This is to certify that the

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PLASMA AMINO ACID RESPONSE TO
INTRAPERITONEAL METHIONINE
LYSINE AND CYSTEINE INJECTIONS
IN HOLSTEIN STEERS

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ROBERTO TOWNS

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PLASMA AMINO ACID RESPONSE TO INTRAPERITONEAL METHIONINE, LYSINE AND CYSTEINE INJECTIONS IN HOLSTEIN STEERS

By

Roberto Towns

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ABSTRACT

PLASMA AMINO ACID RESPONSE TO
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Plasma amino acid (PAA) responses to IP amino acid injections in steers were studied in two experiments. In one experiment 20 g L-lys or 20 g L-met were injected for 14 days. In the met treatment, plasma met peaked by day eight and tended to accumulate. Plasma lys was not affected by met injections. In the lys treatment, plasma lys peaked at about day 8 and did not accumulate. Plasma met was not affected by lys injections.

In the second experiment, sub and supra optimal levels of met and met plus cysteine were injected IP to evaluate the met requirement. PAA 2 phase response curves were evaluated using a two slope regression procedure. Two phase breakpoints occurred at an injection level of about 3.7 g met/day. Breakpoint values plus met in the abomasal flow indicated a met. requirement of 11.41 g/day. A met sparing effect upon administration of cysteine was not observed.

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INTRODUCTION

The capacity of ruminants to utilize cellulose and non protein nitrogen has given them a unique place as a food producing animal. The action of the rumen ecosystem on dietary protein has become an area of intensive study. It is evident that the extensive degration and resynthesis of protein by the rumen microbiota determine the amount and composition of the amino acids reaching the intestine. Hence, the study of amino acid requirements cannot be carried out by merely changing dietary amino acid levels, but has to be done in a way that avoids the action of the rumen ecosystem.

The first experiment of the present work was an attempt to determine the metabolic response of steers to sustained high levels of amino acid administration. The response was measured through changes in plasma amino acid levels with daily intraperitoneal injections of L-methionine and L-lysine.

The second experiment sought to evaluate and quantitate the methionine requirement of growing steers through changes in the plasma amino acid levels in response to intraperitoneal injections of graded levels of methionine alone and methionine plus cysteine.

The results of this work should aid in understanding the amino acid metabolism of the ruminant. Nevertheless, it is hoped that the questions this study answers and the interest it might generate will constitute a meaningful contribution in the area of ruminant nutrition.

LITERATURE REVIEW

A. Protein Metabolism in the Ruminant

Nitrogen Metabolism in the Ruminant

The capacity of ruminants to utilize protein and non-protein nitrogen is now a well recognized feature. Loosli et al (1949) showed that rumen microbes are able to synthesize all amino acids. Dietary proteins are broken down into peptides, amino acids and ammonia according to their degradability which in turn is largely determined by the rumen solubility of the protein, although the morphological characteristics, rate of passage and interactions among the diverse feedstuffs in the diet also influence the degree to which the protein is degraded. (Bull et al, 1977; Satter et al, 1977).

Protein entering the abomasum is thus composed of the undegraded dietary protein that by passed rumen and the microbial protein synthesized by the microbiota (Satter, 1977). The amount of microbial protein produced is regulated by a series of factors; the most important of which is the capacity of rumen microbes to utilize ammonia and carbon skeletons for amino acid synthesis (McDonald, 1948; Loosli et al,

1949). There is however, a limit in the capacity of the microbes to utilize the ammonia for protein synthesis, Roeffler and Satter (1975) have calculated that ammonia in excess of a concentration of 5 mg/dl of rumen fluid, is not utilized for ruminal protein synthesis, although this limit may be regulated by factors such as the type and availability of dietary carbohydrates.

Excess ammonia is absorbed across the rumen epithelium and is transported to the liver where it is transformed into urea, which is partly recycled to the rumen through salivary secretions (McDonald, 1948).

The factors influencing and limiting the growth of rumen microorganisms have been reviewed by Bergen and Yokoyama (1977). The fermentation of carbohydrates into volatile fatty acids in the rumen is the main source of energy. Microbial growth depends on energy metabolism and is related to substrate disappearance, thus it is possible to evaluate the potential for cell production from the amount of ATP generated in the fermentative pathways. The relationship between cell growth and energy production, expressed as growth yield per mole of ATP is known as Y_{ATP}. Early work produced a consensus that the Y_{ATP} was constant for the different rumen microorganisms. A Y_{ATP} of 10.5 was accepted (Bauchop and Eldsen, 1960); however, more recent studies,

taking into account other aspects of rumen psysiology such as dilution rate, additional pathways for ATP generation (i.e. cytochrome linked electron transfer) and the shifts in microbial populations occupying a specific metabolic niche have calculated Y_{ATP} values ranging from 7 to 25, the main factor influencing the fluctuation in values appears to be the specific growth rates (or dilution rates of the microbes).

Sulfer: Nitrogen Relationship in the Rumen

The amount of sulfur (S) in the rumen is a limiting factor for protein synthesis (Moir et al, 1967; Gil et al, 1973; Kennedy et al, 1975). In order to be incorporated into microbial proteins, sulfur must first be reduced to sulfide (Bray and Till, 1975). Rumen microorganisms have a sulfur: nitrogen ratio of approximately 1:15 and optional microbial growth occurs when the dietary proportion is about 1:10 (Moir et al, 1967). The source of sulfur can influence the growth of rumen microbes. Gil et al (1973) working with a culture of rumen microorganisms fermenting glucose and with urea as the sole protein source, found that the addition of methionine hydroxy analog (MHA) elicited an increase of 2.5 times in the logarithmic growth rate of the culture. The addition of other sulfur amino acids also had a stimulant effect but when inorganic sulfur or non sulfur amino acids were added,

the growth rate was not stimulated. It was concluded that sulfur amino acids are easily interconverted and that the incorporation of inorganic sulfur into protein is a rate limiting process. High sulfur may also influence microbial protein production through reducing the availability of hydrogen for the growth of methanogens (Bryant et al, 1977).

Postruminal Fate of Nitrogenous Compounds

Bergen (1978) has reviewed postruminal nitrogen metabolism. Microbial protein, together with dietary protein that escaped ruminal degradation and some free amino acids pass through the omasum and abomasum into the small intestine. The proportion of dietary nitrogen reaching the intestine varies with nitrogen in the ration. When high levels of soluble protein are fed, nitrogen entering the abomasum might be less than the dietary intake. On the other hand, if the ration is low in protein and high in energy, the amount of nitrogen reaching the small intestine might be greater than dietary intake (Clarke et al, 1966). Weller et al (1971) fed sheep a ration containing 8-10% CP and found no net dietary nitrogen loss. These results are in agreement with those of Fenderson and Bergen (1975) who fed a 9.5% CP ration to growing steers and reported a ruminal nitrogen loss of only 2 percent.

Post ruminal enzymatic activity resembles that found in non-ruminants although the neutralization of

the ingesta in transit is slower in ruminants and the highest proteolytic activity takes place in mid jejunum (Bergen, 1978). The digestibility of nitrogenous compounds ranges from 65 to 85% with nucleic acids being among the more digestible compounds. Hogan (1973) reported a digestibility coefficient of 70% for bulk protein in the small intestine of ruminants. Absorptive processes are also similar to those of non-ruminants, but the highest rate of absorption takes place in the posterior part of the ileum (Ben Ghendalia et al, 1974).

According to their absorptive pathways, amino acids can compete for absorption. Hume et al (1972) working with sheep, showed an inhibitory effect of leucine on lysine. Johns and Bergen (1973) confirmed this finding in vitro working with sheep tissue.

Rumen Microbial Protein

Depending on the degradability of dietary protein and the limitations in energy, nitrogen and sulfur; microbial protein can provide a large proportion of the protein requirement of the ruminant. Buchholtz and Bergen (1973) measured microbial protein synthesis as a function of microbial phopholipid synthesis and found that for a 4 liter rumen, the rate of true protein synthesis was 16.1 g Protein/100 g of organic matter digested, enough to meet the needs of growing calves and lambs.

Rumen microbial protein has a high biological value (Bergen et al, 1967; Bergen et al, 1968), with protozoa providing the protein of the highest quality (Bergen et al, 1968). The rumen ecosystem provides a buffering effect in regard to protein quality and essential amino acid composition. Specially in the microbial protein since the protein quality and bulk essential amino acid make up of rumen bacteria and protozoa tend to remain constant regardless of level or source of dietary nitrogen (Bergen et al, 1968; Fenderson and Bergen, 1972; Williams and Dinusson, 1973).

There is however, a dietary effect upon several features of the rumen ecosystem. Protein free diets where all the nitrogen is supplied as nonprotein nitrogen (NPN), have a depressing effect on the protozoal populations. Oltjen and Putnam (1966) studied the effect of NPN or performed protein with purified rations on the rumen microbial population of steers. They found that purified diets containing NPN, reduced the protozoa numbers and nitrogen retention. Plasma levels of branched chain amino acids (val, leu and ile) were also lower in these steers. The rumen contents contained almost no branched chain volatile fatty acids (isobutyrate, isovalerate and valerate) to serve as precursors for branched chain amino acid synthesis. Under such conditions microbial protein synthesis is depressed resulting

in lower plasma levels of branched chain amino acids (Bergen et al, 1973).

Oltjen et al (1971) studied the effects of the addition of branched chain volatile fatty acids in steers fed urea or soy protein as protein source and found that steers feed urea had lower plasma levels of valine, isoleucine, leucine and phenylalanime than the steers fed soy protein. The addition of branched chain volatile fatty acids improved nitrogen retention and raised plasma branched chain amino acid levels; however, numbers of rumen protozoa and viable celluloytic bacteria were not altered.

Klopfenstein et al (1966) assessed the role of faunation on rumen metabolism in sheep and reported that faunation improved dry matter digestion, reduced viable bacteria numbers and increased rumen ammonia but not blood urea. These workers concluded that dietary nitrogen was digested more readily and utilized more efficiently by faunated sheep.

Although the bulk amino acid composition of the microbiota is not influenced by crude protein intake or source, there can be differences in protein quality and amino acid availability within the diverse rumen microbes. Bergen et al (1967) found significant differences in the protein quality of 22 different strains of rumen bacteria whose amino acid compositions were similar, and suggested

that the nitrogen status of the ruminant may be influenced by alterations in the rumen bacterial population. In a later work with rats, Bergen et al (1968) showed that histidine was first limiting in protozoal protein and sulfur amino acids were limiting for bacterial protein. These results contrast with those of Klopfenstein et al (1966) who, using the "energy induced plasma amino acid technique" in vivo, reported that lysine was first limiting in defaunated sheep, although there was a high proportion of corn in the diet, whose lysine content is usually low.

