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PLASMA AMINO ACID STUDIES IN GROWING CATTLE

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# PLASMA AMINO ACID STUDIES IN GROWING CATTLE

By

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ABSTRACT

PLASMA AMINO ACID STUDIES IN
GROWING CATTLE

By

Barakat M. Ahmed

Three experiments were conducted to study requirements and metabolism of certain amino acids (AA) in growing cattle.

The first experiment was designed to determine total sulfur amino acid (TSAA) requirements using plasma methionine concentration responses to intaperitoneal injection of incremental amounts of methionine or methionine plus cysteine. Eight crossbred steers with an average body weight (BW) of 333 kg were fed a 9.5% crude protein semipurified diet once daily at 2% of their BW. The steers were divided into two groups, each group was assigned to a split-plot design. The first group was injected with graded levels of methionine (0, 2, 4, 6, 8, 10, 12 and 14) while the second group was injected with graded levels of methionine (0, 2, 4, 6, 8, 10, 12, and 14) plus 7 g of cysteine. Plasma methionine response curves were constructed and the data revealed that the supplemental methionine requirement was 6.8 g/d under the given experimental conditions. The plasma methionine response curves also showed that cysteine can supply part of the TSAA needs and thus can spare methionine. In this study the TSAA (absorbable) requirement was 17.82 g/d; at least forty two percent of the TSAA requirement must be met by L-methionine.

The second experiment was conducted to examine the use of the esophogeal groove reflex as a mechanism of rumen bypass to study AA requirements of young ruminants after the rumen had developed. Six male Holstein calves (BW of 88 kg) were used in this study. The calves were weaned, placed on dry feed to encourage rumen development. During this period the esophegeal groove was kept functioning by feeding milk once daily. The animals were randomly assigned to two groups fed a low protein diet (at 40% of the dry matter intake), and milk replacer (at 60% of the dry matter intake) supplemented with or without lysine (at level of 0.7% on the dry matter basis). Animal performance and plasma lysine concentrations were criteria employed to evaluate rumen bypass. Results showed that calves fed the lysine-supplemented diet had higher ($P<.05$) average daily gain (.84 vs .63 kg/d), and higher ($P<.05$) gain/feed ratio (.29 vs .20) than the unsupplemented group. The lysine-supplemented group also exhibited a higher ($P<.05$) plasma lysine level after feeding. From these results it can be concluded that a maintained esophageal groove reflex appears to be a satisfactory approach to encourage rumen bypass of dietary ingredients and may be easily applied to study AA requirement of young growing ruminants.

The third experiment was conducted to investigate the AA fluxes across the hind limb in steers during various physiological states. Eight steers were used to study the effect of dietary protein on arteriovenous (AV) concentration differences. Another four steers were used to study the effect of exogenous insulin injection and starvation on AV difference. The saphenous artery and vein were canulated in both legs of all animals five days before blood sampling and blood

flow measurements were conducted.

Plasma flow was reduced ($P<.01$) in the low protein fed and starved groups. The high protein diet markedly increased the plasma flow, while insulin had no effect on plasma flow. Arterial concentrations of almost all AA increased ($P<.05$) after feeding for all dietary treatments. Alanine was the only AA which was released from the hind limb before and after feeding at almost the same rate, while glutamine was released in higher amounts after feeding than before feeding. There were no significant differences in the arterial AA concentrations between the low protein and control diets. Some AA showed a net uptake by hind limbs in animals fed the low protein diet, but this net uptake was smaller than that observed for control diet fed animals. In steers fed the high protein diet a higher arterial AA concentration ($P<.05$) and net uptake across the hind limbs was observed for almost all AAs. Alanine and glutamine showed a net release in steers fed the high protein diet. Exogenous insulin injections had no effect on AA concentration in the arterial blood, but the hormone increased ($P<.05$) the hind limb uptake of AA at two hours after injection. Four hours after injection the net uptake of both essential and nonessential AA started to diminish. When steers were starved for either 24 or 48 hours a net release from the hind limbs of almost all AAs was observed.

To

# EGYPT

With Love

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To my wife and my two lovely children, for their patient love and loving patience.

For all those who, by accident or be design, did in anyway touch my life, I hope the encounters were positive; as for me. I hope the experiences would help to make me a better human being.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Amino acid |
| BCAA | Branched-chain amino acid |
| BCKA | Branched-chain keto acid |
| BF | Blood flow |
| BW | Body weight |
| BWG | Body wieght gain |
| CP | Crude protein |
| EAA | Essential amino acid |
| F/G | Feed/Gain ratio |
| IP | Intraperitoneal |
| NEAA | Non essential amino acid |
| PAA | Plasma amino acid |
| PAH | Para amino hippuric acid |
| PCV | Packed cell volume |
| PF | Plasma flow |
| PUN | Plasma urea nitrogen |

# INTRODUCTION

The extent to which a diet meets requirements of tissue for amino acids in the ruminant depends upon a variety of factors such as amino acid composition of the dietary protein, amount of nonprotein nitrogen in the diet, energy concentration of the diet, quantity of ruminally undegraded protein reaching the small intestine, and quantity and composition of amino acids of ruminally synthesized microbial protein. The potential for improvement in ruminant performance has been demonstrated by increased nitrogen retention from postruminal administration of limiting amino acids in ruminants fed low protein diets (Oltjen et al., 1970; Chalupa et al., 1972; Chalupa et al., 1973; Schelling et al., 1973; and Burris et al., 1976). However, nitrogen retention and amino acid responses in plasma to postruminal administration of single and specific combinations of amino acids have been variable. Lysine and methionine frequently have been identified as limiting amino acids for ruminants. Lysine concentrations in plasma were lower in steers fed urea-supplemented diets than in steers fed soybean meal-supplemented diets (Little et al., 1966; Young et al., 1973; Cross et al., 1974; Burris et al., 1975; and Hill et al., 1980), suggesting that this amino acid may be limiting in cattle fed urea diet. Methionine has also been implicated as limiting amino acid in sheep (Nimrick et al., 1970; and Schelling et al., 1973) and in steers (Chalupa et al., 1973; Fenderson and Bergen, 1975; Richardson and Hatfield, 1975a,b; Towns and Bergen, 1979) when infused or intraperitoneally injected alone or in combination with other amino acids.

Animal growth is a direct function of tissue growth. Tissue growth in turn is dependent upon the rate and extent of hypertrophy and hyperplasia of cell comprising the respective tissue. Cellular enlargment occurs as the result of nutrient uptake by the cell and the balance between anabolic and catabolic processes that regulate accretion of component and structural material of the cell.

It is now firmly established that skeletal muscle is very active in the catabolism of several amino acids, particularly leucine, isoleucine and valine and in the synthesis of others, specifically alanine and glutamine (Young, 1970; Felig, 1975; and Goldberg and Chang, 1978). Considerable interorgan transport of free amino acids thus must be occurring in the ruminant as well as in other species (Schepartz, 1973) and this transport undoubtedly is altered by diet, hormonal injection or starvation.

The first experiment in this study was designed to quantitate the methionine requirement of growing steers. The second was to study the effect of lysine on the performance and plasma amino acid and plasma urea nitrogen of preruminant calves. The third experiment was conducted to investigate the AA fluxes in the steer hind quarters during various physiological states with special emphasis on alanine, glutamine and branched-chain amino acids.

The information obtained from this study will hopefully be of benefit in increasing the animal performance.

# LITERATURE REVIEW

## I. Amino Acid Requirement

### A. Plasma Amino Acid Approach

According to Munro (1970) the plasma contains a very small proportion of the total body free amino acid pool varying from 0.2 to 6% for individual amino acid. Plasma free amino acids are rapidly renewed since the daily amino acid intake is larger than the plasma pool. Change in plasma free amino acids therefore may not reflect changes in body free amino acids as a whole (Munro, 1970). However, Bergen (1979) in his review on physiological and nutritional regulation of the free amino acid in blood of ruminants, pointed out that plasma amino acid (PAA) profiles under well defined experimental conditions, is a useful method to assess a limiting amino acid (AA) and essential AA (EAA) requirements when direct methods cannot be used. Although PAA profiles are technically easy to obtain, physiological implications of these profiles are often difficult to ascertain due to the constant flux and turnover of free AA in plasma. The sum total of all factors affecting total body AA flux (protein synthesis, protein degradation, tissue uptake and efflux and influx from the small intestine and AA catabolism) are reflected by concentrations of AA in the plasma (Bergen, 1979). In nonruminants, variations in dietary AA levels, whether from various protein sources of differing AA compositions, from excess intakes of single AA, or from AA imbalances, are reflected, at least during the absorptive phase, by AA profiles in the peripheral blood (Harper

et al., 1970). Because of microbial cell AA synthesis in the rumen (Loosli et al., 1949) it has long been recognized that there is no known dietary requirement (at least for maintenance and for a reasonable level of production) for AA in ruminants. Tissue requirements of preformed AA are similar for ruminants and nonruminants (Bergen, 1979).

The most useful aspect of PAA profiles in ruminants is not for a general assessment of protein status and animal performance but rather for studying requirements of individual EAA. When experiments are properly designed, the two-phase broken line PAA response curve becomes a useful criterion for AA adequacy as has been shown for nonruminants (Zimmerman and Scott, 1965: Mitchell et al., 1968; and Stockland et al., 1970). At least one EAA from the basal digesta from the rumen must be available at a level less than the animal's requirement so that supplemented EAA can be administered in proper fashion to use the two-phase PAA response curve (Fenderson and Bergen, 1975; and Towns and Bergen, 1979). Nimrick et al.(1970 a,b) determined only the quantity of supplemental EAA needs above those provided by the digesta flow.

Purser (1970) 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. Many workers (McLaughlan, 1963 and 1964; McLaughlan and Illman, 1967; and McLaughlan et al., 1973) 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. Cecyre et al. (1973) indicated that PAA levels decrease

gradually with time after feeding. Hogan et al. (1968), and Potter and Bergen (1974) observed that the total plasma EAA of sheep increased with each successive level of protein infused into the abomasum. Bergen et al. (1973) indicated that the changes observed in PAA papameters 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. Plasma AAs (except methionine and histidine), for the first four days of infusion, did not reflect the amino acid pattern of protein sources infused into the duodenum of sheep (Potter et al., 1972).

