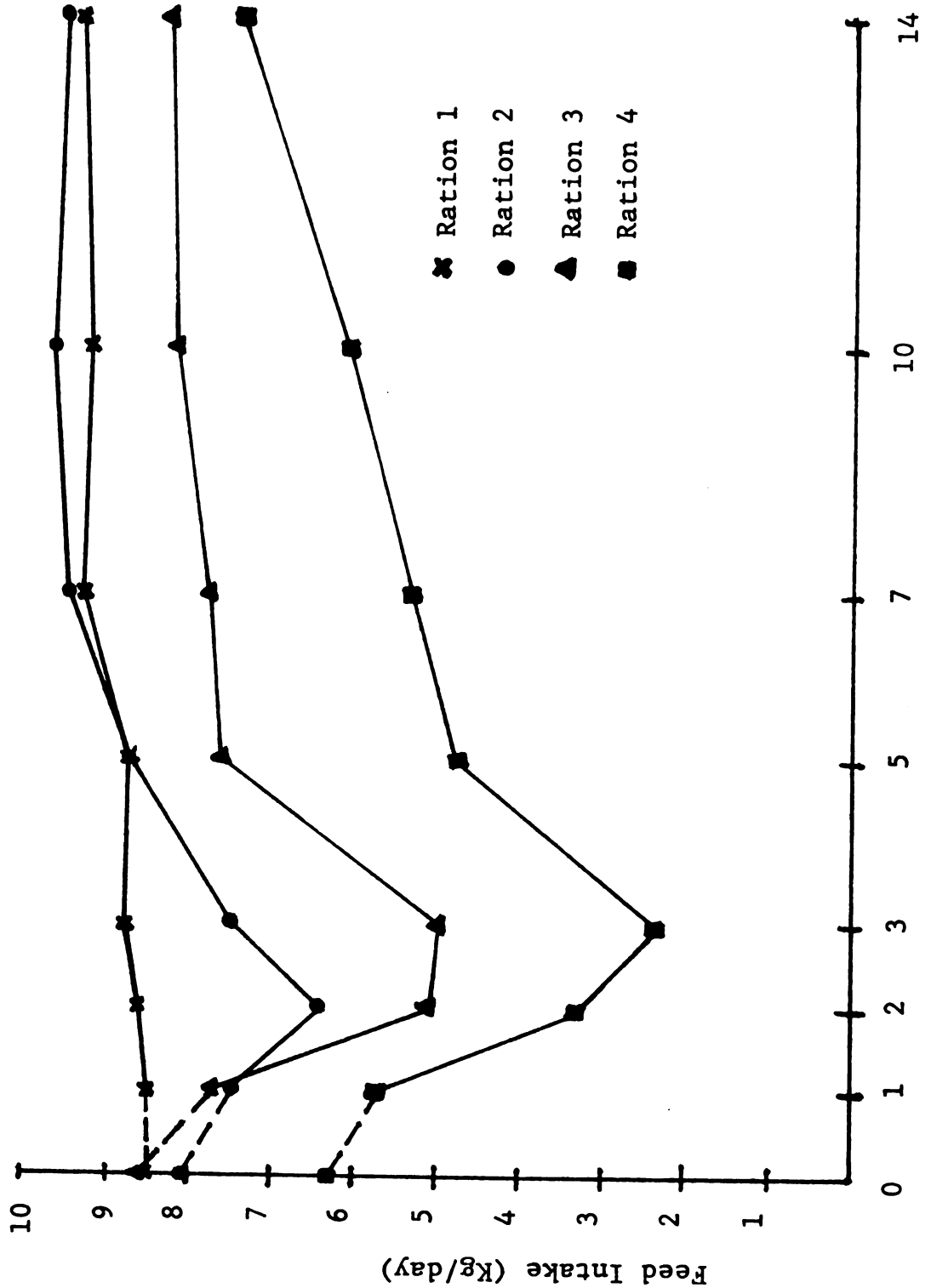

FIGURE 7. Fourteen day patterns of daily feed intake

Daily protein consumptions of steers fed the four rations are presented in Table 11. Protein intake was highest among the steers fed ration 3 ($P<.01$). Steers fed rations 3 and 4 had a significantly higher daily protein intake than steers fed ration 1 ($P<.05$) but protein intakes of steers fed rations 1 and 2 were not significantly different at $P<.05$. Differences in protein intakes of steers fed rations 2, 3 and 4 were not significant ($P>.05$). The fourteen-day patterns of protein intake for steers fed the four rations (Table 12 and Figure 8) are similar to those of total feed intake.

Ruminal Nitrogenous Fractions

Total ruminal nitrogen concentrations of steers fed the four rations are presented in Table 11. Total ruminal nitrogen concentration was highest in steers fed ration 3 and lowest on ration 1. However, differences between treatments were not significant. The fourteen day patterns of total ruminal nitrogen for steers fed the four rations are presented in Table 13. Rumen nitrogen of steers fed ration 1 did not differ during the 14 day period ($P>.05$). Rumen nitrogen of steers fed rations 2 and 3 increased linearly ($P<.05$) throughout the period. Rumen nitrogen of steers fed ration 4 decreased significantly on day 3 ($P<.01$) and increased ($P<.01$) thereafter throughout the remainder of the period.