The enzymatic release pattern of amino acids from microbial protein in the small intestine may be influenced by the dietary protein source. Burris et al (1974) found variations with the source of dietary protein in the enzymatic release pattern of threonine, valine, methionine, phenylalanime and lysine in microbial preparations isolated from the rumen of steers. This may be of physiological importance since the amino acids present at a given absorption site may affect rate of uptake. Competitive interaction during absorption between amino acids of the same transport class, has been studied both in vivo (Hume et al, 1972) and in vitro (Bergen and Johns, 1973). After assessing the diverse factors affecting protein digestion and the length of the intestinal tract, Johns and Bergen (1973) concluded that competitive

inhibition of amino acid uptake is not likely to occur *in vivo* and suggested an unlimited absorptive capacity throughout the intestinal tract.

B. Plasma Amino Acids

Plasma amino acids (PAA) represent a small fraction of the total amino acids of the body. The plasma amino acid pool is a product of the dynamics of amino acid metabolism, affected by amino acids absorbed across the gastrointestinal wall, by those catabolized from tissues and by amino acid synthesis. Plasma amino acids are readily renewed since the daily inflow of dietary amino acids is much larger than the plasma pool. Removal of amino acids from the plasma pool occurs as a result of protein synthesis and amino acid catabolism. The responsiveness of the plasma pool to the dynamics of amino acid metabolism, as well as its dependence on dietary amino acid supply, have made it a basic instrument in the study of amino acid nutrition.

The evaluation of amino acid nutrition through PAA is a well established technique. Longenecker and Hause (1959) working with dogs, studied the relationship of postprandrial changes in PAA to evaluate the amino acid composition of different protein sources. In a later work, Zimmerman and Scott (1965) measured the amino acid requirements of chickens according to changes in the PAA levels. The chickens were fed increments of the first

limiting amino acids both below and above the supposed requirement. When the dietary amino acid intakes were below the requirement, plasma concentrations of that essential amino acid remained unchanged, but once the requirement was met, the plasma concentration started to rise linearly with each dietary increment of that amino acid. Zimmerman and Scott (1965) indicated that this break point at which the PAA level started to increase, coincided closely with the dietary level of the essential amino acid in question above which dietary increments would no longer produce improvements in growth rate.

The break point method was also implemented by Mitchell et al (1968) who measured the requirements of four essential amino acids in pigs according to the plasma amino acid concentrations and nitrogen retention. Mitchell et al (1968) concluded that changes in plasma amino acid concentration offered a more accurate parameter for the evaluation of amino acid requirements than nitrogen retention. These workers further indicated that there must be a period of adaptation to a diet deficient in an essential amino acid (below requirement) before increases of the limiting amino acid can produce a clear break point in the PAA concentrations.

Plasma Amino Acids in Ruminants

The rumen ecosystem significantly changes dietary protein and it is necessary to take into account these modifications in order to evaluate amino acid needs in ruminants. Bergen et al (1973) have postulated a unifying hypothesis on the effect of dietary nitrogen source and level of PAA in ruminants and suggested that changes in PAA patterns can be best explained by the quantity of protein reaching the intestine of the ruminant. This hypothesis by Bergen et al (1973) assumes that amino acid absorption across the rumen epithelium (Leibholz, 1969) is of minor quantitative importance.

The behavior of PAA is influenced by a number of factors in addition to protein. Source and level of energy have been shown to induce a decrease in the plasma amino acid concentrations in ruminants (Potter et al, 1968; Reilly and Ford, 1971; Fenderson and Bergen, 1972; Eskeland et al, 1974).

The effect of several energy sources on the PAA of sheep was studied by Potter et al (1968). Glucose, propionate, acetate and butyrate decreased PAA levels. The sharpest decrease was caused by glucose, followed in order of efficiency by propionate, acetate and butyrate. The above results on energy induced PAA depression were confirmed by Eskeland et al (1974) who also found that glucose was more effective than propionate, acetate and butyrate in depressing PAA concentrations.

Energy induced PAA depressions may be a reflection of the close relationship between protein and energy metabolisms, very probably at the hormonal level. PAA are known to contribute significantly to gluconeogenesis. Reilly and Ford (1971), using labelled amino acids in sheep, found that 28% of the glucose was derived from amino acids. The stimulatory effect of amino acids on insulin secretion is well known (McAtee and Trenkle, 1971); Tae et al, 1974), as is the capacity of insulin to increase amino acid uptake by muscle cells (Munro, 1964; Wool, 1965; Bergen, 1978). These facts suggest that the energy induced depression on PAA levels arises from increased tissue uptake of amino acids due to stimulation of insulin secretion. The theory is further substantiated by the fact that when fat is used as source of energy, uptake of amino acids by tissue is not affected, probably due to lack of stimulus for insulin secretion (Munro, 1964).

Starvation and low nitrogen intakes also affect the concentration of PAA in ruminants. Leibholz (1970) studied the effects of these factors in sheep and found that starvation for a period of 12 to 20 days resulted in a decrease in the concentration of serine, glutamine, glycine, alamine, histidine and arginine, while the levels of lysine, 3-methylhistidine and isoleucine increased markedly. The ratio of essential to nonessential

amino acids increased from .35 to .56 in the starved group. When sheep were fed a low nitrogen diet, the ratio decreased from .40 to .27. Leibholz (1970) suggested that starvation elicited the utilization of non-essential amino acids for energy while a low nitrogen intake favored the utilization of essential amino acids for energy.

Several relationships have been found among the amino acids in plasma. Zimmerman and Scott (1965) found that lysine tended to accumulate when arginine was deficient in the diet, and that threonine was affected by large excesses or deficiencies of lysine, hence it was suggested that a reduction in the plasma levels of threonine does not necessarily mean limiting status. Snyderman and Holt (1967) reported negative relationship between high levels of leucine and the plasma concentrations of valine, isoleucine, threonine and tyrosine. In a number of experiments Oltjen and Lehmann, 1968; Oltjen *et al*, 1970; Leibholz, 1970) glycine and serine have been associated with low plasma essential amino acids and poor nitrogen utilization. This tendency suggests that glycine and serine have an intrinsic negative effect on nitrogen utilization.

The relationship between all sulfur containing amino acids (SAA) is well known. Methionine can be used to synthesize cysteine through the transulfuration pathway

(Radcliffe and Egan, 1978) or can also be oxidated for cystine production (Stipanuk and Benevenga, 1977). Although methionine can fulfill the SAA dietary requirement by itself, part of its requirement can be supplied by both cysteine and cystine (Block et al, 1969; National Research Council, 1973; Stipanuk and Benevenga, 1977). The National Research Council (1973) summaries suggest that cystine can supply 50 to 70% of the SAA dietary requirement for normal growth in pigs. Although the proportion may vary between species, methionine must be present, Byington and Howe (1972) found in chicks that a methionine: cystine ratio of 70M:30C was superior to 30M:70C. These results concur with the observations of Featherson and Rogler (1978) who also found an antagonistic effect of cystine on methionine in chicks when the dietary level of methionine is suboptimal.

Another common feature of SAA is their relatively high toxicity; methionine has been found to be the most toxic nutritionally important amino acid (Sauberlich, 1961, Benevenga, 1974). Methionine can be toxic at levels of only four times its requirements (Benevenga, 1974). Symptoms are varied but are usually reflected in growth depression and tissue damage to organs with high metabolic rates (Benevenga, 1974; Benevenga et al, 1976), the toxicity appears to be caused by aberrations in the metabolism of the methyl group and its conversion to CO_2 (Benevenga, 1974; Benevenga, 1976).

C. Amino Acid Requirements of Ruminants

Qualitative Studies

Due to the action of the rumen ecosystem, it has not been possible to establish a dietary amino acid requirement for ruminants; however, the type and amount of amino acids required at the absorption sites for optimal nitrogen utilization, have been studied.

Techniques such as, plasma amino acid response curves (Brookes et al, 1973; Reis et al, 1973; Tao et al, 1974; Broderick and Satter, 1974; Fenderson and Bergen, 1975; Tao et al, 1974; Williams and Smith, 1975; Foldager et al, 1977), nitrogen balance studies (Nimrick et al, 1970a; Nimrick et al, 1970b; Fenderson and Bergen, 1975; Hall et al, 1974; Tao et al, 1974; Richardson and Hatfield, 1978), plasma urea nitrogen (PUN) response curves (Tae et al, 1974; Williams and Smith, 1975), urinary nitrogen (Tae et al, 1974; Richardson and Hatfield, 1978) and amino acid oxidation levels (Brookes et al, 1973) have produced consistent results in regard to the quality and quantity of amino acid requirements in ruminants.

Nimrick et al (1970) measured changes in nitrogen retention of growing lambs in response to abomasal infusion of amino acids, methionine was the only single amino acid to consistently increase nitrogen retention, lysine improved nitrogen retention only when methionine

was also supplemented, and threonine increased nitrogen retention only after lysine and methionine were supplemented; the combination of these three amino acids improved nitrogen retention by 60% over the urea infused controls. Tryptophan, histidine and leucine did not improve nitrogen retention when infused with methionine, lysine and threonine. Methionine also appeared as the first limiting amino acid when glutamic acid supplied the non specific nitrogen requirement. Nimrick et al (1970) concluded that the limiting order of essential amino acids was methionine, lysine and threonine in sheep on a NPN diet.

The effect of intraperitoneal infusions of amino acids on nitrogen balance and PAA patterns of calves was studied by Hall et al (1974) who found that nitrogen balance was improved by a mixture of methionine, lysine, tryptophan, histidine and arginine but not by any single amino acid; the PAA patterns after intraperitoneal infusion of non specific nitrogen suggested that the limiting amino acids were lysine, methionine and histidine thus, they concluded that it was a group of amino acids and not any single one that was limiting. The exclusion of tryptophan as a limiting amino acid confirms the results of Fenderson and Bergen (1972) who concluded that tryptophan was not a limiting amino acid for growing cattle.

Nitrogen retention, urinary nitrogen and PAA were measured by Richardson and Hatfield (1978) to determine the limiting amino acids in growing cattle after abomasal infusions of amino acids. Methionine was the single amino acid to cause the lowest urinary nitrogen value, the infusion of a combination of lysine and methionine improved nitrogen retention over the infusion of methionine alone, and methionine, lysine and threonine combined, improved nitrogen retention over the combination of methionine and lysine. Since the infusion of tryptophan alone or in combination, or histidine in combination with methionine and lysine gave lower responses than the other amino acids, the authors concluded that methionine, lysine and threonine, in that order, are the first three limiting amino acids in growing steers.