Young et al. (1981) conducted a series of experiments to evaluate the efficacy of measuring PAA response to abomasal infusion of amino acids as a technique for identifying limiting amino acids in growing lambs fed a basal diet. Their results indicate that all EAAs except arginine increased in response to the treatment and then returned to pretreatment level by 48 hrs postinfusion. They concluded that the utility of this method appears promising.

Independent of the dietary protein the PAA level will be affected by the metabolic state of the animal (Munro, 1964). The effect of starvation and hormonal-injection will be extensively reviewed later.

## B. The Amino Acid Requirements of Growing Animals

The amino acid requirements of nonruminants as well as ruminants have been a subject of considerable investigations for a long time and by a variety of methods. Regardless of the method used, however, the fundamental criterion for any AA requirement determination is that the AA under investigation be limiting. The limiting AA of a protein

can be defined according to Bergen (1979) as the EAA available in the least amount in relation to tissue requirement for the given AA. In ruminants the limiting AA does not relate to the EAA profile and content of dietary protein, but rather refers to the EAA profile and content of the bulk protein (composed of microbial protein, partially degraded and undegraded feed protein in various proportions) passing to the abomasum (Bergen, 1978).

The qualitative requirement of AA has to be considered as well as the quantitative requirement, therefore, we could get a better figure to use the available protein sources economically to get the best performance of the animal.

Plasma free amino acid profiles have been used as a tool to study AA requirements (McLaughlan, 1964; Zimmerman and Scott, 1965; McLaughlan and Illman, 1967; Nimrick et al., 1970a,b; Stockland et al., 1970; Young et al., 1971; Tontisirin et al., 1972; Young et al., 1972; McLaughlan et al., 1973; Brookes et al., 1973; Reis et al., 1973; Tao et al., 1974; Broderick et al., 1974; Fenderson and Bergen, 1975; Williams and Smith, 1975; Foldager et al., 1977; Towns and Bergen, 1979; Williams and Hewitt, 1979; Moore et al., 1980; Rousselow and Speer, 1980; Tzeng and Davis, 1980; and Young et al., 1981). These workers found that the first limiting AA in the diet remained at a low and constant level in plasma irrespective of the severity of the deficiency. The intersection of the two response curves represents the requirement. They concluded that the plasma technique can be used to determine the AA requirements.

The data of Morrison et al., (1961) indicate that lysine remains at a low level in the plasma of rats when it is deficient in the diet,

but increases rapidly when the dietary level is moderately in excess of that required to maximize weight gain. McLaughlan and Illman (1967) considered the requirement to be the dietary level at which the corresponding level of plasma free lysine equaled the normal fasting level. As indicated by Stockland et al. (1970) the level of free lysine in the plasma remained at a low and constant level until dietary lysine was increased to the level at which average daily gain and gain/feed ratio were maximal at which point it increased rapidly and linearly as dietary lysine was increased. The lysine requirement based on plasma free lysine was somewhat less than that obtained using average daily gain and gain/feed ratio as the response criteria.

Nimrick et al. (1970a,b) fed growing lambs semipurified diets containing urea as the sole nitrogen source. Individual EAAs were infused into the abomasum in graded amounts and nitrogen retention and PAA patterns were used as response criteria. Interpretation of nitrogen balance and PAA data were based on the well documented response of animals to the addition of the first limiting AA. Upon incremental addition of the limiting AA, nitrogen retention increases until a plateau is reached, at this point plasma concentration of the AA in question also begins to elevate rapidly. This phenomenon is similar to the broken-line response of AA in plasma described for nonruminants when requirement of an AA has been met (Zimmerman and Scott, 1965; and Stockland et al., 1970).

Essential AA requirements observed in this fashion are influenced extensively by the growth of the animals. Often animals under acute experimental conditions in metabolism stalls do not grow well, hence EAA requirements may be underestimated (Bergen, 1979). Fenderson and

and Bergen (1975) showed that with a certain ration, the supplemental or infused methionine requirement of steers growing approximately 750 g/d was 7 g/d. Another group of steers were fed the same diet (Towns and Bergen, 1979) but these steers gained only about 350 g/d. The infused methionine requirement was only 4 g/d. Fenderson and Bergen (1975) suggested that it is possible to extrapolate the requirements of the other AAs from the methionine requirement, according to the proportion of methionine to the requirements of the other AAs as reported for Swine (NRC, 1973).

Strath and Shelford (1978) infused ewe lambs abomasally with varying levels of methionine. The plasma methionine response curve was represented by two straight lines suggesting that the methionine supply was limiting for optimal production. However, using the same procedure with steers and in the presence of adequate lysine Hill et al. (1980) found that infusion of 4, 8, or 12 g/d of methionine with constant lysine (24 g/d) did not alter nitrogen retained from lysine infused alone. Plasma methionine increased linearly with infusion of incremental quantities, so they concluded that methionine was not limiting when infused postruminally with adequate lysine to growing steers fed the urea-supplemented diet with sulfur adequate. However, results of Wakeling et al. (1970) and Fenderson and Bergen (1975) showed that methionine was the first limiting AA in the ingesta reaching the small intestine of the steers.

Balance studies which measure the amount of nitrogen intake and the amount in the feces and urine, have also been used to estimate the adequacy of diets and AA requirements (Nimrick et al., 1970a,b; Boila and Davlin, 1972; Hall et al., 1974; Tao et al., 1974; Fenderson and

Bergen, 1975; Richardson and Hatfield, 1978; Anderson et al., 1980a,b; Moore et al., 1980; Rousselow and Speer, 1980; Tzeng and Davis, 1980; and Young et al., 1981). The level of AA intake or intake plus infusion which coincided with the point of inflection on the nitrogen retention curve was regarded as the requirement of that AA (Nimrick et al., 1970a,b; Nimrick and Kaminiski, 1970; Baila and Devlin, 1972; and Schelling et al., 1973). Nimrick et al., (1970a,b) found a good relationship between nitrogen retention, plasma methionine and methionine supplementation. Nitrogen retention, urea-N, and PAA were measured by Richardson and Hatfield (1978) to determine the limiting AAs, methionine was the only AA to cause the lowest urea-N 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. Abomasal infusion of graded levels of lysine to growing steers fed the urea supplemented diet resulted in increased nitrogen retention (Burris et al., 1976). This response was apparently due to lysine per se.

Plasma urea-N concentration as an indicator for AA requirement has been used by Mercer and Miller (1973). They found that the methionine requirement of lambs was 2.63 g/d. Using urinary $^{35}S$ as an indicator to confirm their results these workers obtained a value of 2.35 g/d. Tao et al. (1974) compared diverse parameters to evaluate the methionine requirement of sheep (prepared in special way for that experiment). Amounts required were calculated through the responses of urinary-N, plasma urea-N, urinary urea-N and N retention to intravenous infusion of methionine. The response curves of nitrogen

utilization indicated a methionine requirement of 4.81 to 5.0 g/d; whereas the PAA level curve showed a requirement of 3.63 g/d. In preruminant calves, Williams and Smith (1975) evaluate the PAA and plasma urea-N responses to dietary supplementation of methionine and cysteine. Response curves indicated a methionine requirement of 4.5 g/d with the PAA response and 3.9 g/d when the plasma urea-N response was used as criterion. Williams and Hewitt (1979) reported the lysine requirement of the preruminant calf as 8.5, 7.5, and 7.2 g/d using plasma urea-N, PAA and nitrogen retention, respectively. They suggested that the plasma urea-N may be an overestimated criterion. However, Phillips and Walker (1980) indicated that the lysine requirement of preruminant calves estimated by plasma urea-N technique was similar to that based on nitrogen retention. Rousselow and Speer (1980) studied valine requirement of the lactating sow. They obtained different values with the different criteria used. Valine requirement was .68% using average daily gain (ADG) whereas it was .53% when they used nitrogen retention and plasma valine response curve, the least value was .38% using plasma urea-N concentration as an indicator. Lewis et al. (1980) investigated the lysine requirement of pigs using PAA and plasma urea-N as indicators. They found that the lysine requirement was 1.25% using both criteria.

Brookes et al. (1972) investigated the AA requirements using the oxidation rate of the AA as an indicator. They 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/feed ratio were maximal. The oxidation rate of lysine was

affected by previous CP intake. They concluded that the oxidation of AA technique is a useful method for determining the dietary AA requirement of a growing rat.

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 HCl. After plotting the oxidation response against the graded levels of infusion, a breakpoint was calculated at the infusion level of 2.1 g/d of lysine. Plasma methionine response curves were in close agreement with a breakpoint at 2.4 g lysine /d. Since the amount of dietary lysine reaching the abomasum was calculated as 4.4 g/d, the lysine requirement for sheep was estimated to be between 6.5 and 6.8 g/d.

Maximum weight gain has been used as an indicator in AA requirement in growing animals (Stockland et al., 1970; Boomgaardt and Baker, 1973a,b; McLaughlan et al., 1973; Foldager et al., 1977; Anderson et al., 1980; and Rousselow and Speer, 1980). The level of AA intake or infusion which corresponds to the point of inflection on the weight gain curve is the requirement of that AA. Thus, Stockland et al. (1970) found the lysine requirement for the maximum weight gain in rats to be 0.6% of the diet which contains 10% dietary crude protein. Boomgaardt and Baker (1973a) reported that the lysine requirement for the maximum growth in chickens fed 14, 18.5 and 23% dietary protein was 4.73, 4.72, and 4.62% of the protein. Boomgaardt and Baker (1973b) found that lysine requirement for maximum growth rate in chicken to be a constant 4.62% of the dietary protein with increasing age. McLaughlan

et al. (1973) found that the lysine requirement for rat obtained by weight gain and that obtained by PAA techniques were identical (0.12% of the diet). 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 gain, nitrogen retention and plasma methionine concentration indicated a requirement for sulfur containing AA, of 3.8 to 4 g/16g N. Tzeng and Davis (1980) fed a semipurified diet containing 14 crystalline AA as the sole source of nitrogen to determine dietary needs of the young calf for lysine and methionine. Body weight gain, nitrogen balance and PAA were the criteria to assess the response of young calves to the diet. Both the dietary lysine and methionine requirement estimated from body weight gain and nitrogen balance agreed while the lysine requirement estimated by the PAA was higher and the methionine requirement was lower.