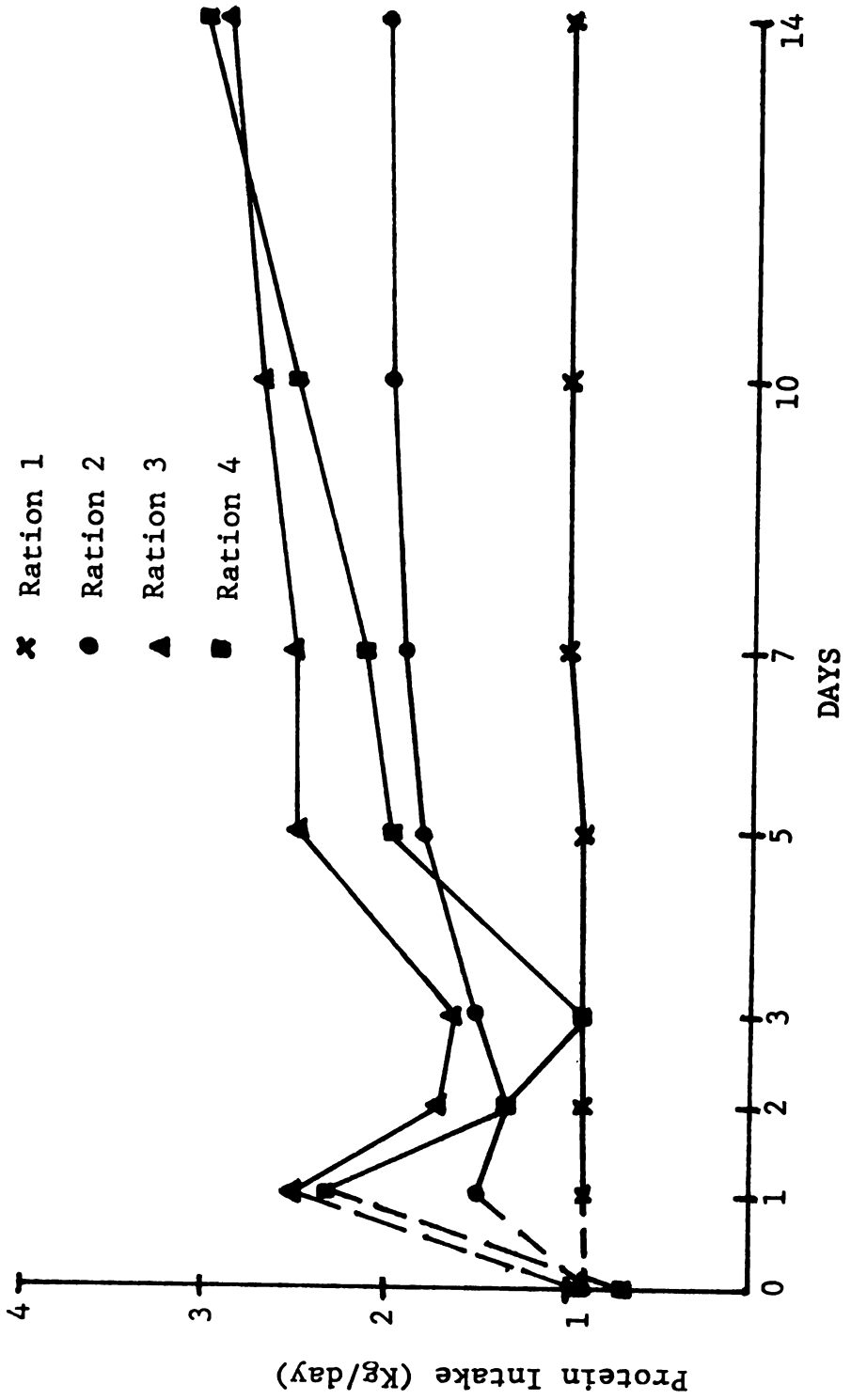

FIGURE 8. Fourteen day patterns of daily protein intake.

Soluble rumen nitrogen of steers fed the four rations are presented in Table 11. Concentrations were highest in steers fed ration 4 and lowest for ration 1 ($P<.01$). The fourteen day patterns of soluble rumen nitrogen for steers fed the four rations are presented in Table 13. There were no significant changes in the patterns of steers fed ration 1. Although soluble rumen nitrogen of steers fed rations 2 and 3 increased significantly ($P<.05$) after day 1 and changed markedly during the period, there was no obvious pattern of change. Steers fed ration 4 had a decrease in rumen soluble nitrogen on day 3. Thereafter, concentrations increased linearly ($P<.01$) for the rest of the period.

Insoluble rumen nitrogen (Table 11) was highest in steers fed ration 2 and lowest in steers fed ration 4. However, differences were not significant at $P<.05$. The fourteen day patterns of insoluble rumen nitrogen of steers fed the four rations are presented in Table 13. Insoluble ruminal nitrogen of steers fed rations 3 and 4 decreased on days 2 and 3, respectively, and then increased linearly throughout the rest of the period, while that of those fed rations 1 and 2 increased over the entire 14 day period, but daily differences were not significant.