Quantitative Studies

Most qualitative studies reviewed heretofore have shown methionine, lysine and threonine to be the first three limiting amino acids in ruminants under most common dietary conditions. As a result, quantitative assessments of amino acid requirements in ruminants, have usually been oriented at establishing the requirements of these three amino acids.

Nimrick et al (1970b) infused graded levels of amino acids into the abomasum of growing lambs and evaluated the effect on nitrogen retention to determine

the quantitative requirements. They found maximal nitrogen retention at levels equivalent to dietary proportions of .40% glutamic acid (a non essential -- amino acid), .10% methionine, .10% lysine-HCI and .10% threonine. The response curves confirmed the earlier findings (Nimrick et al, 1970a) that the limiting order of essential amino acids for this species was methionine, lysine and threonine.

The lysine requirement of sheep with a weight of 45 kg was evaluated by Brookes et al (1973) who measured lysine oxidation after abomasal infusions of graded levels of lysine, the oxidation of lysine was measured as expired radioactivity from the oxidation of radioactive L-lysine hydrochloride. After plotting the oxidation response against the graded levels of infusion, a break point was calculated at the infusion level of 2.1 g/d of lysine. Plasma response curves were in close agreement with a break point at 2.4 g/day of lysine. Since the amount of dietary lysine reaching the abomasum was calculated as 4.4 g/day, the lysine requirement for sheep was estimated to be between 6.5 and 6.8 g/day.

In another study, Tao et al (1974) compared diverse parameters to evaluate the methionine requirement of sheep, the amounts required were calculated through the

responses of urinary nitrogen (UN), plasma urea nitrogen (PUN), urinary urea nitrogen (UUN), nitrogen balance (NB), plasma insulin (PI) to intravenous infusions of methionine. The response curves of nitrogen utilization indicated a methionine requirement of 4.81 to 5.0 g/day; whereas the PAA level curve showed a requirement of 3.63 g/day. The plasma insulin level was influenced by the amounts of methionine infused and was similar to the response in nitrogen utilization.

The methionine requirement in preruminant calves was investigated by Williams and Smith (1975), who evaluated the PAA and plasma urea (PU) responses to dietary supplementation of methionine and cysteine. Both PAA and PU were affected within 4 hours after ingestion, the response curves indicated a methionine requirement of 4.5 g/day with the PAA response and 3.9 g/day when the PU response was used as criterion. More recently Foldager et al, (1977) supplemented the milk replacer in nursing Holstein calves with graded increments of methionine; the regression analysis of the response in daily gains, nitrogen retention and plasma methionine concentrations indicated a requirement of sulfur containing amino acids of 3.8 to 4.0 g of SAA/16g of nitrogen. The researchers indicated that only three days on diets were necessary to predict the requirements.

The amino acid requirements of growing steers were studied by Fenderson and Bergen (1975); PAA levels and nitrogen balance trials were used to evaluate the response to abomasal infusions of graded amounts of methionine, lysine, threonine and tryptophan. The analysis of the response curves indicated a break point at the infusion level of 7 g/day for methionine. No break point was observed in the plasma responses of lysine, threonine and tryptophan but linear increases in plasma concentrations were recorded with every increment in infusions, suggesting that the requirement for these was met by the digesta entering the abomasum. Considering the amino acid composition of the digesta in the abomasum as well as the methionine required to produce a break point in the response curve, the methionine requirement was set at 14.9 g/day, and the total sulfur amino acid requirement at 18.7 g/day for steers fed a 9.5% protein ration. The investigators suggested that it is possible to extrapolate the requirements of the other amino acids from the methionine requirement, according to the proportion of methionine to the requirements of the other amino acids as reported for swine by the National Research Council (1973). This approach seems to be supported by the fact that the tissue requirements and amino acid composition of pigs and cattle are similar (Black et al, 1957; Dowes, 1961), and that

the proportion of the requirement of an amino acid per 16 g of nitrogen remains constant regardless of the protein level (Boomgaardt and Baker 1973).

MATERIALS AND METHODS

A. Experiment One

General Design

Eight Holstein steers with an average body weight of 270 kg. were fed a 9.5% C.P. ration (Table 1) once daily at noon, at 2.5% (6.8 kg) of their body weight. The steers were housed individually in 91 x 244 cm. metal metabolism stalls with free access to water.

Prior to the start of the experimental period, the steers were adapted to the diet for a 21-day period (Figure 1). In the experimental period, 20 g. of amino acid (L-lysine HCI or L-methionine; obtained from Sigma Chemical Co.) were injected intraperitoneally (IP) once daily, at 8:00 a.m. for 14 days. The amino acids were diluted in distilled water before the injections, in proportions of 1:10 (w/v) for lysine and 1:20 (w/v) for methionine. Blood samples were taken from the jugular vein for amino acid (AA) and blood urea nitrogen (BUN) determinations on days 1, 2, 5, 8, 11 and 14. On days 1, 8 and 14, blood samples were taken at 0, 1, 4 and 8 hr., after the IP injection. On days 2, 5 and 11, blood samples were taken at 0 and 1 hr. after injection.

TABLE I. RATION USED IN THE EXPERIMENTS

Ingredients	%
Oats, grain (4) 4-03-309	10.00
Wheat, bran (4) 4-05-191	5.00
Corn, dent yellow grain gr 2 US mm wt 54 (4) 4-02-931	51.55
Soybean seeds, solv-ext, grnd mx 7% fiber (5) 5-04-604	3.75
Corn, cobs, grnd (1) 1-02-782	20.00
Sugarcane, molasses, mm 48% invert sugar mm 79.5 degrees br/x (4) 4-04-696	5.00
Wheat, flour by product, fine sifted mx 4% fiber (4) 4-05-203	1.00
Urea (45% N)	0.25
Limestone, grnd[a], mn 33% calcium (6) 6-02-632	1.45
Trace mineral salt[bc]	2.00
Vitamin A[d] 2,000,000 IV/ton	
Vitamin D[e] 250,000 IV/ton	
Vitamin E[f] 55,000 IV/ton	
Crude Protein (NX6.25)	9.50

[a]Calcium Carbonate Co., Quincy Illinois

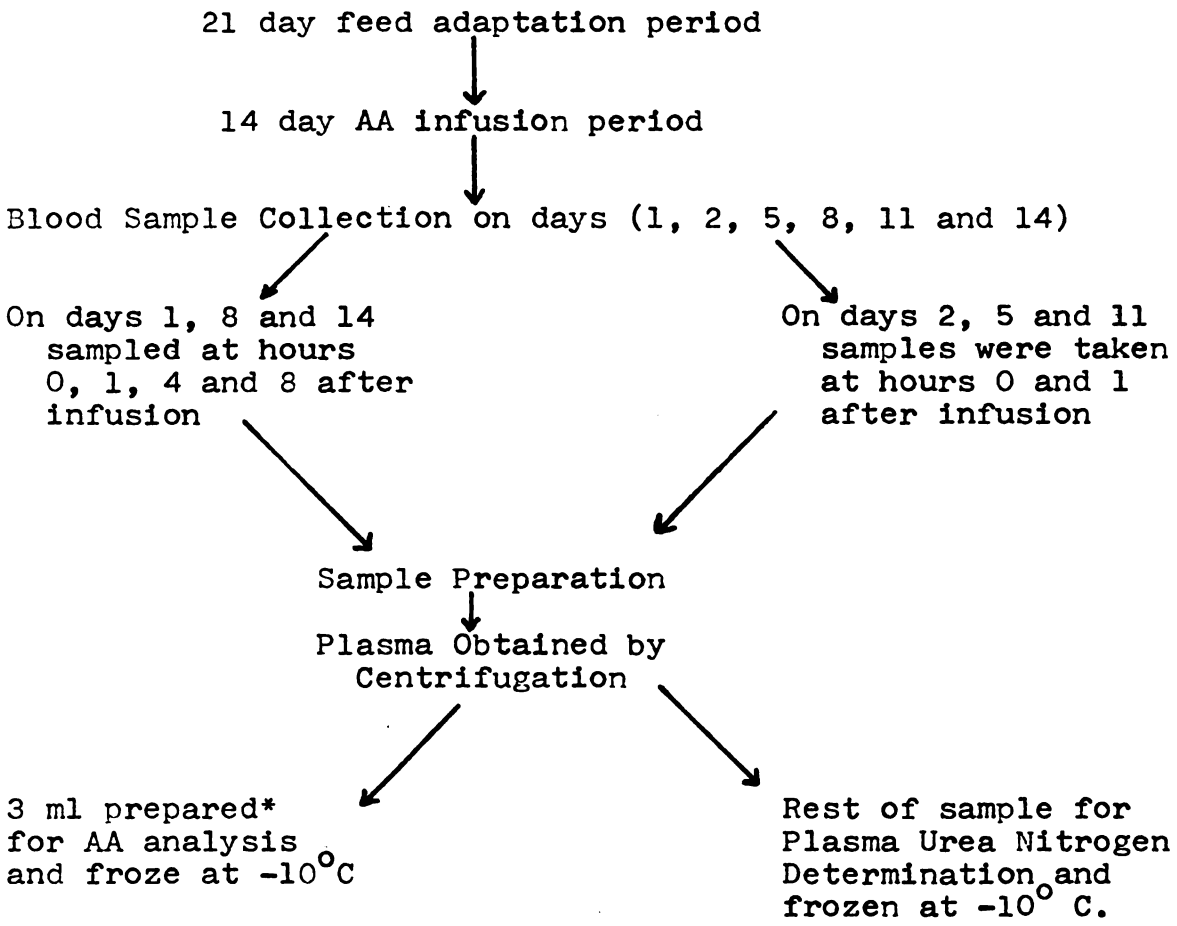
[b]Contained in %: Zn, mn 0.35; Mn, mn 0.2; Fe, mn 0.2; Mg, mn 0.15; Cu, mn 0.03; Co,mn 0.05; I_2, mn 0.007; NaCl, mx 98.5

[c]International Mineral Co.

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

[e]Ergocalciferol (Fleichman Irradiated Dried Yeast)

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



*According to the procedure described by Bergen et al (1973)

Figure 1. Flow chart of Experiment One.

The steers were divided into 2 groups of four steers each; of these, 2 steers were given Lysine injections and two were given methionine injections simultaneously. The first group under went the treatment in January 1978. The animals were injected with the amino acids and the blood samples obtained by using an outdoor squeeze chute. The second group was treated in April 1978 and all IP injections and blood samplings were performed in metabolic stalls. During the above experimental period, the steers had an average daily gain of .52 kg/day.