C. Amino Acid Degradation and Rumen Bypass

The main problem in studying the AA requirement in ruminant animals is the AA degradation in the rumen. Chalupa (1975) concluded that as little as 40% or as much as 80% of the dietary protein normally might be degraded in the rumen into peptides and AAs which in turn are catabolized to ammonia and carbon sources for microbial growth, or directly incorporated into microbial protein. Some of the $NH_3$-N is also lost through the rumen wall. Chalupa (1974) has investigated EAA degradation in both in vitro and in vivo experiments. The V max values indicated that arginine and threonine were rapidly degraded; lysine, phenylalanine, leucine and isoleucine formed an intermediate group; and valine and methionine were AAs least rapidly degraded.

From this viewpoint we could conclude that to get an accurate AA requirement value, AA under investigation must bypass the microbial degradation in the rumen. Many ways were suggested to bypass the rumen such as abomasal infusion, intraperitoneal injection, and esophageal groove closure.

As it has been reviewed earlier, abomasal infusion and intraperitoneal injection have been extensively used. However, several researchers have given consideration to the esophageal groove as a means to obtain rumen bypass (Wise and Anderson, 1939; Ørskovand Benzie, 1969; Ørskov et al., 1970; Ørskov, 1972; Robinson et al., 1977; Guilhermet et al., 1977; Abe et al., 1979; Standaert, 1979). The closure of the esophageal groove involves a series of coordinated reactions of the caudal thoracic esophagus, the reticular groove and the reticulo-omasal orifice (Titchen and Newhook, 1975). These reactions, which are normal functions in young ruminants, facilitate the passage of suckled liquid from the esophagus through the reticular groove and omasal canal into the abomasum. This direct passage of suckled liquid into the abomasum is associated with the contraction of the reticular groove. Factors believed to influence groove closure are age, temperature of the liquid, posture of the animal while suckling and composition of the suckled liquid (Ørskov, 1972). A little earlier Ørskov and Benzie (1969) found that the nature of the fluids tested had no effect on the groove function. Ørskov et al. (1970) indicated that if lambs are trained to suck from a bottle at weaning and do so voluntarily and eagerly, milk will continue to pass to the abomasum for months or years. Standaert (1979) was able to train two year old heifers to re-initiate suckling. The suckling habit continued for up to 10 months

in some of his animals. Wise and Anderson (1939) concluded that with the dairy calf, suckling from a nipple pail promoted groove closure while drinking from an open pail failed to enhance the passage of milk to the abomasum. Abe et al. (1979), however, found no difference in functioning of the groove reflex between the nipple-feeding method and the bucket-feeding method.

Several workers have shown improvement in growth rate and feed efficiency when nutrients bypassed the rumen via closure of the esophageal groove (Ørskov, 1972). Guilhermet et al. (1977) observed improvements in growth and feed efficiency with liquid feeding of casein and soya flour to eight week old calves. However, Robinson et al. (1977) observed no significant differences in performance of calves fed the same protein included in the basal ration. They also observed a trend towards reduced weight gains and dry matter intake with nipple fed calves.

## II. Amino Acid Metabolism in Skeletal Muscle

All protein depots are very active in the degradation of certain AA and the synthesis of others. It is now well established that skeletal muscle is the main site of catabolism of (BCAA) (leucine, Iso leucine and valine) (at least in rats), and in the synthesis of alanine and glutamine (Young, 1970; Felig, 1975; and Goldberg and Chang, 1978). Odessey and Goldberg (1972 and 1979) demonstrated that isolated rat diaphragm can rapidly degrade the three BCAA as well as several non-essential AA (NEAA), including alanine, glutamate and aspartate. By contrast, muscle does not degrade to any significant extent the carbon skeletons of other amino acids found in plasma, such as lysine, serine,

proline, threonine, methionine, cysteine, phenylalanine, histidine, tyrosine, and tryptophan (Odessey and Goldberg, 1972).

Amino acid metabolism in ruminant is of importance, not only for protein synthesis, but also for gluconeogenesis. Ruminants are known to absorb only limited quantities of glucose and thus rely heavily upon glucose synthesis by the liver (about 85%) and kidneys (about 15%) (Bergman and Heitmann, 1978). Muscle, as a reservoir for AAs, can remove or release free AAs in net amounts. Considerable interorgan transport of free AAs thus must be occurring in the ruminant as well as in other species (Schepartz, 1973). This transport is undoubtedly altered by diet, hormones or stresses such as starvation (Bergman and Heitmann, 1978).

A. Origin of Alanine and Glutamine Released by Muscle

The breakdown of BCAA in muscle generates amino groups whose accumulation in the organism could be toxic. Unlike liver, skeletal muscle lacks the enzymes to dispose of ammonia as urea. Felig (1975), and Goldberg and Chang (1978) found that the pattern of amino acid released from muscle in the postabsorptive state or during starvation was not similar to the AA composition of muscle protein. Instead, alanine and glutamine are released in much larger amounts than would be anticipated simply by the net breakdown of muscle proteins. These two amino acids are synthesized in muscle de novo (Odessey and Goldberg, 1972; and Felig, 1975) and this process appears to utilize amino groups generated by amino acid degradation. Under these conditions, muscle releases leucine, isoleucine, valine and aspartate in lesser amounts than would be expected from their frequency in tissue protein.

Alanine production by muscle seems to play an important role in the maintenance of blood glucose. In the liver, alanine represents the most important amino acid precursor for gluconeogenesis. On this basis Felig (1973 and 1975) proposed the "glucose-alanine cycle", in which alanine would carry amino groups derived from amino acid metabolism in muscle and pyruvate to the liver for conversion into urea and glucose. Accordingly the glucose synthesized by the liver can then be taken up again by muscle and be converted back to alanine. Alanine production in muscle is coupled to the degradation of the BCAA (Odessey et al., 1974). Addition of any of the BCAA to incubated muscle stimulate the production of alanine and glutamine. In starvation, when the capacity of muscle to degrade these AAs increases several times (Goldberg and Chang, 1979) alanine production also increases (Odessey et al., 1974).

Many investigators (Odessey et al., 1974; Garber et al., 1976; Grubb, 1976; Ruderman et al., 1977; Chang and Goldberg, 1978a,b; Heitmann and Bergman, 1978, Galim et al., 1980; and Tischler and Goldberg, 1980) indicated that alanine and glutamine are synthesized in muscle by the pathways summarized in Figure 1.

Transamination of the BCAA occurs almost exclusively with α-k-glutarate to form glutamate, which may either donate its amino group to pyruvate to form alanine or may incorporate free ammonia to form glutamine. The relative amounts of glutamine and alanine produced depend largely on the concentration of ammonia within the tissue. Increased levels of $NH_3$ will promote glutamine production and decrease alanine synthesis (Chang and Goldberg, 1978a). Free $NH_3$ in muscle may arise from extracellular sources or by degradation of purines and AAs (Goldberg and Chang, 1978). Thus, the BCAA appear to contribute only amino groups for the production

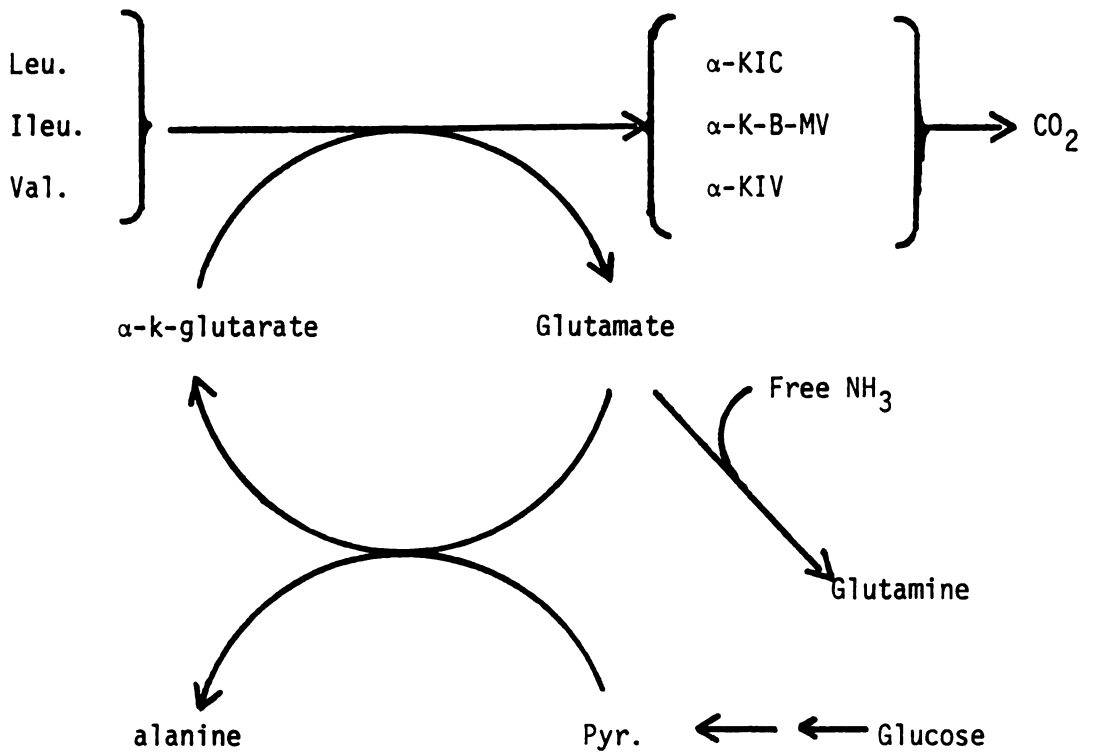


Figure 1. The pathway of alanine and glutamine synthesis in muscle.

of alanine, while exogenous glucose or muscle glycogen provides the pyruvate (Chang and Goldberg, 1978a).