Ruminal non protein nitrogen (rumen NPN) of steers fed the four rations are presented in Table 11. Rumen NPN was highest in steers fed ration 4 ($P<.01$) and lowest in

TABLE 13

Average Daily Concentrations of Ruminal Nitrogenous Fractions[1], Experiment Two

Parameter	Days							S.E.
	1	2	3	5	7	10	14	
Total Rumen Nitrogen								
Ration 1	515^{a}	531^{a}	582^{a}	639^{a}	633^{a}	646^{a}	580^{a}	22.7
Ration 2	615^{a}	724^{a}	734^{a}	758^{a}	752^{a}	821^{b}	828^{b}	22.7
Ration 3	610^{aA}	578^{aA}	608^{aA}	678^{aAB}	940^{bc}	910^{bBC}	868^{bBC}	22.7
Ration 4	590^{abAC}	572^{aAB}	473^{aA}	690^{bcAC}	765^{bcBC}	744^{bcBC}	831^{cC}	22.7
Soluble Rumen Nitrogen								
Ration 1	130^{a}	114^{a}	117^{a}	117^{a}	124^{a}	124^{a}	115^{a}	4.8
Ration 2	110^{aA}	153^{bAB}	176^{bB}	167^{bB}	159^{bAB}	164^{bB}	170^{bB}	4.8
Ration 3	138^{aA}	166^{abAB}	184^{bcABC}	183^{bcABC}	224^{dC}	210^{cdBC}	209^{cdBC}	4.8
Ration 4	136^{aA}	186^{bcABC}	157^{abAB}	210^{cBCD}	222^{cdCD}	239^{dD}	252^{dD}	4.8
Insoluble Rumen Nitrogen								
Ration 1	385^{a}	417^{a}	465^{a}	522^{a}	510^{a}	522^{a}	465^{a}	22.1
Ration 2	505^{aAC}	571^{aA}	559^{aAB}	591^{abAB}	593^{dD}	657^{dCD}	659^{bdBCD}	22.1
Ration 3	471^{abcAB}	412^{abAB}	424^{aA}	495^{abcAB}	716^{bcAB}	699^{bcAB}	659^{cB}	22.1
Ration 4	453	387	316	488	543	505	579	
Non Protein Nitrogen								
Ration 1	53^{a}	56^{a}	58^{a}	61^{a}	61^{a}	51^{a}	52^{a}	3.0
Ration 2	58^{a}	70^{ab}	83^{ab}	81^{ab}	85^{b}	72^{ab}	83^{ab}	3.0
Ration 3	62^{aA}	70^{abAB}	84^{abcABC}	91^{bcdABC}	114^{cdeC}	102^{cdeB}	116^{aC}	3.0
Ration 4	52^{aA}	86^{bcBC}	74^{abAB}	103^{cdBC}	112^{dCD}	135^{eDE}	148^{eE}	3.0

Table 13 (Continued . . .)

Parameter	Days							S.E.
	1	2	3	5	7	10	14	
Rumen Ammonia								
Ration 1	13^{aaA}	12^{abAB}	12^{abB}	11^{abB}	13^{abB}	11^{abB}	9^{abB}	2.9
Ration 2	12^{aaA}	33^{bbB}	48^{bcBC}	44^{bcBC}	45^{bdC}	44^{bcdC}	52^{bdC}	2.9
Ration 3	15^{aaA}	49^{bbBC}	70^{bcB}	70^{bcCD}	94^{cdCD}	91^{bdD}	94^{bdD}	2.9
Ration 4	18^{aaA}	69^{bbBC}	57^{bcB}	92^{cCD}	98^{cdCD}	119^{dD}	120^{dD}	2.9
Peptide and Amino Acid Nitrogen								
Ration 1	40^{aa}	44^{aa}	46^{aa}	50^{aa}	48^{aa}	41^{aa}	43^{aa}	2.3
Ration 2	47^{aB}	37^{aA}	35^{aA}	37^{aA}	40^{aA}	28^{aA}	31^{aA}	2.3
Ration 3	47^{bA}	21^{abA}	14^{abA}	21^{aaA}	20^{aA}	11^{abA}	22^{abA}	2.3
Ration 4	34^{bA}	17^{abA}	16^{abA}	11^{aaA}	14^{aaA}	16^{abA}	28^{abA}	2.3
Precipitable Nitrogen								
Ration 1	77^{aa}	58^{abAB}	59^{abB}	56^{abB}	63^{abAB}	73^{abB}	63^{abB}	3.1
Ration 2	51^{aaA}	83^{bbAB}	93^{bbB}	86^{bbB}	74^{bAB}	92^{bbB}	87^{bbB}	3.1
Ration 3	77^{aa}	96^{abab}	99^{abab}	92^{abab}	100^{bb}	109^{bb}	92^{abab}	3.1
Ration 4	85^{aa}	99^{aa}	83^{aa}	98^{aa}	110^{aa}	104^{aa}	104^{aa}	3.1

[1]Mg nitrogen per 100 ml rumen content.

Values with different superscripts in the same row are significantly different

^{a,b,c,d,e} $P<.05$; ^{A,B,C,D,E} $P<.01$

steers fed ration 1 ($P<.01$). Fourteen day patterns are presented in Table 13. There were no significant daily changes in rumen NPN of steers fed ration 1 throughout the 14 day period while that of steers fed rations 2 and 3 increased over the entire 14 day period but decreased slightly on day 10. Rumen NPN of steers fed ration 4 decreased on day 3, then increased linearly throughout the rest of the period and was closely related to the pattern of protein intake.