Sample Processing

Approximately 18 ml. of blood were collected in heparinized vacutainer tubes by jugular puncture. Plasma was then obtained by centrifugation and 3 ml were deproteinized and prepared for AA analysis according to the procedures described in Bergen et al (1973) and then frozen at -10° C. until analysis. The remaining plasma was also frozen at -10° C. for the determination of BUN.

Chemical Analyses

a. Plasma lysine and methionine

Plasma lysine and methionine concentrations were determined from the plasma protein free filtrate by means of ion exchange chromatography (with a Technicon TSM Amino Acid Analyser) as described by Bergen et al (1973).

b. Plasma urea nitrogen (PUN)

PUN levels were determined according to the micro diffusion technique as detailed by Conway (1960).

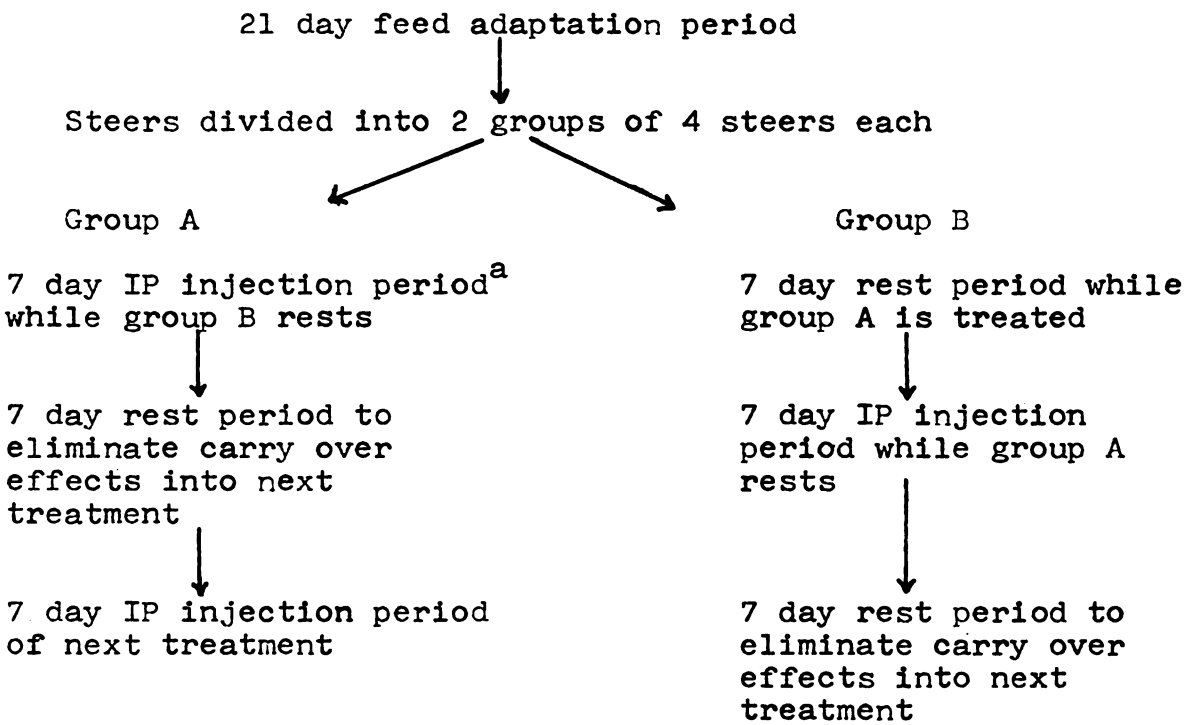
B. Experiment Two

General Design

Eight Holstein steers with an average body weight of 325 kg were used for this experiment. The steers were housed as in experiment one and were fed 6.8 kg/day of the 9.5% CP semipurified ration fed in experiment one.

The amino acids utilized were L-isomers of methionine and cysteine obtained from Sigma Chemical Company. There was a 21 day period of adaptation to the diet before the start of the experiment (Figure 2). In this study, the steers were injected IP once daily at 8:00 a.m. for 7 days with graded levels of methionine, and cysteine plus methionine; the amounts of amino acids injected during each treatment period are presented in Table 2. Methionine was diluted in distilled water in a proportion of 1:20 (w/v) and cysteine was diluted in a ratio of 1:10 (w/v). The pH of the solutions containing cysteine was raised to 6.5 by addition of 6NNaOH to prevent damage to the peritoneum.

The steers were divided in 2 groups of 4 steers each and the treatments were organized so that one group was on treatment while the steers of the other group



[a]Blood samples taken before and one hour after IP injection on days 6 and 7 of injection periods. Blood samples were processed in the same way as in experiment one.

Figure 2. Flow Chart of Experiment Two.

Table 2. Amino Acid Composition[a] of Treatments in Experiment Two

Treatment	Methionine	Cysteine
0[b]	0	0
1	5	0
2	10	0
3	15	0
4	20	0
5	0	7
6	3	7
7	6	7
8	10	7
9	15	7

[a]grams/day of amino acid

[b]PAA values for this level were obtained from the sample drawn before IP injection of day 1 in the first experiment.

were in a 7 day rest period to eliminate carry-over effects into the next treatment. To minimize this risk even further, a 2 week rest period was given between treatments 1-4 (Methionine alone) and 5-9 (Cysteine plus Methionine).

Blood samples were taken on days 6 and 7 of the treatment period before and one hour after the injection. In this experiment both injections and sampling were done in the metabolic stalls. This experiment took place between June and September 1978; the steers showed an average daily weight gain of 0.52 kg during that period.

Sample Processing

Blood samples were prepared in the same manner described in experiment I.

Chemical Analysis

a. Plasma cysteine, lysine and methionine.

All amino acid concentrations were determined from the protein free filtrate by means of Ion Exchange Chromatography (in a Durrum Chromatography Amino-Acid Analyzer Kit) according to the overall procedures outlined by Bergen et al (1973)

Statistical Analysis

The data obtained were statistically analyzed according to the procedures described by Snedecor and Cochran (1967).

RESULTS

Experiment One

Plasma Amino Acid Response to Long-Term Administration of L-Methionine or L-Lysine

The purpose of this experiment was to evaluate a potential adaptive response of plasma amino acids (PAA) in growing steers to IP injections of high (above requirement) quantities of methionine and lysine. The response was studied by following changes in the plasma levels of methionine, lysine and plasma urea nitrogen concentrations over time, after the daily IP injections during a 14 day experimental period. Special attention was also given to the clinical state of the steers during this study due to the potential toxicity of high levels of methionine.

Throughout the whole experiment feed intake remained unchanged. Daily feed intakes were 6.8 kg per steer with no significant weigh backs. Usually the feed was consumed within the first 4 hours after feeding. The overall condition of the steers was satisfactory throughout the experiment. Unusual animal behavior due to the potential toxicity of methionine was not observed during or after the experimental period.

After the experiment, one of the steers developed an outward convature of the right frong leg but this condition was probably due to lack of exercise and the slotted floor of the metabolic stalls. This steer was subsequently replaced for experiment two.

Tables 3 through 6 depict the effect of IP injections of lysine or methionine on the concentration of PAA. The tables include means and standard deviations for all four steers as well as the data grouped according to the type of weather and other environmental effects during the experimental period. Steers A and B were treated in January at temperatures below -10^{o} C as they were led outdoors to a squeeze chute for the IP injections and blood samples, whereas steers C and D were treated in April at temperatures above 0^{o} C.

The effect of IP methionine injections on plasma methionine appears in Table 3. The daily plasma methionine response at the 0 hr. sample shows an increase over the 0 hr. values for the previous days during the first week of the trial. Plasma methionine concentrations at 0 hr. peaked at about day eight and then decrease slightly for days 11 and 14. Changes in the post-injection levels of plasma methionine (1, 4, 8 hr. samples) reveal that the methionine concentrations peaked during the first four hours and then declined by eight hours after the injection.

Table 3. MEAN AND STANDARD DEVIATION OF PLASMA METHIONINE[a] WITH IP INJECTION OF 20 GRAMS METHIONINE PER DAY

Steer & Sample[b]		Days of Experiment 1	2	5	8	11	14
A-B[c]	0[b]	2.7 ± 00.1	2.4 ± 00.3	24.4 ± 00.3	47.6 ± 00.3	42.8 ± 31.7	27.9 ± 24.5
	1	8.1 ± 00.9	2.2 ± 00.8	40.6 ± 10.9	67.9 ± 09.0	67.3 ± 22.1	57.5 ± 30.0
	4	2.4 ± 01.0			116.4 ± 18.1		82.8 ± 51.0
	8	1.9 ± 01.0			46.6 ± 20.6		68.4 ± 36.7
C-D[d]	0	1.8 ± 00.1	4.2 ± 02.7	6.0 ± ----[e]	4.2 ± 00.9	6.9 ± 03.4	6.9 ± 00.0
	1	29.6 ± 00.3	61.6 ± 54.1	47.9 ± 00.4	59.5 ± 25.1	42.6 ± 03.8	47.2 ± 12.6
	4	28.0 ± 00.7			46.2 ± 13.2		2.0 ± 06.4
	8	20.7 ± 00.3			28.2 ± 05.8		13.4 ± 06.1
All	0	2.3 ± 00.5	3.3 ± 01.9	18.3 ± 10.6[f]	25.9 ± 25.0	24.9 ± 27.7	17.4 ± 18.6
	1	18.8 ± 12.4	17.0 ± 18.4	44.2 ± 07.5	63.6 ± 16.1	55.0 ± 19.2	52.3 ± 19.7
	4	15.2 ± 14.8			81.3 ± 42.5		51.4 ± 46.8
	8	11.3 ± 10.8			37.4 ± 16.3		40.9 ± 38.3

[a] Micromoles/dl
[b] Hours after injection
[c] Steers A and B injected in January
[d] Steers C and D injected in April
[e] Value from steer C only
[f] Mean and standard deviation of 3 steers

There was a degree of methionine accumulation in the plasma pool over each 24 hr. period during the first week of the trial as evidenced by the increments in pre-injection levels (Sample 0) of days 1, 2, 5 and 8. Pre-injection values then decreased in the last two samplings (days 11 and 14) of the experiment.

Steers A and B, treated outdoors in January, showed the highest increases in plasma methionine with a strong accumulative effect over a 24 hour period. Steers C and D, treated indoors in April showed smaller increases in plasma methionine. Daily values peaked between days 5 and 8 and post-injection levels were highest in the samples taken one hour after the injections.

Table 4 depicits the effect of IP lysine injections on plasma lysine. The response pattern showed an increase in plasma lysine concentrations (1 hr sample) during the first five days of the experiment and then decreased gradually during the remainder of the experiment. Changes in the post-injection levels indicate that plasma lysine peaked during the first hour post-injection. During the lysine experiment there was no accumulation in plasma lysine in the 0 hr. sample. Plasma lysine levels returned to preinjection values within each 24 hour period.