Tishler and Goldberg (1980) indicated that under certain conditions, muscle may release even greater amounts of glutamine than alanine. Odessey et al. (1974), and Chang and Goldberg (1978b) believe that the origin of the carbon skeleton of glutamine are those AAs that are generated by muscle protein degradation and then enter the tricarboxylic acid cycle (i.e., valine, isoleucine, aspartate, asparagine and glutamate). When rat diaphragm is incubated in vitro, it releases much lower amounts of these five amino acids than would be anticipated from the composition of muscle protein. The missing amounts of these AAs together equaled the amount of glutamine synthesized de novo by the muscle. These findings suggested conversion of the carbon skeletons of these AAs into glutamine.

B. Alteration in AA Metabolsim in Skeletal Muscle

In nonruminant as well as in ruminant, starvation, hormonal administration and altered protein intake can result in significant alterations in amino acid concentration in plasma (Clark et al., 1968; Munro, 1979; Felig et al., 1970; Adibi, 1971; Hambraeus et al., 1976; Bergman and Heitmann, 1978; Hutson and Harper, 1981; and Motil et al., 1981), as well as the arteriovenous concentration differences (Aoki et al., 1973; Bell et al., 1975; Ballard et al., 1976; and Heitmann and bergman, 1978) which can be attributed to change in AA metabolism in the tissues.

1. Effect of dietary protein on AA metabolism in skeletal muscle

Clearly, one would anticipate marked changes in amino acid metabolism

following the ingestion of a protein meal. Hutson and Harper (1981) showed that blood BCAA concentrations of rats fed high protein diet were elevated two- to three-fold. Branched-chain keto acid concentrations were also increased about two-fold.

With respect to the effect of protein ingestion on muscle AA exchange, data are not available in ruminants. However, studies in the rat have demonstrated a net uptake by peripheral tissues of the BCAA in the absorptive period (Yamamoto et al., 1974). Studies with normal humans reveal selective splanchnic release of BCAA and uptake by muscle tissue which persists for at least three hours after protein ingestion (Felig, 1975). In contrast, alanine output from muscle continued unchanged or is reduced for only one hour after protein intake (Aoki et al., 1973; Yamamoto et al., 1974; and Felig, 1975).

Working with young men, Hambraeus et al. (1976) suggested that dietary leucine facilitate both tissue uptake of BCAA and their intracellular metabolism.

Motil et al. (1981) fed a group of young men three different levels of protein (a surfeit level, a level approximating maintenance requirements, or a grossly inadequate level). The change in protein intake from a marginal to a surfeit level was associated with an increased leucine flux and incorporation of leucine into body protein. In the fed state, oxidation of leucine increased sharply and release of leucine from tissue protein diminished. When dietary protein intake was reduced from the requirement to an inadequate level, leucine flux and body protein synthesis and protein breakdown were reduced, also a smaller reduction in leucine oxidation was noted.

When AAs are infused intravenously rather than ingested, thus

bypassing the gut and liver, the importance of alanine in AA catabolism and nitrogen disposal is also readily apparent (Felig, 1975). In the rat, infusion of each of 20 AAs resulted in increases in the alanine content of a variety of tissues (liver, kidney and muscle) (Coulson and Hernandez, 1968). From these observations it is clear that alanine is the major vehicle of α-amino nitrogen output from gut and muscle in the fed as well as the fasted state.

2. Effect of starvation on AA metabolism in skeletal muscle.

The metabolic response to starvation has been described as biphasic, the change in body fuel metabolism differing in the early and late stages of fasting (Felig, 1975). The initial response is directed at maintaining hepatic glucose output by increasing gluconeogenesis, whereas the late response is directed at maintaining body protein reserves by minimizing protein catabolism. Since stored liver glycogen is rapidly depleted in fasting (Felig, 1975), initially there is an augmented hepatic uptake of glucose precursors, notably alanine, to maintain hepatic glucose output. This increase in gluconeogenesis observed during the first three days of starvation is a consequence of augmented splanchnic fractional extraction of alanine as well as increased release of this amino acid from muscle (Odessey and Goldberg, 1972; Odessey et al., 1974; Felig, 1975; Ballard et al., 1976; and Bergman and Heitmann, 1978) which in turn comes from the breakdown of BCAA in skeletal muscle, as reviewed earlier.

During starvation, plasma level of the EAA in sheep increased significantly (Leibholz and Cook, 1967; Hogan et al., 1968; and Leibholz; 1970). They attributed this large increase in plasma free AA to tissue

breakdown and AA release during starvation.

In nonruminants skeletal muscle rapidly degrades the BCAA, This tissue is probably the major site for the degradation of these amino acids in the organism (Odessey and Goldberg, 1972, 1979; Adibi, 1976; Chang and Goldberg, 1978a,b; and Goldberg and Chang, 1978). Starvation enhances the catabolism of these AAs in man (Aoki et al., 1973; Felig, 1975; Hambraeus, 1976; and Motil et al., 1981), in dogs (Nissen and Haymond, 1981) and in rats (Buse et al., 1973; Chang and Goldberg, 1978 a,c; Goldberg and Chang, 1978; Odessey and Goldberg, 1979; Hutson et al., 1980; Goodman et al., 1981; and Hutson and Harper, 1981). However, working with fasted sheep, Ballard et al. (1976) and Bergman and Heitmann (1978) concluded that the concentration of the BCAA either decreased or remained unchanged after fasting. The arteriovenous differences noted by Ballard et al. (1976) showed that, after fasting, there was no real alanine release, while Bergman and Heitmann (1972) showed an overall release of AAs by the hindquarters especially of alanine and glutamine, which is similar to non-ruminant species. However, unlike nonruminant, BCAA are not totally catabolized in ruminant muscle. Bergman and Pell (1982) concluded that the rise in blood leucine concentration in starved sheep occurred because net leucine production by peripheral tissues overcompensated for the negligible leucine absorption by the portal-drained viscera. Also, there was little or no concomitant increase in leucine utilization in other tissues.

3. Effect of insulin on AA metabolism in skeletal muscle.

Amino acid metabolism in skeletal muscle is controlled by hormones (Manchester, 1970; Young, 1970; Call et al., 1972; and McLaughlan, 1974).

Insulin is thought to be one of the major regulators of muscle protein metabolism. Insulin and growth hormone are both necessary for growth of mammals and are usually considered to have the greatest effect on protein synthesis in muscle (Trenkle, 1974). The effect of insulin on AA metabolism has received considerable attention with respect to stimulation of protein synthesis (Munro, 1964; Manchester, 1970; McLaughlan, 1974; Bergman and Heitmann, 1978; and Goldberg et al., 1980), transport of amino acids into cells (Grubb, 1976; Hutson et al., 1980; Young, 1980; Fehlmann et al., 1981; and Horowitz and Pearson, 1981) and inhibition of protein breakdown (Mortimore and Mondon, 1970; Fulks et al., 1975; Rannels et al., 1975; Li and Goldberg, 1976; Rannels et al., 1977; and Goldberg, 1979).

The predominant effect of insulin is to increase synthesis of all proteins in about the same proportion as in normal animals (Trenkle, 1974). The level of insulin is probably the most important factor regulating protein balance in skeletal muscle (Cahill et al., 1972). After food intake, the elevated plasma levels of this hormone promote a net uptake of AAs by muscle and their incorporation into protein, while upon fasting, the fall in insulin leads to a net release of AA from muscle (Cahill et al., 1972; Felig, 1975; and Ruderman, 1975). The increase in plasma insulin level after a meal (Fajans and Floyd, 1972) or increase with diets containing protein would stimulate the uptake of amino acids by muscle, thereby protecting them from catabolism by the liver and result in overall improved utilization of AAs for protein synthesis rather than the stimulation of AA uptake by insulin being a prerequisite for protein synthesis.

Effect of three days of starvation on catabolism of leucine and α-keto isocaproic acid in skeletal muscle have been examined using a perfused hindquarter preparation of rats (Hutson et al., 1980). There was net release into the perfusate of total leucine carbon by hindquarter from starved rats, and no change with fed controls. After 30 minutes insulin addition resulted in net uptake of leucine from a reduced accumulation of α-keto isocaproic in the perfusate. With fed rat hindquarter, insulin addition resulted in a slower constant rate of oxidative decarboxylation. In both fasted and fed groups leucine incorporation increased with insulin addition. A little earlier, Grubb (1976), working with the same perfusate, found that addition of insulin resulted in a significant increase in the rate of glucose uptake and de novo alanine production.

Effect of insulin on AA metabolism has been also investigated with ruminants. Call et al. (1972) working with sheep, indicated that plasma free amino-nitrogen was slightly lower after insulin injection. The NEAA were reduced to 83% of the initial level and EAA to 66% of the initial level. Isoleucine, leucine, tyrosine, lysine, histidine, proline and arginine were significantly depressed. Apparent depression of alanine, valine, methionine and phenylalanine by insulin was less conclusive. Aspartic acid, threonine, serine and glycine were not influenced by insulin.

Brockman and Bergman (1975) and Brockman et al. (1975) studied the effects of insulin on sheep. Insulin had no effect on net hepatic removal or concentrations of the above amino acids. It did, however, decrease the concentrations of the BCAA indicating increased protein synthesis in muscle.

According to the review of Munro (1970) AA levels in the plasma can influence secretion of insulin. An oral dose of leucine causes hypoglycemia due to release of insulin. After a meal containing protein, the three BCAA often show the largest increments in the peripheral blood, which may have relevance for the increase in blood insulin level found after meals of protein. He also reported that insulin secretion is much more enhanced by a meal containing carbohydrate and protein than by giving carbohydrate or protein alone. In ruminant, however, Forbes (1980) reported that propionate plays the main role in stimulating insulin secretion. Bhattacharya and Alulu (1975) injected salts of the three major volatile fatty acids intraruminally in sheep and found that, although subsequent food intake was depressed by all treatments, only propionate significantly stimulated the secretion of insulin as measured in both portal and jugular blood.