As presented in Table 11, rumen ammonia nitrogen was highest for steers fed ration 4 ($P<.01$) and lowest for steers fed ration 1 ($P<.01$). Steers fed ration 4 had significantly higher concentrations ($P<.05$) than those fed 1 and 2, and steers fed ration 3 higher ($P<.05$) than those fed rations 1 and 2, but differences between rations 3 and 4 were not significant. Fourteen day patterns are presented in Table 13. Differences in daily rumen ammonia nitrogen were not significant for steers fed rations 1 and 2. Rumen ammonia of steers fed rations 3 and 4 increased linearly ($P<.05$) throughout the 14 day period with a slight depression on day 3 for steers fed ration 4.

Rumen peptide and amino acid nitrogen (RPAAN) of steers fed the four rations are presented in Table 11. Steers fed ration 1 had higher RPAAN ($P<.05$) than those fed rations 3 and 4 but not significantly higher than those fed ration 2. Also, steers fed ration 2 had higher RPAAN

(P<.05) than those fed ration 4 but not significantly higher than steers fed ration 3. Fourteen day RPAAN patterns are presented in Table 13. Differences in daily RPAAN of steers fed rations 1 and 2 were not significant. Steers fed rations 3 and 4 had their highest RPAAN on day 1 (P<.01) with a significant depression (P<.05) on day 4 for steers fed ration 4.

Rumen tungstic acid precipitable nitrogen (RPN) of steers fed the four rations are presented in Table 11. RPN nitrogen was highest in steers fed ration 4 and lowest in steers fed ration 1 (P<.05) but differences for steers fed rations 2, 3 and 4 were not significant. Fourteen day patterns are presented in Table 13. Differences in daily RPN of steers fed rations 1 and 4 were not significant. However, the random significant differences noted in steers fed rations 2 and 3 were not part of any consistent pattern.

Rumen Volatile Fatty Acids

Rumen acetate concentrations of steers fed the four rations are presented in Table 11. Although rumen acetate was higher in steers fed rations 2 and 3, differences among treatments were not significant. Fourteen day patterns are presented in Table 14. There were no significant differences in daily rumen acetate of steers fed ration 1 but that of steers fed rations 2, 3 and 4 increased daily throughout the entire 14 day period with the exception of a significant depression on day 3 for steers fed ration 4.

Rumen propionate of steers fed the four rations are presented in Table 11. Steers fed ration 2 had higher ($P<.05$) rumen propionate than steers fed rations 3 or 4 but the differences between that of steers fed rations 2 and 1 were not significant. Fourteen day patterns are presented in Table 14. Differences in the daily ruminal propionate of steers fed ration 2 were not significant but steers fed ration 1 had their highest rumen propionate level on day 14 ($P<.05$). Rumen propionate of steers fed rations 3 and 4 decreased significantly on day 3 ($P<.05$) and then increased daily during the remainder of the period.

Rumen butyrate of steers fed the four rations are presented in Table 11. Steers fed ration 2 had the highest rumen butyrate ($P<.05$) but differences in concentrations for steers fed the other 3 rations were not significant. The fourteen day patterns are presented in Table 14. Differences in daily rumen butyrate of steers fed rations 1 and 2 were not significant. Rumen butyrate in steers fed rations 3 and 4 decreased slightly on days 2 and 3, but increased linearly ($P<.05$) throughout the remainder of the period.

Rumen isovalerate (Table 11) was higher ($P<.05$) in steers fed ration 4 than steers fed rations 1 and 2, but was not significantly different from steers fed ration 3. Rumen isovalerate of steers fed ration 3 was higher than that of steers fed ration 1 ($P<.05$) but not significantly higher than that of steers fed ration 2. Differences in concentrations

TABLE 14

Average Daily Rumen Volatile Fatty Acid Concentrations[1], Experiment Two

Parameter	Days							S.E.
	1	2	3	5	7	10	14	
Rumen Acetate								
Ration 1	50^{a}	52^{a}	53^{a}	49^{a}	49^{a}	42^{a}	49^{a}	2.0
Ration 2	54^{aA}	64^{ab}	67^{ab}	66^{bcAB}	68^{cB}	70^{b}	72^{b}	2.0
Ration 3	50^{ab}	48^{aA}	57^{abA}	68^{abA}	79^{bc}	78^{cB}	79^{cB}	2.0
Ration 4	55^{ab}	48^{ab}	42^{a}	54^{ab}	63^{bc}	64^{bc}	68^{c}	2.0
Rumen Propionate								
Ration 1	28^{a}	29^{a}	34^{ab}	32^{ab}	33^{ab}	33^{ab}	48^{b}	1.9
Ration 2	40^{acA}	43^{abA}	44^{aA}	45^{abA}	43^{cA}	41^{bcA}	45^{cA}	1.9
Ration 3	32^{bB}	24^{abAB}	21^{aA}	23^{abAB}	42^{aAB}	38^{aAB}	43^{abAB}	1.9
Ration 4	36^{bB}	23^{abAB}	14^{aA}	15^{abAB}	19^{aAB}	20^{aAB}	29^{abAB}	1.9
Rumen Butyrate								
Ration 1	15^{a}	16^{a}	15^{a}	13^{a}	17^{a}	13^{a}	16^{a}	0.8
Ration 2	17^{aAB}	20^{aA}	21^{aA}	21^{aAB}	20^{bC}	21^{bBC}	24^{bC}	0.8
Ration 3	12^{abcABC}	10^{abAB}	10^{aA}	13^{abAB}	22^{abcABC}	20^{cdBC}	22^{dC}	0.8
Ration 4	12^{abcABC}	9^{abAB}	8^{aA}	10^{abAB}	15^{abcABC}	17^{cdBC}	21^{dC}	0.8
Rumen Isovalerate								
Ration 1	4.1^{a}	4.5^{a}	3.5^{a}	2.9^{a}	3.4^{a}	2.0^{a}	1.9^{a}	0.3
Ration 2	3.2^{a}	4.0^{a}	5.0^{a}	3.7^{a}	4.3^{a}	4.0^{a}	4.0^{a}	0.3
Ration 3	4.5^{a}	5.0^{a}	5.5^{a}	5.2^{a}	6.5^{a}	6.0^{a}	6.6^{a}	0.3
Ration 4	3.5^{a}	6.6^{a}	5.4^{a}	7.3^{a}	7.0^{a}	8.1^{a}	6.9^{a}	0.3

Table 14 (Continued . . .)