Unlike the methionine response, there were no differences in the peak values of plasma lysine of

Table 4. MEAN AND STANDARD DEVIATION OF PLASMA LYSINE[a] WITH IP INJECTION OF 20 GRAMS LYSINE PER DAY

Steer & Sample[b]		Days of Experiment 1	2	5	8	11	14
A-B[c]	0[b]	8.3 ± 1.4	6.4 ± 2.8	9.9 ± .2	8.1 ± 1.5	6.5 ± .4	6.0 ± .0
	1	9.2 ± 1.6	8.4 ± 5.2	36.5 ± 12.2	35.1 ± 2.1	36.5 ± .2	35.1 ± .5
	4	6.4 ± 1.2			24.6 ± 7.2		17.4 ± 1.1
	8	5.9 ± 1.6			12.5 ± 5.6		19.8 ± 12.0
C-D[d]	0	4.5 ± .7	5.7 ± 4.0	5.7 ± 1.7	6.7 ± 1.0	5.2 ± .9	4.5 ± ----[e]
	1	20.6 ± 1.7	30.2 ± 5.1	33.7 ± 3.0	33.5 ± 5.0	17.7 ± 20.0	35.8 ± 5.4
	4	16.9 ± 1.7			15.8 ± 2.4		12.7 ± 12.5
	8	10.6 ± .5			10.3 ± 4.1		4.5 ± 2.4
All	0	6.6 ± 2.4	6.0 ± 2.8	7.8 ± 2.6	7.4 ± 1.3	5.9 ± .9	5.5 ± .8[f]
	1	14.9 ± 6.6	19.3 ± 13.2	35.1 ± 7.4	34.3 ± 3.3	27.11 ± 16.4	35.4 ± 3.1
	4	11.6 ± 6.2			20.2 ± 6.7		15.0 ± 7.7
	8	8.3 ± 2.8			11.4 ± 4.2		12.4 ± 11.1

[a] Micromoles/dl
[b] Hours after injection
[c] Steers A and B injected in January
[d] Steers C and D injected in April
[e] Value from steer D only
[f] Means and standard deviation of 3 steers

steers A-B and steers C-D. Steers C-D (treated in April) showed a much quicker response as plasma lysine did not increase in steers A-B until after the second day, while steers C-D showed a post-injection increase on the first day of the experiment.

Table 5 contains plasma lysine values for the methionine injection study on the days at which plasma methionine peaked (Days 5 and 8). All levels appear within the normal concentrations as there was no evidence of influence on plasma lysine by high levels of methionine. Table 6 contains plasma methionine values for the lysine injection study in the days at which plasma lysine peaked (Days 5 and 8). All levels fall within normal ranges and there was no evidence of influence on plasma methionine by high levels of lysine.

Plasma urea nitrogen concentrations (See Appendices 1 and 2) were not influenced by either of IP methionine or lysine injections. PUN analyses were suspended after having analyzed one third of the total samples with no evidence of influence by IP methionine or lysine on PUN levels. Pun levels remained consistently low throughout the experimental period with values ranging from 4.8 mg/dl to 8.4 mg/dl.

Table 5. MEAN AND STANDARD DEVIATION OF PLASMA LYSINE[a] WITH IP INJECTION OF 20 GRAMS METHIONINE PER DAY

Steer & Sample[b]		Days of Experiment 5	8
A-B[c]	0	5.4 ± ----[e]	7.8 ± .4
	1	7.5 ± 3.3	8.4 ± .2
	4		6.8 ± .4
	8		6.8 ± .4
C-D[d]	0	4.6 ± ----[f]	6.6 ± .3
	1	5.3 ± 1.6	7.4 ± 1.8
	4		6.9 ± 1.1
	8		8.1 ± .2
All	0	5.0 ± .5[g]	7.2 ± .7
	1	6.4 ± 2.4	7.9 ± 1.2
	4		6.8 ± .7
	8		7.4 ± .8

[a] Micromoles/dl

[b] Hours after injection

[c] Steers A and B injected in January

[d] Steers C and D injected in April

[e] Value from Steer B only

[f] Value from Steer C only

[g] Means and standard deviation of 2 steers

Table 6. MEAN AND STANDARD DEVIATION OF PLASMA METHIONINE[a] WITH IP INJECTION OF 20 GRAMS LYSINE PER DAY

Steer & Sample[b]		Days of Experiment 5	8
	0	3.7 ± .1	3.5 ± .0
	1	2.6 ± ----[e]	2.8 ± .1
A-B[c]	4		3.0 ± .6
	8		4.0 ± .1
	0	3.1 ± .0	4.1 ± 2.3
	1	3.2 ± .5	3.6 ± .6
C-D[e]	4		4.3 ± 1.8
	8		5.3 ± 1.0
	0	3.4 ± .3	3.8 ± 1.3
	1	3.0 ± .5[f]	3.2 ± .5
All	4		3.6 ± 1.3
	8		4.6 ± 1.1

[a] Micromoles/dl

[b] Hours after injection

[c] Steers A and B injected in January

[d] Steers C and D injected in April

[e] Value from steer B only

[f] Mean and standard deviation of 3 steers

Experiment Two

Plasma Amino Acid Response to IP Injections of Amino Acids as Criterion of Amino Acid Requirements

The purpose of this study was to measure plasma methionine, lysine and cystine responses to incremental levels of IP injections of methionine and cysteine plus methionine in order to evaluate the methionine requirement in growing steers in a manner similar to Fenderson and Bergen (1975). Plasma methionine should remain at a constant basal level as long as the methionine supply from the abomasal flow supplemented with the IP injections was below the requirement. Once the IP injections caused the methionine supply (abomasal flow plus IP injections) to meet the requirement, plasma methionine would be expected to increase with each additional increment of the methionine injection level. The point at which the plasma methionine concentration starts to accumulate is considered the requirement.

The effect of graded levels of methionine on the concentration of plasma methionine, lysine, and cystine appear in Table 7. Plasma methionine started to accumulate at the injection level of 5 g/day with a linear increase after each successive increment. Since the same steers were used, and the same ration was fed, preinjection plasma methionine levels from the first day of experiment one were considered to represent the basal

Table 7. PAA[ab] RESPONSE TO IP INJECTIONS OF GRADED LEVELS OF METHIONINE

Amino acid	Treatment[c] 0 Met	5 Met	10 Met	15 Met	20 Met
Methionine	2.30 ± .5[d]	5.85 ± .3	11.85 ± 1.5	20.79 ± 2.0	31.74 ± 4.0
Lysine	6.65 ± 2.4[d]	5.05 ± 1.0	5.85 ± 1.4	5.56 ± 1.4	6.07 ± .7
Cystine	----------[e]	1.31 ± .1	1.71 ± .2	1.70 ± .2	2.08 ± .3

[a]Micromoles/dl

[b]Mean and standard deviations of four steers

[c]Grams per day per steer

[d]Values obtained from experiment one (first day, sampled before IP injection)

[e]Not obtained

concentration of plasma methionine. The point at which a line representing this basal level was intersected by a regression line obtained from the incremental levels of plasma methionine was the breakpoint at which plasma methionine would start to accumulate above the basal line with each increase in the IP methionine injections and was therefore considered to represent the requirement. The breakpoint was equivalent to a IP injection level of 3.6 g of methionine per day.

The plasma methionine response to incremental IP injections of methionine is displayed graphically in Figure 3.

The effect of IP injections of graded levels of methionine on plasma lysine and plasma cystine appears in Figure 4. Regression analyses and correlation coefficients were used as criteria to evaluate the response. No significant correlation was found between the graded increments of IP injections of methionine and plasma lysine or cystine concentrations.

Table 8 depicts the effect of IP injections of 7 g of cysteine per day plus graded amounts of methionine on plasma concentrations of methionine, lysine and cystine. Plasma methionine levels for treatments 7 g cysteine-0 g methionine and 7 g cysteine-0 g methionine were considered to represent the basal methionine level since their mean concentrations in the two treatments

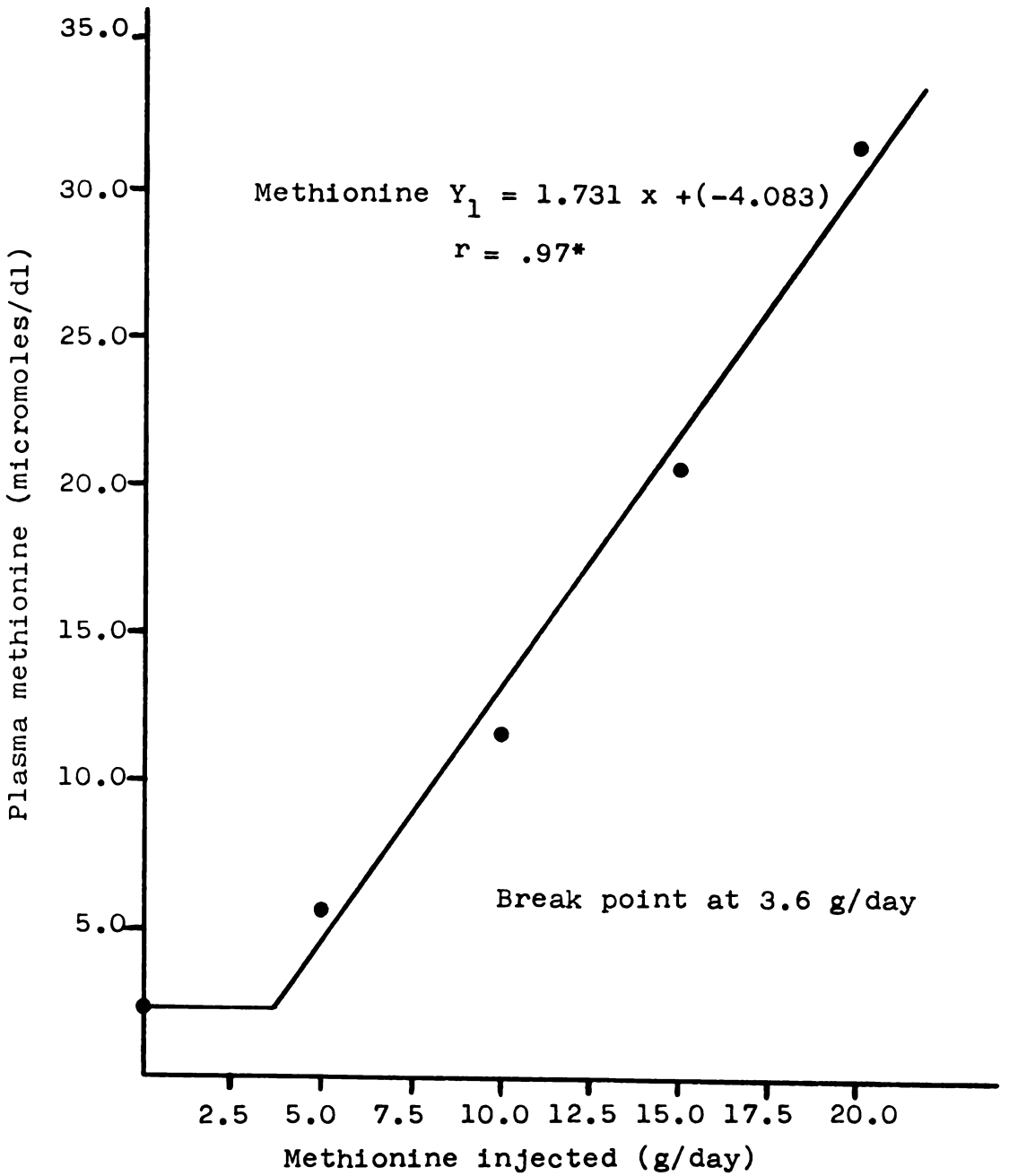


Figure 3. Plasma methionine response to IP injections of graded levels of methionine