Insulin has been shown to increase transport of AAs into skeletal muscle cells (Snipes, 1967) as well as in freshly isolated rat hepatocytes (LeCam and Freychet, 1978; Fehlmann et al., 1979; and Fehlmann et al., 1981). Membrane active transport was initiated by insulin (Horowitz and Pearson, 1981).

Goldstein and Reddy (1970) found no stimulatory effect from insulin when muscle tissue was incubated in high concentrations of AAs and suggested that insulin exerts its effects on protein synthesis in muscle almost entirely on amino acid transport.

Binding of insulin to specific receptors on the plasma membrane of target cells is important because of its role in mediation of hormone action (Etherton, 1982). Although insulin binding is essential for hormone action, binding may not represent the rate-limiting step in

the action of insulin.

In his review, Trenkle (1974) reported that all muscle proteins continue to be synthesized, but in reduced amounts, in the absence of insulin. Also, this hormone is not always essential for an increase in protein synthesis to occur. Because in its absence, there is a significant increase in protein content of muscle during work-induced growth (Goldberg 1967; 1968).

# MATERIALS AND METHODS

## I. Experiment One A

### General Design

Eight crossbred steers with an average body weight (BW) of 333 kg were fed a 9.5 % crude protein (CP) semipurified ration (Table 1) once daily at 9 a.m., at 2% (6.7 kg) of their BW. The steers were housed individually in 2 x 2 m 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. In the experimental period the steers were divided into two groups of four steers each. Each group was assigned to a split-plot design (animals as incomplete blocks) Gill,(1978; 1980) (Table 2).

The amino acids utilized were L-isomers of methionine and cysteine obtained from Sigma Chemical Company. In this study, the steers were injected intraperitoneally (IP) twice daily at 9:00 a.m. and 9:00 p.m. for five days with graded levels of methionine and methionine plus cysteine; the amounts of amino acids injected during each treatment period are presented in Table 2. Methionine was diluted in sterile saline solution 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 were raised to 6.5 using 6 N NaOH to prevent damage to the peritoneum.

The steers were given a five-day rest period after each treatment period to eliminate carryover effects from the previous treatment.

Blood samples were collected from the right jugular vein of each

TABLE 1. Composition of the diet used in Experiments One and Two

| Ingredients | International reference no. | % |
|---|---|---|
| Oats, grain | (4) 4-03-309 | 10.00 |
| Wheat, bran | (4) 4-05-191 | 5.00 |
| Corn, dent yellow grain grnd 2 US mm wt 54 | (4) 4-02-931 | 51.55 |
| Soybean seeds, solv-ext, grnd ms 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 brix | (4) 4-04-696 | 5.00 |
| Wheat, flour byproduct, fine sifted ms 4% fiber | (4) 4-05-203 | 1.00 |
| Urea (45% N) | | 0.25 |
| Limestone, grnd, mn 33% calcium[a] | (6) 6-02-632 | 1.45 |
| Trace mineral and vitamin mix[b] | | 2.00 |
| Crude Protein (N x 6.25) | | 9.50 |

[a]Calcium Carbonate.

[b]Contained in %: Zn, mn 0.35; Mn, mn 0.2: Fe, mn 0.2: mg, mn .15: Cu, mn 0.03; Co, mn 0.05; $I_2$, mn 0.007; NaCl, mx 98.5, Vitamin A palmitate, 2,000,000 IU/ton of final diet; Vitamin D, 250,000 IU/ton; Vitamin E, 55,000 IU/ton.

TABLE 2. Experimental Design of Experiment One

| Period | Steer Number | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 1 | A | H | B | E |
| 2 | B | D | E | C |
| 3 | F | B | G | A |
| 4 | G | E | A | D |
| 5 | C | F | H | G |
| 6 | H | C | D | F |

Treatments

| AA injection level (g/d) | | | | |
|---|---|---|---|---|
| Group I | | Group II | | |
| Met. | Cyst. | Met. | Cyst. | Code |
| 0 | 0 | 0 | 7 | H |
| 2 | 0 | 2 | 7 | G |
| 4 | 0 | 4 | 7 | C |
| 6 | 0 | 6 | 7 | D |
| 8 | 0 | 8 | 7 | B |
| 10 | 0 | 10 | 7 | A |
| 12 | 0 | 12 | 7 | F |
| 14 | 0 | 14 | 7 | E |

steer at the fifth day of the treatment period, two hours after the morning injection. This experiment took place between August and December 1979, the steers showed an average daily weight gain of .34 kg during that period.

## Sample Processing

Approximately 12 ml. of blood were collected in heparinized vacutainer tubes. Plasma was then obtained by centrifugation and 2 ml were deproteinized and prepared for AA analysis according to the procedures described by 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 plasma urea nitrogen (PUN).

## Chemical Analyses

### A. Plasma methionine

Methionine concentration was determined from the protein free filtrate by means of Ion Exchange Chromatography (with a Durrum Chromatography Amino Acid Analyzer Kit) according to Bergen et al. (1973).

### B. Plasma urea nitrogen (PUN)

Plasma urea nitrogen levels were determined by Conway Microdiffusion method (1960) as outlined by Fenderson (1972).

## Statistical Analysis

The data obtained were statistically analyzed according to Gill (1978).

## II. Experiment One B

### General Design

This study was carried out at the dairy farm between September and November 1980. Six male Holstein calves with an average body weight of 88 kg and approximately two months of age were used in this experiment. These animals were weaned and their rumens were developed, but kept with their active esophageal groove as means of rumen bypass by feeding milk once a day for at least two weeks before the experiment started.

The calves were divided into two groups of three each, in a completely randomized design (Gill, 1978). They were housed individually in 1.5 x 1.8 m metal stalls with free access to water.

Due to disease one calf of the group fed milk replacer supplemented with lysine died during the last week of the experimental period, therefore, the data for the last week is an average of two observations.

Milk replacer (Table 3) supplied by Milk Specialties Co., Dundee, Illinois, were fed at 60% of the DM requirements (NRC, 1978). To complete the DM requirement the ration shown in Table 1 was weighed and offered twice daily at 7:00 a.m. and 4:00 p.m. Feed refusals, if any, were collected and weighed daily before the next feeding for DM intake measurement.

Milk replacers were diluted to 13% solids with water ($37^{o}$ C), mixed with a hand beater, and fed to the animals by nipple pail in two equal meals at 7:00 a.m. and 4:00 p.m.

All animals were weighed at biweekly intervals and the amount of feed intake were adjusted according to body weight changes to calculate

TABLE 3. The composition of milk replacers used in Experiment Two.

| | Milk replacer | |
|---|---|---|
| Ingredients | A | B |
| Dried skim milk | 50 | 50 |
| Protein-fat mix (12/50) | 20 | 20 |
| Corn gluten meal | 20 | 20 |
| Dextrose | 8 | 8 |
| Premix of vit. and minerals | 2 | 2 |
| Lysine* | - | + |

*Lysine was added to milk replacer B at level of .7% on the DM basis.

the G/F ratio.

At weeks 2, 5, and 8 blood samples (approximately 12 ml) were collected immediately before morning feeding and then at 1, 2, 4, and 6 hrs after feeding. All samples were processed as described in experiment one.

Plasma lysine determination

Plasma lysine concentration was determined from the protein free filtrate by means of Ion Exchange chromatography (with a Durrum Chromatography Amino Acid Analyzer Kit) according to Bergen et al. (1973).

Statistical Analysis

The data obtained were statistically analyzed according to Gill (1978).

## III. Experiment Two

General Design

Eight crossbred steers were used to study the effect of dietary protein on the arteriovenous concentration difference. Animals were approximately 15 months old with an average BW of 510 kg. All animals were accustomed to metabolism cages and to frequent handling.

Prior to the start of the experimental period, the steers were adapted for two weeks to the control diet No. 1 (Table 4). They were then divided into two groups of four steers each. Each group was fed either high or low protein diet (Table 4) for at least two weeks before sampling. Feed intake was restricted to 90% of the ad lib. to insure cleanup. All animals had free access to water.

TABLE 4. Composition of diets used in the Third Experiment

| Ingredient | International reference no. | Diet 1 | Diet 2 | Diet 3 |
|---|---|---|---|---|
| | | - - - - - | % of DM | - - - |
| Corn, aerial pt, W. ears ensiled, mature, well-eared, mx 50% mn, 30% dry matter | 3-08-153 | 72.5 | 72.5 | 72.5 |
| Corn, dent, yellow grain, gr 2 US | (4) 4-02-931 | 20.0 | 24.0 | 12.0 |
| Soybean, seeds, Solv-ext. grnd, ms 7% fiber | (5) 5-04-604 | 4.0 | - | 12.0 |
| Limestone, grnd, mn 33% calcium | (6) 6-02-632[a] | 1.5 | 1.5 | 1.5 |
| Trace mineral and vitamin mix | | 2.0 | 2.0 | 2.0 |
| Analysis | | | | |
| Crude protein (%) | | 12.8 | 9.4 | 19.74 |
| Total Digestible Nutrient (%) | | 79.0 | 79.4 | 77.50 |
| Dry Matter (%) | | 43.81 | 42.91 | 42.91 |

[a]Calcium Carbonate.

[b]Contained in %: An, 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, mn 98.5, Vitamin A palmitate, 2,000,000 IU/ton of final diet; Vitamin D, 250,000 IU/ton; Vitamin E, 55,000 IU/ton.

Five days before sampling, steers under went surgical procedures.

Surgical Procedures

At least five days before sampling the steers were placed under general anesthesia for surgical implantation of the saphenous artery and vein cannulas. On the inside of the thigh an incision was made over the saphenous artery and vein. The two vessels were exposed and a polyvinyl tube (1.5 mm ID, 2.32 mm OD) was inserted into each vessel and passed under the skin for a distance of 30 cm to the outside of the thigh. These cannulas thus gave an arterial and venous blood sample draining the hind limb. All cannulas were filled with sterile saline containing 100 U of Heparin/ml and were flushed daily.

Both sides were cannulated, so if one side was clotted the other side could be patent.

Blood Sampling and Processing

All animals fed control, high protein or low protein diets were sampled immediately before the morning feeding and then 2, 4 hours after feeding. The insulin treated animals were sampled right before the injection and then 1, 2 and 4 hours after injection. The starved animals were sampled once after 24 hour starvation, the second sampling was taken after 48 hour starvation.