Parameter	Days							
	1	2	3	5	7	10	14	S.E.
Rumen Valerate								
Ration 1	3.2^{a}	3.1^{a}	3.6^{a}	3.0^{a}	3.6^{a}	2.7^{a}	3.3^{a}	0.3
Ration 2	4.1^{a}	3.8^{a}	4.7^{a}	4.0^{a}	3.6^{a}	4.8^{a}	5.0^{a}	0.3
Ration 3	4.9^{a}	3.5^{a}	3.3^{a}	3.2^{a}	5.1^{a}	5.5^{a}	5.6^{a}	0.3
Ration 4	4.5^{a}	3.7^{a}	2.8^{a}	3.3^{a}	3.4^{a}	4.4^{a}	5.1^{a}	0.3

[1]Micromoles of VFA per ml of rumen liquor

Values with different superscript in the same row are significantly different

^{a,b,c} $P<.05$; ^{A,B,C} $P<.01$

between steers fed ration 1 and ration 2 were not significant. Fourteen day patterns for steers fed the 4 rations are presented in Table 14. Daily differences in concentrations for steers fed each ration were not significant.

Rumen valerate of steers fed the four rations are presented in Table 11. Steers fed ration 3 had higher rumen valerate ($P<.05$) than steers fed ration 1 but differences between steers fed the other rations were not significant. The fourteen day patterns for steers fed the four rations are presented in Table 14. Daily differences in concentrations of steers fed each ration were not significant.

Plasma Parameters

Plasma urea nitrogen (PUN) of steers fed the four rations are presented in Table 11. Steers fed ration 4 had higher PUN ($P<.05$) than those fed rations 1 and 2 but not significantly higher ($P<.05$) than steers fed ration 3. Steers fed ration 3 had higher PUN ($P<.05$) than steers fed rations 1 and 2 but differences between steers fed rations 1 and 2 were not significant. The fourteen day patterns of PUN are presented in Table 15 and Figure 9. There were no significant differences in the daily PUN of steers fed ration 1. PUN of steers fed rations 2 and 3 increased significantly ($P<.01$) on day 3 and then remained constant during the remainder of the 14 day period; whereas that of

TABLE 15

Plasma Urea Nitrogen and Amino Acid Concentrations, Experiment Two

Parameter	Days							S.E.
	1	2	3	5	7	10	14	
Plasma Urea Nitrogen[1]								
Ration 1	9^{aA}	7^{bB}	7^{cB}	6^{bcB}	7^{bcB}	7^{cB}	7^{cB}	0.5
Ration 2	6^{A}	13^{B}	17^{C}	16^{BC}	16^{C}	17^{C}	18^{C}	0.5
Ration 3	6^{aA}	20^{bBC}	19^{bB}	24^{cCD}	24^{cCD}	27^{cC}	27^{cD}	0.5
Ration 4	7^{aA}	18^{bBC}	25^{cC}	21^{cCD}	23^{cC}	24^{cC}	23^{cD}	0.5
Total EAA[2]								
Ration 1	0.85^{aA}	0.74^{aA}	0.75^{aAB}	0.99^{aAB}	0.79^{aAB}	1.00^{aAB}	1.04^{bB}	0.05
Ration 2	0.81^{a}	0.97^{a}	1.07^{a}	1.14^{a}	1.04^{a}	1.09^{a}	1.54^{b}	0.05
Ration 3	0.90^{a}	0.84^{a}	0.89^{ab}	0.76^{ab}	0.93^{ab}	1.16^{a}	0.99^{b}	0.05
Ration 4	0.77^{a}	0.77^{a}	0.91^{ab}	0.91^{ab}	1.04^{ab}	0.80^{a}	1.23^{b}	0.05
Total NEAA[2]								
Ration 1	1.06^{a}	1.01^{a}	1.15^{a}	1.02^{a}	1.05^{a}	1.00^{a}	1.22^{a}	0.03
Ration 2	1.08^{bB}	0.97^{abAB}	1.11^{abAB}	1.09^{aA}	0.91^{aAB}	0.95^{aA}	1.06^{abAB}	0.03
Ration 3	1.12^{bA}	0.89^{abA}	0.88^{abA}	0.72^{abA}	0.78^{abA}	0.70^{aA}	0.86^{abA}	0.03
Ration 4	1.12^{bA}	0.89^{abA}	1.08^{abA}	0.98^{abA}	0.88^{abA}	0.79^{aA}	0.87^{abA}	0.03
EAA/NEAA[3]								
Ration 1	0.83^{aA}	0.75^{aAB}	0.67^{aAB}	0.95^{aAB}	0.76^{abAB}	1.07^{abAB}	0.91^{bB}	0.06
Ration 2	0.76^{A}	1.01^{A}	0.95^{A}	1.02^{A}	1.15^{A}	1.19^{B}	1.54^{B}	0.06
Ration 3	0.80^{aA}	0.96^{aAB}	1.01^{aAB}	1.05^{abAB}	1.21^{abAB}	1.89^{abAB}	1.14^{bB}	0.06
Ration 4	0.71^{aA}	0.88^{aAB}	0.86^{aAB}	0.99^{abAB}	1.17^{abAB}	1.00^{abAB}	1.38^{bB}	0.06