*Correlation statistically significant ($p < .05$)

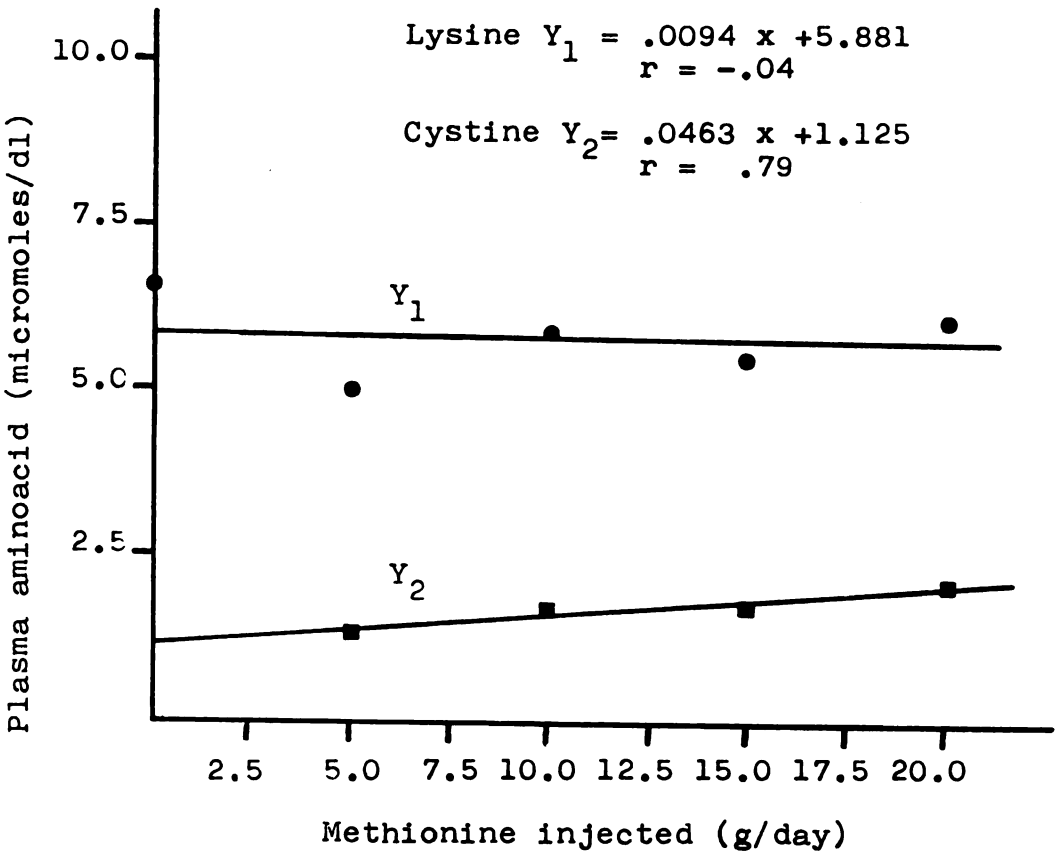


Figure 4. Plasma lysine and plasma cysteine response to IP injections of graded levels of methionine.

Table 8. PAA[ab] RESPONSE TO IP INJECTIONS OF CYSTEINE PLUS GRADED LEVELS OF METHIONINE

	Treatment[c]				
Amino acid	7 Cys - 0 Met	7 cys - 3 Met	7 Cys - 6 Met	7 Cys - 10 Met	7 Cys-15 Met
Methionine	2.58 ± .2[d]	2.59 ± .2[d]	6.47 ± .8	13.67 ± 1.6	16.21 ± 1.9
Lysine	5.86 ± 1.3	4.82 ± .7	5.46 ± 1.1	6.61 ± .6	3.96 ± .6
Cystine	2.35 ± .2	2.52 ± .4	3.18 ± .3	3.66 ± .2	2.82 ± .35

[a]Micromoles/dl

[b]Mean and standard deviation of four steers

[c]Grams per steer per day

[d]Means not different significantly ($p < .10$)

did not differ statistically ($p < .10$). These basal methionine levels were similar to those found in experiment one. Plasma methionine started to increase with treatment 7 g cysteine-6 g methionine and continued to rise for treatments 7 g cysteine-10 g methionine and 7 g cysteine-15 g methionine; however, this latter treatment did not produce a linear increase in plasma methionine but a smaller increment which resulted in a sigmoid-type response curve for this series of treatments. Figure 5 includes the graph of the response pattern as well as the correlation coefficient and regression equation of plasma methionine levels. The regression equation in this case was obtained from the plasma methionine levels of the 4 steers at the treatment levels of 7 g cysteine + 6 g methionine and 7 g cysteine + 10 g methionine which were the levels at which plasma methionine increased linearly with each incremental level of amino acid injection. Thus for this series of treatments, the break point occurred at a value representing an IP injection level of 7 g cysteine + 3.8 g methionine per day.

The graph of the effect of IP injections of cysteine plus methionine on the plasma levels of lysine and cystine appears as Figure 6. The response was evaluated through regression and correlation analyses. No significant correlations were found between the IP injections

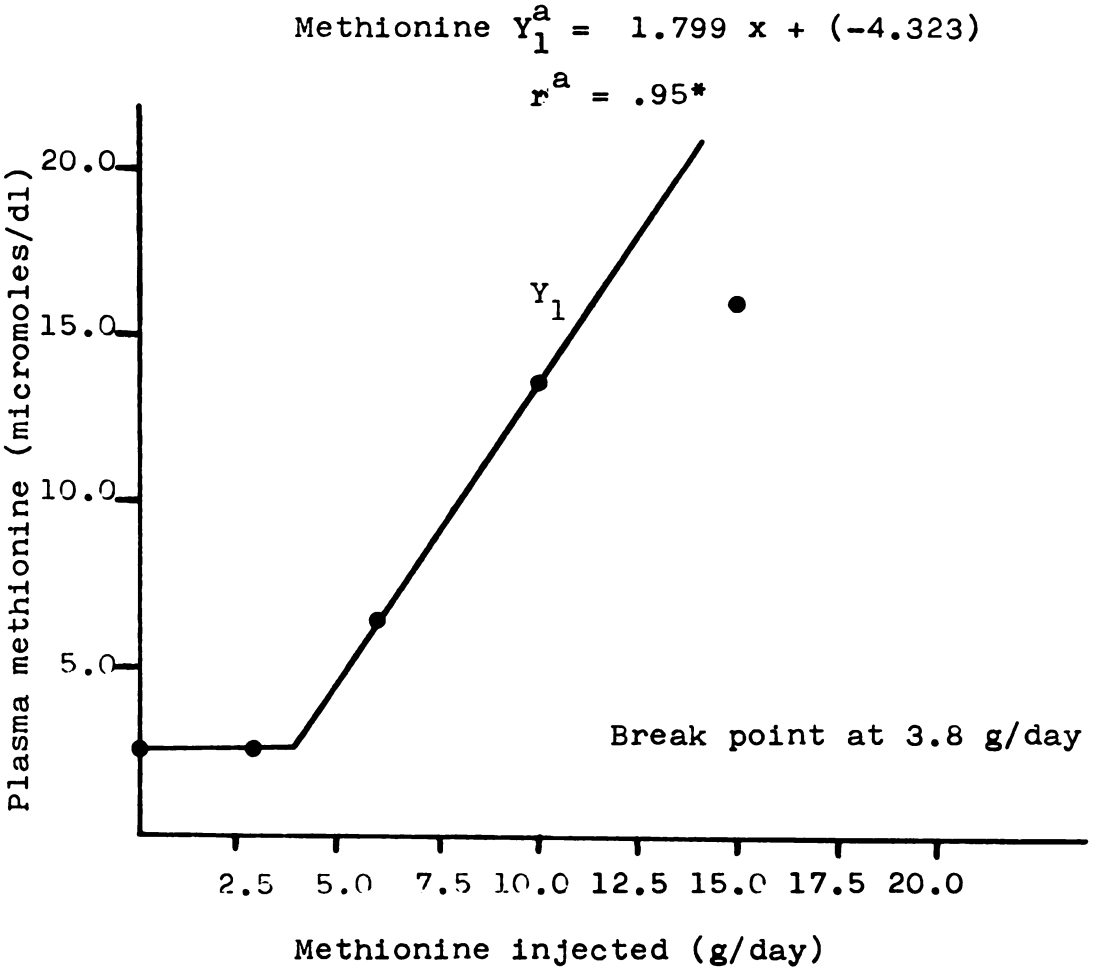


Figure 5. Plasma methionine response to IP injections of seven g/day cysteine plus graded levels of methionine

[a]Regression and correlation obtained from the plasma methionine response of 4 steers at levels of injection 7 cysteine-6 met and 7 cysteine-10 met.

* Correlation statistically significant ($p < .05$).