Twenty ml blood were taken from each of the two vessels at the same time and transferred to heparinized tubes and placed in ice. The plasma was obtained and prepared for AA analysis as described in Experiment 1.

After the blood sampling, only two steers were kept with their cannulas open, and they were used as control for this group. They

went through the same feeding procedure but on the control diet.

Another group of four crossbred steers were used through the same procedure to study the effect of insulin injection and starvation on the arteriovenous concentration difference. They were fed the control diet No. 1 (Table 4) for two weeks, then went under the cannulation and sampling procedures as control for this study. Two days later all four steers were injected with insulin (.20 units/kg BW) and the blood sampling repeated. The animals were then starved for 24 and 48 hours and the sampling procedure repeated.

## Plasma Flow Measurement

### A. Blood Samples

Para-amino hippuric acid (PAH) was infused into the jugular vein as a blood flow marker (Katz and Bergman, 1969). The PAH solution used was 10% (w/v); a primer dose of 15 ml was administered and was followed by continuous infusion at 45.8 mg/hr for three hours. Blood samples (5 ml each) were taken simultaneously from both cannulas at 100, 120, 140, 160 and 180 min. after the start of the PAH infusion. The blood was transferred to heparinized tubes and placed in ice.

Immediatedly after the collection of blood samples, packed cell volume (PCV) was determined by centrifugation of capillary tubes containing whole blood. The remainder of the sample was used for PAH determination.

### B. Para Amino Hippuric Acid Determination

Para amino hippuric acid concentration in blood samples was determined by procedures described by Smith et al. (1945) and modified

by Katz and Bergman (1969). (Appendix A).

C. Calculations

$$F_B = \frac{I}{C_V - C_A}$$

$$F_p = F_B \times \frac{100 - PCV}{100}$$

Net uptake ($\mu$ mole/hr) = $F_p (C_{xA} - C_{xV})$

where:

$F_B$ = Blood flow (ml/min)

I = Infusion rate of PAH (mg/min)

$C_v$ and $C_A$ = The concentration of PAH (mg/ml) in the venous and arterial blood respectively.

$F_p$ = Plasma flow (ml/min)

PCV = Packed cell volume (%)

$C_{xV}$ and $C_{xA}$ = AA concentration in venous and arterial plasma (n moles/ml) respectively.

Statistical Analysis

The data were statistically analyzed according to Gill, (1978).

# RESULTS

## AMINO ACID REQUIREMENT STUDIES
## EXPERIMENT ONE
## A

### Plasma methionine response to IP injections of methionine and methionine plus cysteine as criterion of methionine requirement:

The purpose of this study was to measure plasma methionine response to incremental levels of IP injection of methionine and methionine plus cysteine in order to evaluate the methionine requirement in growing steers in a manner similar to Fenderson and Bergen (1975) and Towns and Bergen (1979). Plasma methionine was expected to remain at a low, relatively constant level until the requirement was reached, after which it increased rapidly with higher injection level. The injection level, at which the plasma methionine concentrations begin to deflect upward, is considered the requirement.

The effect of incremental levels of methionine on its respective plasma concentration is presented in Table 5. Plasma methionine started to increase at the injection level of 4 g/d with a linear but low rate, and started to accumulate at the injection level of 8 g/d with a linear and higher rate after each successive increment. Since the same steers fed the same diet throughout the study were used, pre-injection plasma levels from the first day of the experiment were considered to represent the basal 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

TABLE 5. Plasma Methionine and Plasma Urea-N Responses to IP Injection of Graded Levels of Methionine

| Met. Injection Level[a] | Plasma Methionine μ mole/dl[b] | | Plasma Urea-N mg/dl[b] | |
|---|---|---|---|---|
| 0 | 1.25 ± 0.26 | | 7.84 ± 0.55 | |
| 2 | 1.57 ± 0.12 | NS | 7.39 ± 0.75 | NS |
| 4 | 2.20 ± 0.06 | * | 8.45 ± 0.31 | NS |
| 6 | 2.52 ± 0.16 | * | 7.20 ± 0.51 | NS |
| 8 | 3.36 ± 0.31 | ** | 7.36 ± 0.61 | NS |
| 10 | 5.06 ± 0.36 | ** | 7.28 ± 0.81 | NS |
| 12 | 7.24 ± 0.55 | ** | 7.84 ± 0.55 | NS |
| 14 | 10.75 ± 2.13 | * | 8.07 ± 0.71 | NS |

[a]Grams per day per steer.

[b]Mean and standard error of four steers.

*Statistically significant (from the zero level) at the 5% level.

**Statistically significant (from the zero level) at the 1% level.

NSNon-significance.

methionine is the so called 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. This breakpoint was equivalent to an IP injection level of 6.8 g of methionine per day (Figure 2).

Incremental levels of methionine along with 7 g of cysteine per day were injected intraperitoneally to determine if cysteine can supply part of the total sulfur amino acid need and can thus spare methionine. Under the conditions of this study plasma methionine increased immediatly (Table 6) with the first injection level (7 g cysteine + 0 g methionine) and then continued to increase rapidly with each successive increment. The biological interpretation of these data (Bergen, 1979) is that methionine could no longer be shown as limiting in the presence of adequate cysteine and that methionine passing to the small intestine from the rumen-reticulum satisfied the requirement of this AA and the cysteine and methionine combined met the TSAA requirement.

The plasma methionine response to incremental IP injection of cysteine plus methionine is presented in Figure 3.

Plasma urea nitrogen concentration (Tables 5, 6) were not influenced by either of IP methionine or methionine plus cysteine. Plasma urea nitrogen levels remained consistantly low through the experimental period with values ranging from 6.72 to 8.68 mg/dl. This was expected because the highest injection level of 21 g/d (14 g methionine plus 7 g cysteine) which represents only 3.3% of the total N intake and administered postruminally would not be expected to affect the plasma urea nitrogen substantially.

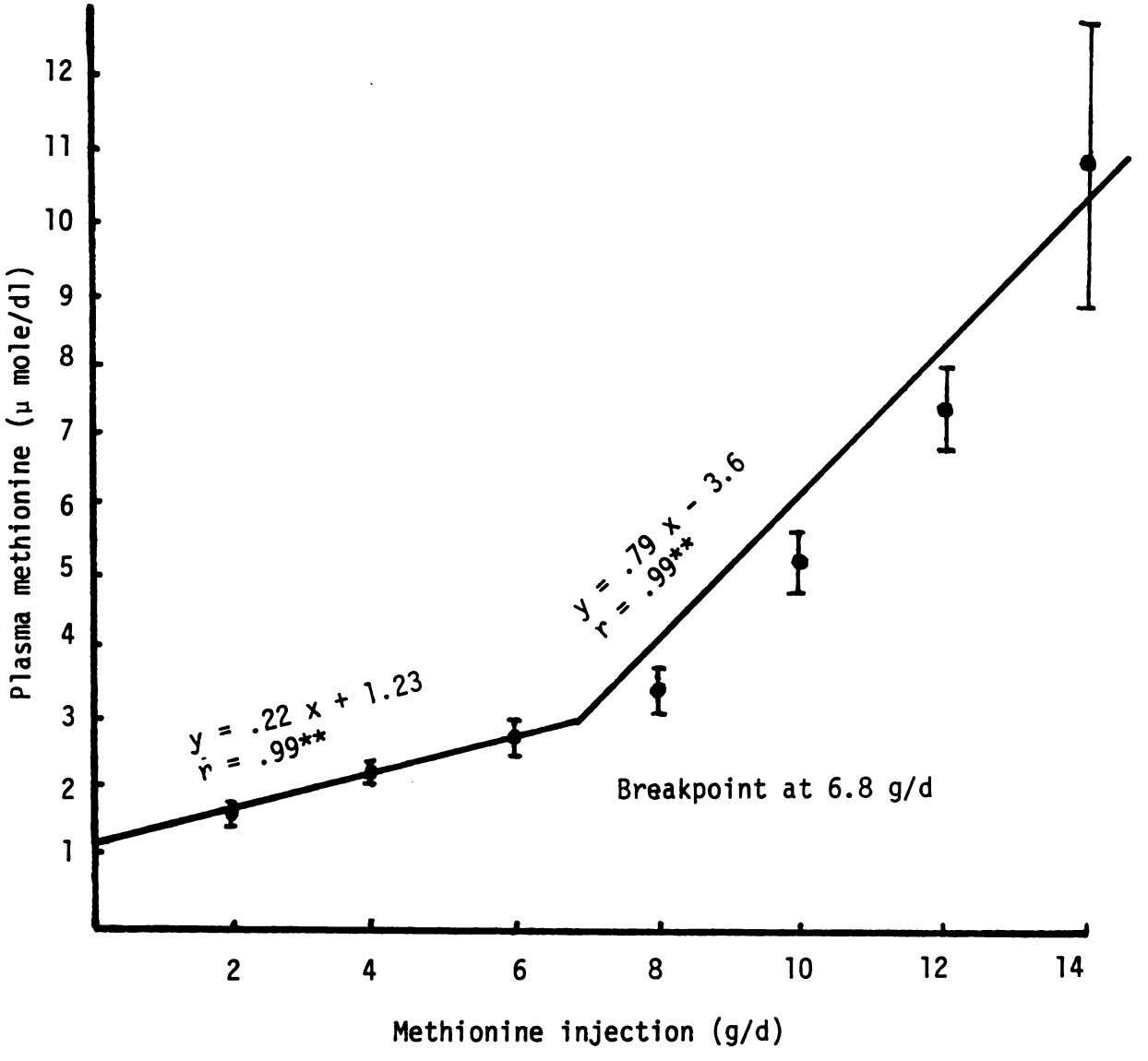


Figure 2. Plasma methionine response to IP injection of graded levels of methionine

**Correlation statistically significant (P<.01).

TABLE 6. Plasma Methionine and Plasma Urea-N Responses to IP Injection of Graded Level of Methionine Plus a Constant Level of Cysteine.

| Treatment | | Plasma Methionine | Plasma Urea-N |
|---|---|---|---|
| Met.[a] | Cyst.[a] | μ mole/dl[b] | mg/dl[b] |
| 0 | 7 | 2.36 ± 0.81 | 8.68 ± 0.27 |
| 2 | 7 | 3.46 ± 1.03 NS | 6.91 ± 0.99 NS |
| 4 | 7 | 7.29 ± 1.06 ** | 7.54 ± 0.26 NS |
| 6 | 7 | 7.47 ± 1.34 ** | 7.47 ± 0.37 NS |
| 8 | 7 | 5.56 ± 1.02 * | 7.65 ± 1.53 NS |
| 10 | 7 | 10.55 ± 3.26 * | 8.40 ± 0.55 NS |
| 12 | 7 | 12.26 ± 2.43 ** | 7.47 ± 0.49 NS |
| 14 | 7 | 9.44 ± 2.42 * | 6.72 ± 1.17 NS |

[a]Grams per day per steer.