[1]Mg nitrogen per 100 ml blood plasma

[2]Micromoles per ml plasma

[3]Essential to nonessential amino acid ratio

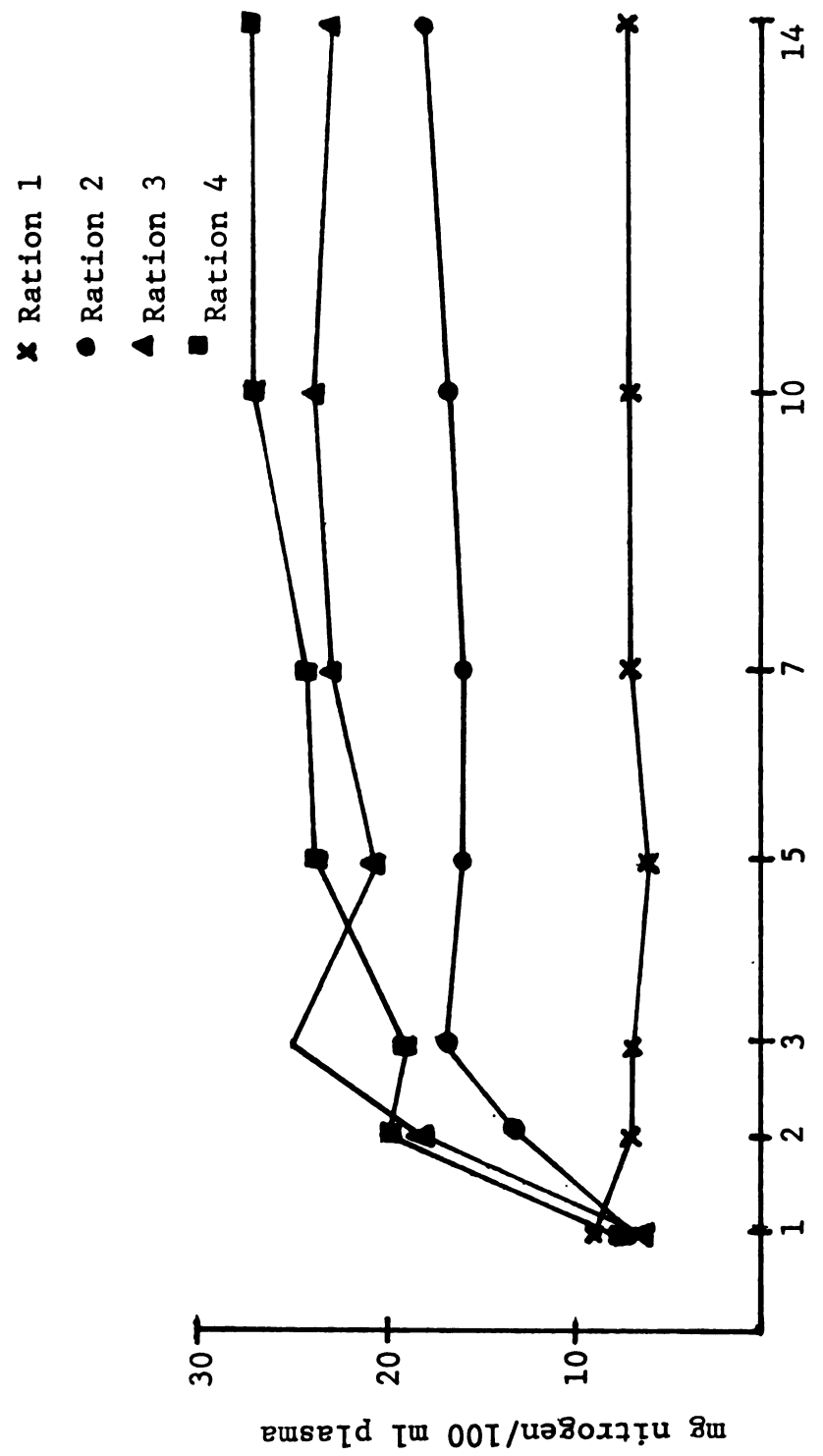

FIGURE 9. Fourteen day patterns of plasma urea nitrogen concentrations

steers fed ration 4 increased significantly ($P<.01$) throughout the period.

Total plasma essential amino acid concentrations (TEAA) of steers fed the four rations are presented in Table 11. Differences in TEAA of steers fed the four rations were not significant. The fourteen day patterns are presented in Table 15 and Figure 10. There were no significant differences in daily TEAA of steers fed ration 1 or ration 3, but concentrations in steers fed ration 2 increased throughout the 14 day period with a significant difference ($P<.05$) only between days 1 and 14. There was no consistent pattern for steers fed ration 4.