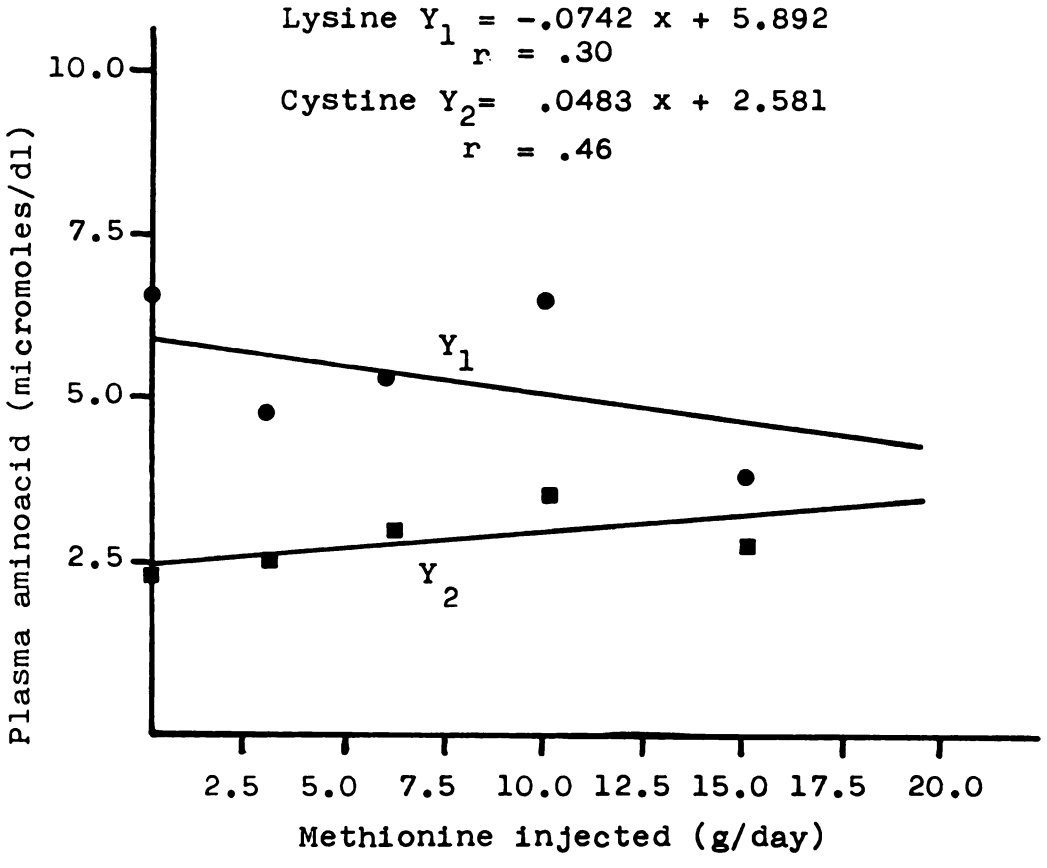


Figure 6. Plasma lysine and plasma cysteine response to IP injections of seven g/day cysteine plus graded levels of methionine

of cysteine plus methionine and the plasma concentrations of lysine or cystine.

DISCUSSION

Experiment One

Proper evaluation of plasma amino acid (PAA) metabolism is closely related to the validity of sampling technique. Plasma amino acid responses can be studied when changes in the PAA levels are more evident, this entails careful examination of the adaptive behavior of PAA. The response over time of PAA concentrations after changes in the level of amino acid intake has been extensively studied (Zimmerman and Scott, 1965; Mitchell *et al*, 1968; Fenderson and Bergen, 1972; Hall *et al*, 1974; Fenderson and Bergen, 1975; Foldager *et al*, 1977). The purpose of this experiment was to evaluate the PAA level response to sustained administrations of L-lysine and L-methionine and to use these data to determine the optimal sampling times for experiment two. (Evaluation of the methionine requirement.)

Mitchell *et al* (1968), working with pigs, suggested that a period of adaptation to changes in amino acid intake is necessary before PAA levels can be used to obtain valid data. Fenderson and Bergen (1975), working with steers, indicated that PAA levels did not become stable until a 4 day period of adaptation to changes in amino acid supply. The results of the daily plasma methionine response to IP injections of

20 g/day of methionine (Table 3) show that plasma values peaked between days 5 and 8 and that increases in the post injection levels of days 1 and 2 were small by comparison. The daily plasma lysine response to IP injections of 20 g/day of lysine (Table 4) also show that an adaptation period of 5 to 8 days is necessary before plasma lysine peaked. Here again, increases in post injection concentrations on days 1 and 2 were smaller than those of days 5 and 8. Considering the high (20 g/day) amount of the amino acids injected, it seems likely that a response to smaller quantities of amino acids would not be clearly evidenced on days 1 and 2. This can be of particular importance when the PAA concentrations are used to evaluate PAA responses to graded levels of amino acid intake.

Basal plasma methionine concentrations (0 hr samples) during the 14 day experimental period (Table 3) started to accumulate after day 2 and remained high for days 5, 8 and 11 but showed a tendency to decline by day 14. Plasma lysine during the 14 day experimental period (Table 4) did not accumulate but returned to normal pre-injection levels within each 24 hour period. Based on the abomasal passage studies of Fenderson and Bergen (1975), with a similar ration the steers in this study had 14.75 g/day of absorable methionine in the lower gut. Therefore, the 20 g of methionine injected daily resulted in a total available level of 34.75 g/day. Since Fenderson and Bergen (1975) estimated that the lower limit of the daily methionine requirement (expressed as 44%

of the total sulfur amino acid requirements) was about 9.8 g of methionine per day. The total methionine intake in this experiment at 34.75 g/day represents an excess of 350% of the lower limit for methionine. In view of the lack of symptoms of toxicity, these results suggest a great capacity to handle high methionine intakes.

With respect to lysine intake, Fenderson and Bergen (1975) estimated that the daily lysine requirement was equal or less than the absorbable lysine of the abomasal flow, which in this experiment was 21.42 g/day. Considering that the total lysine intake of 41.42 g/day was approximately 190% of the requirement level, the lack of accumulation of plasma lysine concentrations in the daily pre-injection levels (0 hr samples in Table 4) suggests a high capacity to adapt to and metabolize excess lysine. The accumulation of methionine levels and the absence of accumulation in the lysine concentrations may be explained by the fact that the methionine intake was 350% of the required level while the lysine intake was 190% of the requirement.

Post-injection plasma methionine concentration after IP methionine injections (Table 3) tended to peak between 1 and 4 hr. Post injection plasma lysine values after IP lysine injections (Table 4) tended to peak at around one hour. These results are in agreement with the optimal sampling times reported in the literature. Hall *et al* (1974) working with IP amino acid injections in calves obtained clear

responses 2 hr after the injections. Foldager et al (1977) in a study of amino acid requirements of preruminant calves concluded that PAA responses to oral amino acid intakes were significant after 1 hr of the amino acid administration.

Plasma lysine levels with IP methionine injections (Table 5) were not affected by the methionine injections on the days when plasma methionine peaked (days 5 and 8). Similarly, plasma methionine levels with IP lysine injections on the days of the highest plasma lysine levels (days 5 and 8) were not affected by the lysine injection. These results differ from those of Richardson and Hatfield (1978) who found that abomasal infusions of methionine had a tendency to lower plasma lysine concentrations.

The differences in plasma methionine behavior observed between steers A-B treated in January, with temperatures of -10°C and led outdoors to a squeeze chute for injections and samplings and steers C-D treated in April in the metabolism stalls with temperatures above 0°C, also merit discussion. Steers A-B showed higher increases in plasma methionine than steers C-D. Although the plasma methionine values for steers C-D were somewhat lower, their response to the IP methionine injections was quicker. Plasma methionine levels started to increase from the first day in steers C-D while plasma methionine in steers A-B did not rise significantly until day 2.

The differences can not be readily explained and they are most likely due to environmental factors. An explanation for the different PAA behavior must take into account both the severity of the weather, the extra excitement incurred by steers A-B as they were moved to the squeeze chute, as well as the hormonal and metabolic factors governing the removal of amino acids from the plasma.

The constantly low plasma urea nitrogen was probably due to the low protein intake, this is in agreement with Leibholz (1970) who showed that low nitrogen intakes and starvation resulted in low PUN concentrations in sheep. Based on the data of Fenderson and Bergen (1975) with a ration of the same composition in growing Holstein steers, the daily absorbable crude supply in this experiment was estimated to be about 433 g. The addition of 20 g of amino acid per day represents only an increase of 4.5% with respect to this amount of daily absorbable crude protein. The IP injection of 20 g of amino acid per day was therefore probably too small to elicit a change in the PUN levels.

Experiment Two

A. Plasma Level Response

Plasma amino acid response curves are a well established procedure in the study of amino acid requirements. (Zimmerman and Scott, 1965; Mitchell *et al*, 1968; Young *et al*, 1971; Brookes *et al*, 1973; Reis *et al*, 1973; Tao *et al*, 1974; Broderick and Satter, 1975; Williams and

Smith, 1975; Fenderson and Bergen, 1975; Foldager et al 1977). This technique requires that the essential amino acid in study be limiting before the plasma level response to incremental AA addition can be used to evaluate the requirement (Zimmerman and Scott, 1965). Further a period of adaptation to this limiting condition must be allowed before a well defined break point in plasma response to incremental AA administration will be achieved (Mitchell et al, 1968). In ruminants, the determination of amino acid requirements must include a estimation of the abomasal flow of amino acids so that the total (abomasal flow plus graded increments) intake can be properly evaluated (Fenderson and Bergen, 1975).

Plasma methionine responses with IP supplementation of methionine alone and cysteine plus methionine (Figures 3 and 5 respectively) confirm the results of Fenderson and Bergen (1975); who, working with growing steers fed a similar ration, found that the methionine supply in abomasal flow was limiting.

The break point in the plasma methionine response curve was equivalent to an IP methionine injection level of 3.6 g/day for graded increments of methionine alone, and 3.8 g of methionine per day for 7 g of cysteine plus graded increments of methionine. The close agreement of the breakpoint levels in the treatments with and without cysteine suggests that the inclusion of cysteine did not

have a methionine sparing effect and that the matabolic requirement for cysteine was already met by the amino acids absorbed from the intestinal flow.

The sigmoid shape of the plasma methionine response curve in the treatment series of daily IP injections of graded levels of methionine plus cysteine (Figure 5) was reported in other studies (Mitchell et al, 1968; Young et al, 1971). This sigmoid type of response curve constitutes an obstacle in the accuracy of the determination of amino acid requirements because it destroys the linearity of the plasma response to incremental levels of amino acid intake after the requirement has been met.

The requirement is usually set as the intersection of a line representing the basal plasma amino acid level with a line obtained from a regression equation based on the linear increase in the plasma amino acid level after the requirement has been met by the increments in the intake of the amino acid in study. Therefore, a sigmoid-type response will disrupt the linearity and cause the intersection of the regression line to be displaced to the left possibly resulting in an underestimation of the amino acid requirement. The analysis of the results of this experiment showed that when the regression analysis included treatments 7 g cysteine + 6 g methionine; 7 g cysteine + 10 g methionine and 7 g cysteine + 15 g

methionine (The latter one causing the sigmoid response), the regression line intersected the basal line at a point representing an IP injection level of approximately 1.2 g methionine per day, an implausible value since the IP injection of 3 g of methionine did not produce an upward deflection in plasma methionine above the basal level.