[b]Mean and standard error of four steers.

*Statistically significant (from the zero level) at the 5% level.

**Statistically significant (from the zero level) at the 1% level.

NSNon-significance.

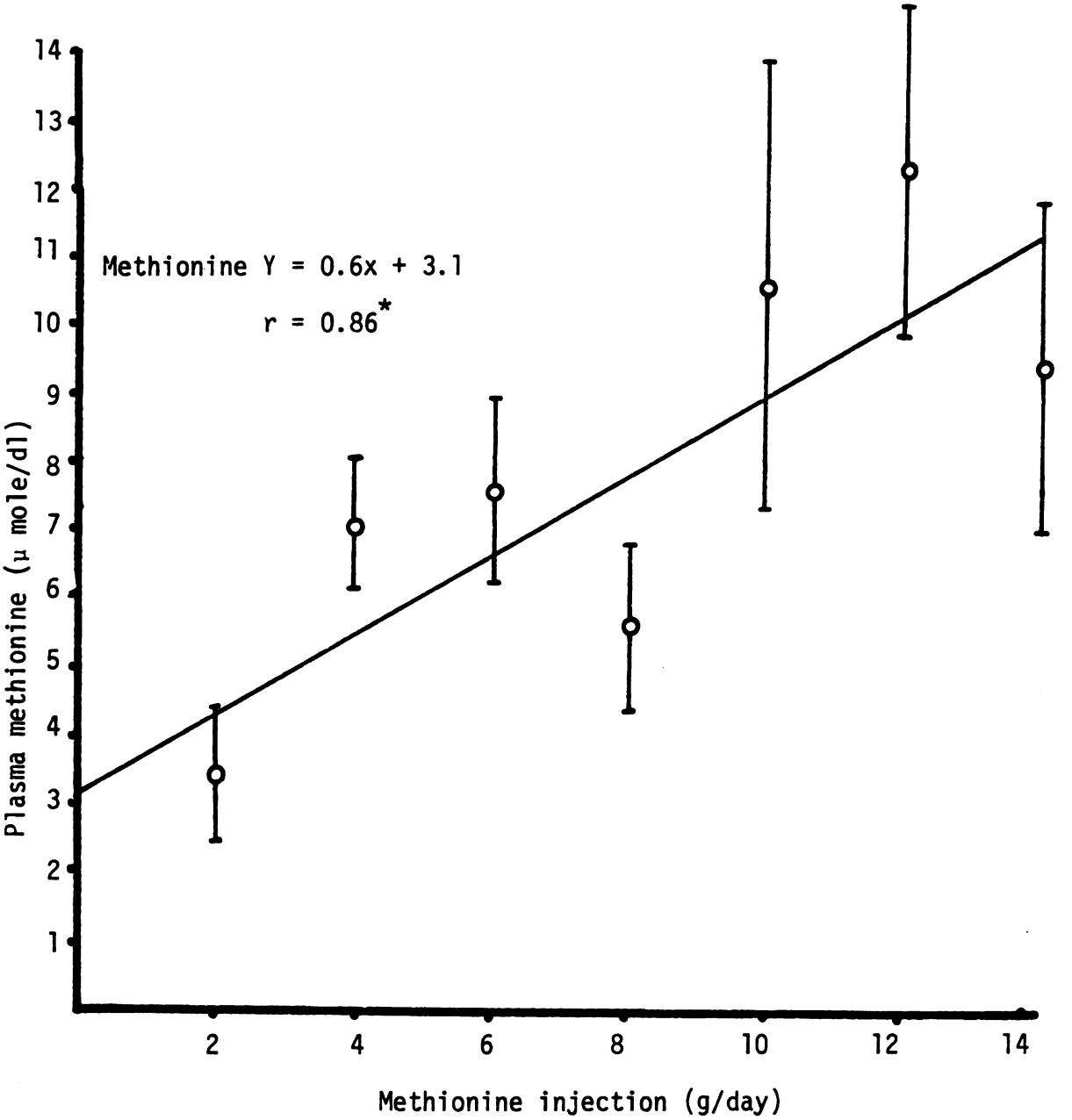


Figure 3. Plasma methionine response to IP injections of graded levels of methionine supplemented with cysteine.

*Correlation statistically significant (P<.05).

## EXPERIMENT ONE
## B

This experiment was conducted to examine the use of the esophageal groove as a method of rumen bypass to study amino acid requirements of young ruminant with developed rumen. The calves used in this study were weaned and fed dry feed to insure rumen development. Lysine addition to a gluten-based milk replacer fed at 60% of the required dry matter (NRC 1978) was used as an experimental variable. Animal performance and plasma lysine concentration were used as indicators of the occurence of rumen by-pass.

Table 7 shows changes in body weight, average daily gain (ADG), dry matter (DM) intake and gain/feed (G/F) ratio of the animals. All animals gained weight during the experiment. However, the lysine-supplemented group gained significantly more weight (.84 kg/d) ($P<.05$) than the unsupplemented group (.63 kg/d). Daily gain was within the range of gain (.36 to .90 kg/d) reported by NRC (1978).

It was also observed (as shown in Table 7) that the DM intake was not significantly different between the two dietary regimes.

The data in the same table indicates that the G/F ratio was significantly ($P<.05$) increased from 0.2 to 0.29 when lysine was added to the milk replacer.

Plasma lysine concentration at different times after feeding are summarized in Table 8. In both groups plasma lysine concentration significantly ($P<.05$) increased after feeding, reaching the maximum level at 2 hours after feeding (Table 8 and Figure 4). However, at all times after feeding plasma lysine concentrations were significantly ($P<.05$)

TABLE 7. Effect of Feeding Milk Replacers Supplemented with or without Lysine on Calf Performance[a]

| Parameter | Milk Replacer with lysine | Milk Replacer without lysine | |
|---|---|---|---|
| No. of animals | 3 | 3 | |
| Initial BW (kg) | 87.1 ± 9.3[b] | 88.6 ± 9.1 | NS |
| Final BW (kg) | 138.3 ± 15.5 | 119.5 ± 10.7 | * |
| Duration of expt. (weeks) | 8 | 8 | |
| ADG (kg/d) | .84 ± .06 | .63 ± .03 | * |
| DM intake (kg/d) | 3.39 ± .29 | 3.38 ± .19 | NS |
| G/F ratio | .29 ± .014 | .20 ± .03 | * |

[a]Calves were weaned and fed dry feed to start rumen development and the esophageal groove reflex was kept operative by feeding milk once daily. Milk replacer constituted 60% of daily dry matter intake.

[b]Means ± SE.

*Statistically significant at the 5% level.

NSNon-significance.

TABLE 8. Plasma Lysine Response to Time after Feeding Milk Replacer Supplemented with or without Lysine[a]

| Parameter | Milk Replacer with lysine | Milk Replacer without lysine |
|---|---|---|
| | μ mole/dl | |
| I. hrs after feeding | | |
| 0 | 1.81 ± 0.21[b] | 1.08 ± 0.17[b] |
| 1 | 8.76 ± 1.50[c,e] | 4.05 ± 1.07[d] |
| 2 | 11.99 ± 1.27[c] | 4.09 ± 0.76[d] |
| 4 | 11.43 ± 1.95[c] | 1.50 ± 0.15[b] |
| 6 | 6.53 ± 1.11[e] | 1.73 ± 0.29[b] |
| II. weeks of feeding | | |
| 2 | 2.72 ± 0.46[b] | 1.99 ± 0.39[b] |
| 5 | 7.76 ± 1.88[c] | 3.18 ± 0.44[b] |
| 8 | 10.27 ± 2.33[c] | 4.93 ± 2.13[b] |

[a]Values are means and standard error of three calves.

[b,c,d,e]Values not sharing common superscript in rows or columns were significantly different ($P<.05$).