Total plasma nonessential amino acid concentrations (TNEAA) of steers fed the four rations are presented in Table 11. Differences in TNEAA of steers fed the four rations were not significant whereas TNEAA of steers fed rations 3 and 4 were highest on day 1 ($P<.05$) but decreased gradually during the remainder of the period.

The plasma essential amino acid nonessential amino acid (E/N) ratios are presented in Table 11. Steers fed ration 2 had a significantly higher ($P<.05$) plasma E/N ratio than steers fed ration 1, but differences in the ratios of steers fed ration 2 and rations 3 and 4 were not significant. The fourteen day patterns are presented in Table 15. Daily differences in the plasma E/N ratios of steers fed ration 1 were not significant. Daily E/N ratios

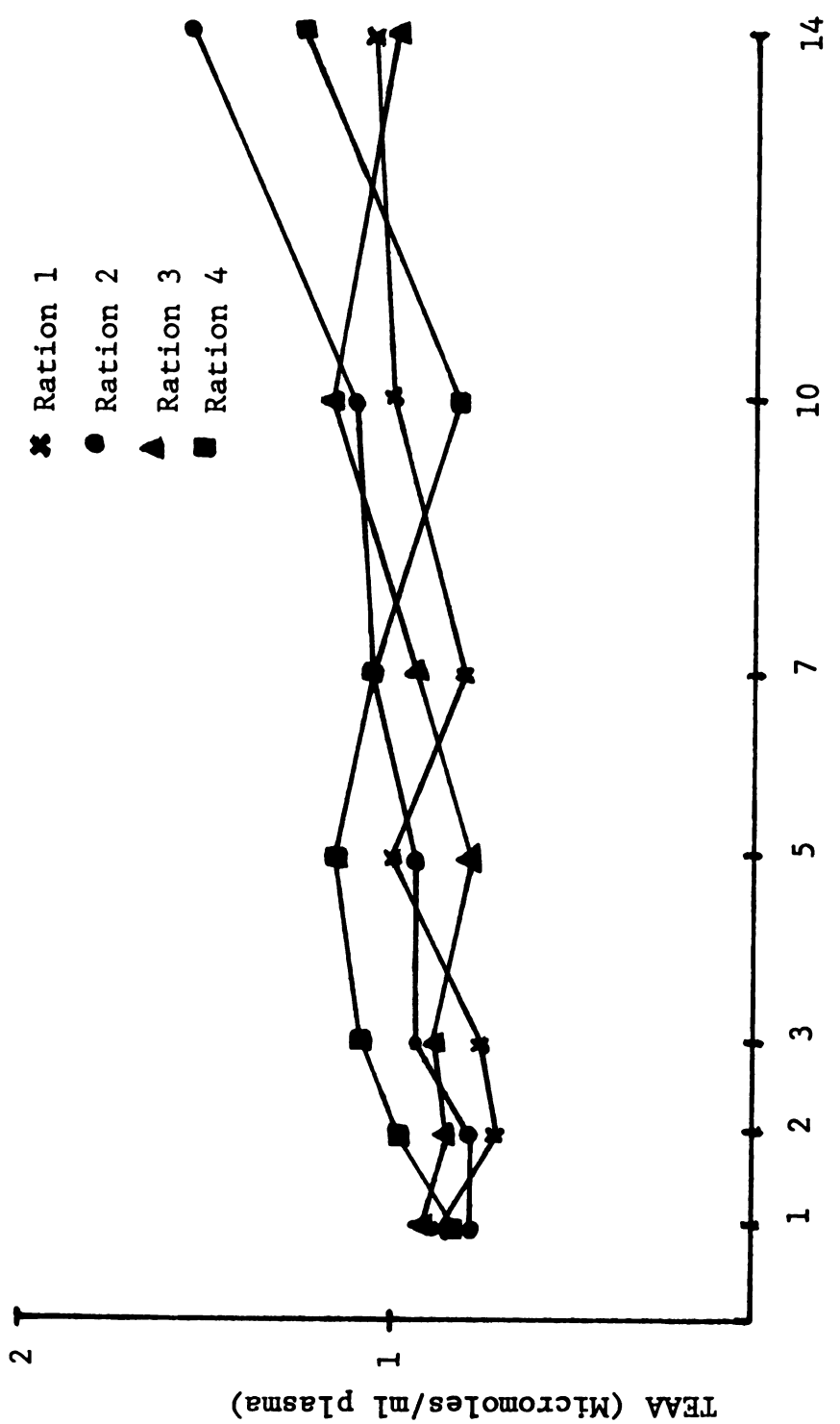

FIGURE 10. Fourteen day patterns of plasma TEAA concentrations.

in steers fed rations 2, 3 and 4 increased throughout the period with their highest values (with the exception of steers fed ration 3) appearing on day 14.

DISCUSSION

Effect of Excess Dietary C rude Protein on Voluntary Feed Intake and Various Ruminal and Plasma Parameters, Experiment Two

The present study was conducted with the basic premise that the large quantity of ammonia produced in the rumen by microbial degradation of excess protein would exert a feedback saturation effect on the proteolytic and deaminating enzymes, so that a greater amount of undergraded protein and amino acids would be reaching the lower gut. Chalmers and Hughes (1969) and Emery (1971) showed that when a large quantity of a single amino acid was administered to the rumen, the rate of deamination of that amino acid was substantially reduced. Thus, a greater quantity of the amino acid was absorbed from the small intestine as evidenced by the increased plasma level (Chalmers and Hughes, 1969). The results of this study (Table 11) demonstrated that there was no apparent upper limit to ruminal proteolysis and deamination.