On the other hand, a regression analysis including only treatments 7 g cysteine 6 met. and 7 g cysteine 10 met. produced a break point at a level equivalent to 3.8 methionine/day. A more logical value since it occurs between the last methionine injection level that failed to increase the plasma methionine concentration about the basal line (3 g of methionine per day) in this series of treatments (Figure 5).

B. Quantitation of the Methionine Requirement

The methionine requirement was calculated by adding the amount of injected methionine needed to produce the break point in the plasma response to the amount of methionine absorbed across the intestinal epithelium. The amounts of dietary nitrogen and amino acids reaching the abomasum in steers in this study appears in Table 9. The data derived in this experiment was based on the passage studies with the same ration as reported by Fenderson and Bergen (1975). Using the dry matter intake of 6.8 kg/day of this study, dietary nitrogen reaching the abomasum was 101.32 g/day which represents 98% of the 103.36

Table 9. NITROGEN AND AMINO ACID PASSAGE IN STEERS FED THE 9.5% CRUDE PROTEIN RATION UTILIZED IN THIS EXPERIMENT[a]

Item	g/kg feed	g/day[b]
Nitrogen	14.9	101.32
Crude Protein[c]	93.1	633.25
Lysine	4.5	30.60
Cystine	.8	5.44
Methionine	1.6	10.88
TSAA	2.4	16.32

[a] Based on data by Fenderson and Bergen (1975)

[b] Dry matter intake of 6.8 kg/day

[c] N x 6.25

g of nitrogen ingested daily. Amino acids reaching the abomasum amounted to 30.60 g/day for lysine, 5.44 g/day for cystine, 10.88 g/day for methionine and 16.32 g/day for the total sulfur amino acids.

Table 10 contains the quantitation of the methionine requirement. Hogan, (1973) determined that the digestibility of bulk protein in the small intestine of ruminants was 70%. In the present calculation it was therefore assumed that 70% of the protein reaching the abomasum and small intestine would be digested and absorbed. Hence, the absorbed values were 7.61 g/day of methionine and 7.14 g/day of cystine, adding up to 14.75 g/day of total sulfur amino acids.

The mean of the break point methionine levels of the treatments with and without cysteine (3.7 g of methionine per day) was considered to represent the amount of methionine needed to produce the break point in the plasma response pattern.

The methionine requirement was set as the sum of the absorbed methionine (7.61 g/day) plus the injected break point level (3.7 g/day) for a daily methionine requirement of 11.31 g/day, the addition of this value to the absorbable of cystine (7.14 g/day) resulted in a total sulfur amino acid (TSAA) requirement of 18.45 g/day.

Table 10. QUANTITATION OF METHIONINE AND TOTAL SULFUR AMINO ACIDS

Amino Acid	Passage to Abomasum[a] (g/day)	Digestibility[b] Coefficient (%)	Absorable Amino Acid (g/day)	Amino acid Injected IP to break point (g/day)	Requirement (g/day)
Methionine	10.88	70	7.61	3.70[d]	11.31
Cystine	5.44	70	3.80	----	----
TSAA[c]	21.08	70	11.41	----	15.11

[a]Based on data by Fenderson and Bergen (1975)

[b]Hogan (1973)

[c]Total sulfur amino acids

[d]Expressed as the mean of the breakpoint methionine levels of the treatments with and without cysteine.

The TSAA value thus obtained was extrapolated to calculate the required amounts of the other essential amino acids (EAA). To do this, the TSAA requirement was taken as a unit. The required amounts, the other EAA were then calculated by multiplying their requirements (as established in the NRC pig requirement pattern, 1973) by their ratio to the TSAA requirement obtained in this study.

This extrapolation is supported by the assumption that tissue requirements are qualitatively similar for pigs and cattle (Black et al, 1957), that gain in carcass nitrogen as a percentage of live weight gain is similar, (2.64% for pigs and 2.40% for steers) (Woodman and Evans, 1951), and that the proportion of the requirement of an amino acid per 16 g of nitrogen remains constant over a wide range of protein levels (Boomgaardt and Baker, 1973).

Fenderson and Bergen (1975) calculated the lower limit of the methionine requirement as 44% of the TSAA requirement. The results of this experiment do not support this assumption. The fact that the addition of 7 g cysteine per day did not have a methionine sparing effect, suggests that the metabolic requirement for non essential sulfur amino acids had already been met by the daily inflow of absorbable cystine (3.8 g) present in the intestine. In order to obtain a methionine

sparing effect by the IP injection of a non essential sulfur amino acid (7 g of cysteine per day in this experiment), the amount of non essential sulfur amino acids being absorbed from the intestine must be below the metabolic requirement. Since in the present experiment the administration of 7 g of cysteine did not have a methionine sparing effect, the substitution of non essential sulfur amino acids for methionine below the level of 11.31 g/day calculated through the plasma level response, might cause a deficiency of methionine. The methionine requirement set as 11.31 g/day represents 74.85% of the TSAA requirement and 9.04% of the total essential amino acid requirement.

The requirement values obtained in this study are lower than those of Fenderson (1974). This difference may be accounted for by the fact that the steers used by Fenderson (1974) had an average daily weight gain of .73 kg while the steers used in this experiment gained only .52 kg/day and therefore had a lower tissue demand.

Throughout the course of this work, PAA responses proved to be a reliable tool in the study of amino acid metabolism. It seems desirable and useful to continue the application of this technique particularly in situations where amino acids other than methionine are limiting and in studies relating rate of weight gain to amino acid requirements.

Table 11. ESTIMATED DAILY ESSENTIAL AMINO ACID REQUIREMENTS[a] OF GROWING STEERS

Amino Acid	Present 9.5% CP	Fenderson (1974) 9.5% CP	Chalupa (1974) 300 kg steer
TSAA[b]	15.11	22.3	----
Methionine	11.31	9.8[e]	12.0
Lysine	21.14[c]	31.2[c]	36.5
Phe & Try	15.10[c]	22.3[c]	21.0
Valine	15.10[c]	22.3[c]	25.0
Isoleucine	15.10[c]	22.3[c]	25.0
Leucine	18.15[c]	26.8[c]	39.0
Threonine	13.61[c]	20.1[c]	20.5
Tryptophan	3.92[c]	5.8[c]	3.0
Histidine	5.42[c]	8.0[c]	13.5
Arginine	6.05[c]	8.9[c]	30.0
TEAA[d]	125.00	177.5	222.5

[a]Grams per day

[b]Total sulfur amino acids

[c]Calculations based on the swine AA requirement pattern (National Research Council, 1973)

[d]Total essential amino acids

[e]Set as 44% of the TSAA requirement

APPENDIX

APPENDIX 1. PLASMA UREA NITROGEN[a] LEVELS WITH IP INJECTIONS OF 20 GRAMS LYSINE PER DAY

Steer & Sample[b]		Days of Experiment 1	8	14
B	0	7.2	8.0	4.9
	1	5.5	7.5	7.8
	4	5.8	6.9	5.4
	8	8.4	7.6	7.3
D	0	5.0	7.2	8.0
	1	4.9	6.4	7.0
	4	4.8	7.5	7.9
	8	6.2	4.8	6.8

[a] mg/dl

[b] Hours after injection

APPENDIX 2. PLASMA UREA NITROGEN[a] LEVELS WITH IP INJECTIONS OF 20 GRAMS METHIONINE PER DAY

Steer & Sample[b]		Days of Experiment 1	8	14
A	0[b]	8.0	5.0	6.2
	1	7.9	6.1	5.5
	4	8.4	5.6	5.4
	8	6.3	7.5	6.8
C	0	7.1	6.3	7.2
	1	7.8	5.2	8.3
	4	5.2	8.4	6.9
	8	6.1	7.3	7.6

[a] mg/dl

[b] Hours after injection

APPENDIX 3. INDIVIDUAL LEVELS OF PLASMA AMINO ACIDS[ab] WITH IP INJECTIONS OF GRADED LEVELS OF METHIONINE

		Amino Acid		
Treatment[c]	Steer	Methionine	Lysine	Cystine
0 Met[d]	A	2.67	9.63	----[e]
	B	2.87	7.29	----
	C	1.76	4.05	----
	D	1.93	5.08	----
5 Met	A	5.77	4.49	1.21
	B	5.47	4.35	1.43
	C	5.87	4.88	1.38
	D	6.32	6.50	1.22
10 Met	A	13.15	6.29	1.78
	B	9.79	5.70	1.45
	C	12.70	6.79	1.74
	D	11.79	4.65	1.90
15 Met	A	18.82	5.19	1.68
	B	23.51	6.29	1.93
	C	19.84	3.77	1.72
	D	21.00	6.99	1.48
20 Met	A	30.10	6.46	2.28
	B	37.81	6.18	2.02
	C	29.54	5.00	1.70
	D	29.51	6.66	2.35

[a]Micromoles/dl

[b]Mean plasma concentration of blood samples obtained 1 hr after the IP amino acid injection on days 6 and 7.

[c]Grams per day per steer

[d]Obtained from experiment 1 (day 1, sample time 0 hours)

[e]Not obtained for this treatment

APPENDIX 4. INDIVIDUAL LEVELS OF PLASMA AMINO ACIDS[ab] WITH IP INJECTIONS OF CYSTEINE PLUS GRADED LEVELS OF METHIONINE

		Amino Acid		
Treatment	Steer	Methionine	Lysine	Cystine
7 Cys 0 Met	A	2.33	4.40	2.19
	B	2.98	6.85	2.36
	C	2.39	5.10	2.66
	D	2.63	7.93	2.21
7 Cys 3 Met	A	2.99	5.85	3.22
	B	2.53	4.30	2.42
	C	2.46	4.70	2.29
	D	2.39	4.45	2.16
7 Cys 6 Met	A	5.87	6.98	3.49
	B	5.74	4.49	2.95
	C	7.38	5.54	3.36
	D	6.90	4.83	2.94
7 Cys 10 Met	A	13.06	6.97	3.81
	B	15.46	5.61	3.82
	C	14.51	6.96	3.27
	D	11.65	6.93	3.76
7 Cys 15 Met	A	18.31	3.33	3.03
	B	13.62	3.65	2.31
	C	16.37	4.91	2.85
	D	16.57	3.96	3.09

[a]Micromoles/dl

[b]Mean plasma concentration of blood samples obtained 1 hr after IP amino acid injection on days 6 and 7

[c]Grams per day per steer

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