It was observed that steers fed the high protein rations (3 and 4) were nibblers (eating a very small quantity of feed at any one time throughout the day) while steers fed the low protein rations (1 and 2) were meal

eaters. Such an irregular pattern of feed consumption of steers fed rations 3 and 4 completely nullified the expected effects. This problem along with an apparent dilution of rumen volume by an observed increased water intake in steers fed high protein rations might have caused the failure to demonstrate an upper limit in ruminal proteolysis and deamination in steers fed the high protein. That is, ruminal amino acid and ammonia concentrations might not have been high enough to impose a feedback saturation effect on the proteolytic and deaminating enzymes.

The overall average daily voluntary feed intake (although not statistically significant) decreased as the protein content of the ration was increased (Table 11). Protein intake, on the other hand, was highest for the high protein rations. An explanation of this is that the high protein content of rations 3 and 4 more than compensated for the decrease in total feed intake. The non significant decrease in feed and protein intakes in steers fed ration 2 on day 2 and the significant decrease in these two parameters in steers fed rations 3 and 4 on day 3 require careful consideration. Palatability is generally one of the contributing factors in intake phenomenon. However, since intake of the high crude protein rations was high on day 1 it is not likely that palatability exerted any significant influence on feed intake in this study. Therefore, some feedback mechanisms might have triggered the depression in

feed intakes on day 3. It is possible that high protein, high ruminal ammonia and high blood urea concentrations inhibited enzyme systems of the rumen or the liver; or they might have stimulated the satiety center of the brain. Russek (1971) suggested that the presence of hepatic receptors which monitor glucose and ammonia levels might be major contributory factors in the reduced voluntary feed intake of high protein rations. Peng et al. (1974), working with rats, explained control of voluntary food intake by protein level as shifts in tissue free amino acid equilibrium towards anabolism when the rats ate low protein diet and towards catabolism when the high protein diet was consumed. Almquist (1954) working with chickens, Mellenkoff (1957) working with humans, Harper et al. (1964) and Harper (1965) working with rats all suggested that voluntary feed intake is monitored by plasma amino acid concentrations. This is not a completely valid explanation for the results of the present study since differences in plasma essential or non-essential amino acid concentrations of steers fed the four rations were not significant. However, differences in amino acid passage to the small intestine were not assessed. It is therefore difficult to pinpoint an aminostatic control that was monitoring the intake phenomenon in this study.

Although total protein intake was significantly higher for the high protein rations, differences in total rumen nitrogen of steers fed the four rations were not

significant. The most logical explanation of this leveling off effect of total ruminal nitrogen is that there was an extensive loss of rumen nitrogen in steers fed rations 3 and 4 due to rapid proteolysis and deamination. This is evidenced by the significantly higher rumen ammonia and plasma urea nitrogen levels in steers fed rations 3 and 4. The fourteen day patterns of daily total rumen nitrogen were similar to those of the daily protein intake. The decrease in total rumen nitrogen of steers fed rations 3 and 4 on day 3 was in response to the decrease in feed and protein intake.

The increase in soluble rumen nitrogen as the level of dietary protein increased in rations 3 and 4 is an indication of the high solubility of the isolated soy protein of these two rations as compared with the high concentration of corn protein in rations 1 and 2. Since rumen ammonia indicate the extent of ruminal protein degradation, and the extent of ruminal protein degradation depends on its solubility in rumen fluid (el-Shazly, 1958; Blackburn and Hobson, 1960; Hendrickx and Martin, 1963), the higher rumen ammonia of steers fed rations 3 and 4 was a function of both the high level of protein and the high solubility of the isolated soy protein. The higher rumen ammonia in the steers fed the high protein rations confirmed the findings of Lewis (1957), Tagari et al. (1964), Preston et al. (1965), Cocimano and Leng (1966) and McIntyre (1970) who

reported a high positive correlation between protein intake and rumen ammonia nitrogen. RPAAN decreased as the crude protein content of the ration increased. Since rations 1 and 2 contained a large proportion of corn and since zein is not extensively degraded by ruminal proteolysis (McDonald 1954), it is possible that a fair amount of the zein might have undergone partial proteolysis resulting in a greater quantity of peptides and small molecular weight compounds which increased the quantity of RPAAN fraction of steers fed rations 1 and 2. The higher RPN of steers fed the high protein rations reflects the high quantity of undegraded soluble soy protein of these rations. According to Hume (1974) only 60% of soy protein is degraded in the rumen.

The significant increase in ruminal isovaleric concentration with increased dietary protein can be explained as the result of the larger proportion of branched chain carbon skeletons resulting from the deamination of the higher level of branched chain amino acids found in the isolated soy protein of rations 3 and 4 (Ørskov and Oltjen, 1967).

The significant increase in PUN with increased dietary protein is in agreement with the findings of Lewis (1957); Tagari et al. (1964); Preston et al. (1965) and Fenderson (1972) who observed a significant positive correlation between dietary protein, rumen ammonia and PUN concentrations.

It can be concluded from this study that a control mechanism was monitoring the depression of feed intake in steers fed rations 3 and 4 on days 2 and 3. However, since daily feed consumption in steers fed these two rations was most irregular, no obvious mechanism was evident. The results also imply that it is not economical to feed high protein rations to growing steers unless ruminal proteolysis and deamination can be controlled.

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