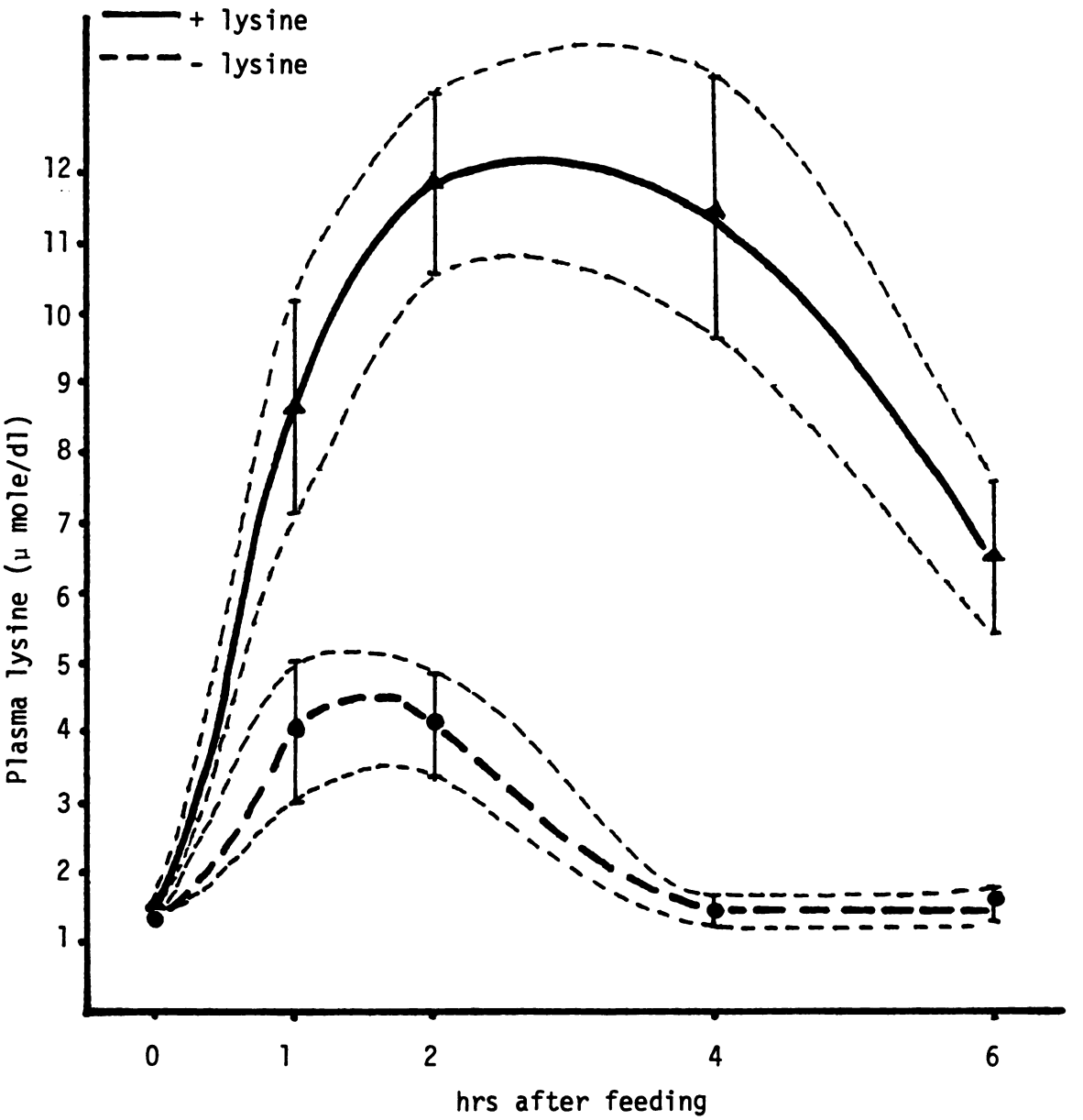


Figure 4. Plasma lysine response to time after feeding of young ruminant calves fed milk replacer (at 60% of DM intake) supplemented with or without lysine.

higher for the lysine-supplemented group than lysine level in plasma of the calves fed the replacer devoid of supplemented lysine. The plasma lysine in the latter group decreased to prefeeding levels four hours post feeding, while in the lysine-supplemented group plasma lysine concentrations did not return to prefeeding levels even after six hours (Figure 4). Table 8 also shows that in both groups, the plasma lysine concentrations, sampled at two hours after feeding increased throughout the experimental period. In the lysine-supplemented group plasma lysine increased at a significantly ($P<.05$) higher rate than the unsupplemented group, which also increased in linear pattern. This indicates that the lysine intakes for both feeding regimes were apparently exceeding the lysine requirements especially at five and eight weeks of the experiment.

# DISCUSSION

## AMINO ACID REQUIREMENT STUDIES
## EXPERMENT ONE A

The plotting of a two-phase plasma amino acid response curves is a well established procedure in the study of AA requirements (Zimmerman and Scott, 1965; Mitchell et al., 1968; Stockland et al., 1970; Young et al., 1971; Brookes et al., 1973; Reis et al., 1973; Broderick et al., 1974; Tao et al., 1974; Fenderson and Bergen, 1975; Williams and Smith, 1975; Foldager et al., 1977; Towns and Bergen, 1979; Tzeng and Davis, 1980).

Plasma met responses with IP supplementation of met alone (Figure 1,2) are in agreement with the results of Fenderson and Bergen (1975) and Towns and Bergen (1979); who, working with growing steers fed a similar ration, found that the methionine supply in abomasal flow was limiting. Methionine was also the first limiting AA in the studies of Nimrick et al. (1970a) for growing lambs, Wakeling et al. (1970) for sheep, Richardson and Hatfield (1978) for growing steers and Tzeng and Davis (1980) for young calves.

The methionine needed above that supplied from digesta flow reported in the literature vary from 2 to 14 g/d. Williams and Hewitt (1979) reported a methionine requirement of the preruminant calf of 2.1 g/d (depended on the lysine/methionine ratio in the carcass), while Williams and Smith (1975) and Towns and Bergen (1979) found the supplemental requirement to range between 3.8 and 4.5 g/d. The methionine requirement

reported by Foldager et al. (1977) for preruminant calf and the supplemental methionine reported by Fenderson and Bergen (1975) for growing steers ranged from 5.9 to 7 g/d. The highest methionine requirement reported for the young calf was by Tzeng and Davis (1980) at 10.2 to 13.8 g/d.

Using the two different accumulation ratios (in the manner of Tzeng and Davis, 1980) the two regression lines (Figure 2) intersected at a breakpoint equivalent to an IP injection level of 6.8 g/d. Therefore, the supplemental methionine requirement over the digesta flow is 6.8 g/d. This value is in agreement with those reported by Fenderson and Bergen (1975) and Foldager et al. (1977).

The immediate and linear increase in plasma methionine during IP injection of cysteine plus methionine indicated that methionine was spared in the presence of adequate (or probably excess) cysteine. Methionine also was not limiting in the study of Hill et al. (1980) when infused postruminally to growing steers fed a urea-supplemented diet adequate in sulfur. This response was similar to that in lambs infused with 3 g methionine or higher daily (Strath and Shelford, 1978). However, the present data disagree with those of Towns and Bergen (1979); who, working with growing steers fed a similar diet, obtained a breakpoint on the plasma methionine response curve at 3.8 g/d when methionine was injected with 7 g cysteine. This disagreement probably was because their last treatment did not produce a linear increase in plasma methionine response curve, but a smaller increment which resulted in a sigmoid-type response. Therefore, not to destroy the linearity of the plasma response curve, these workers avoided taking this point into account. Also, plasma methionine concentrations at the injection

levels of 0 and 3 g/d were the same; the overall linear regression does not apply. This type sigmoid shape was reported by others (Mitchell et al. 1968; Young et al. 1971).

In ruminants, the determination of AA requirements must include an estimation of the abomasal flow of AAs so that the total (abomasal flow plus the injected amount) intake can be properly evaluated (Fenderson and Bergen, 1975). The methionine requirement was calculated by adding the amount of injected methionine needed to produce the breakpoint in the plasma response to the amount of methionine absorbed across the intestinal epithelieum. The amount of dietary N and AA 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 diet as reported by Fenderson and Bergen (1975). Using the DM intake of 6.7 kg/d of the present study, dietary N reaching the abomasum was 99.82 g/d which represents 98% of the 101.84 g of N ingested daily. Amino acids reaching the abomasum amounted to 5.03 g/d for cystine, 10.72 g/d for methionine and 15.75 g/d for TSAA.

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 7.5 g/d of methionine and 3.52 g/d of cystine, adding up to 11.02 g/d of total sulfur amino acids.

Based on the data obtained here with those of Fenderson and Bergen (1975), the absorbable methionine requirement ranges from 7.5 to 14.3 g/d, while that of TSAA (absorbable) was 17.82 g/d. This is in

TABLE 9. Nitrogen and Amino Acid Passage in Steers Fed the 9.5% Crude Protein Ration Utilized in Experiment One.[a]

| Item | g/kg feed | g/day[b] |
|---|---|---|
| Nitrogen | 14.9 | 99.83 |
| Crude Protein[c] | 93.1 | 623.77 |
| Methionine | 1.6 | 10.72 |
| Cystine | .75 | 5.03 |
| TSAA[d] | 2.35 | 15.75 |

[a]Based on data by Fenderson and Bergen (1975).
[b]Dry matter intake 5.7 kg/day.
[c]N x 6.25.
[d]Total sulfur amino acids.

TABLE 10. Quantitation of Methionine and Total Sulfur Amino Acids.

| AA | Passage to Abomasum[a] (g/d) | Digestibility coefficient[b] % | Absorbable AA (g/d) | AA injected IP to breakpoint (g/d) | | Requirement (g/d) based on | |
|---|---|---|---|---|---|---|---|
| | | | | Met. | Met & Cys. | Met. | Met & Cys. |
| Methionine | 10.72 | 70 | 7.50 | 6.8 | - | 14.3 | 7.50 |
| Cystine | 5.03 | 70 | 3.52 | - | 7.0 | 3.52 | 10.32 |
| TSAA | 15.75 | 70 | 11.02 | 6.8 | 7.0 | 17.82 | 17.82 |

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

[b]Hogan (1973)

[c]Total sulfur amino acids.

agreement with Fenderson and Bergen (1975), who obtained a value of 18.6 g/d. According to these values (Table 10), 7.5 g methionine and 3.52 g cystine out of the TSAA of 17.82 were recovered from the digesta flow. The remaining 6.8 g/d need to be supplemented. Therefore, the 7 g cysteine injected at the first level (Figure 3) covered this need and a linear increase might be expected with each incremental level of methionine and/or cysteine injected.

The minimum methionine requirement obtained from this work represents 42% of TSAA. This is in agreement with the estimation of Fenderson and Bergen (1975) which was 41% of TSAA.

## EXPERIMENT ONE
## B

After the rumen has developed, the esophageal groove reflex can be kept actively working by suckling milk (or other liquids) to older ruminants (Standaert, 1979) as well as the young ruminants (Abe et al., 1979). So this approach can be used as a mechanism of rumen bypass to study amino acid requirements.

There are practical and economic limitations to the estimation of AA requirements. It is very expensive to formulate a diet that is markedly limiting in one EAA in which all or almost all of the AAs are provided in crystalline form. It is cheaper to replace some of the milk protein with an alternative protein which is markedly deficient in one EAA to produce a diet which is palatable and does not result in digestive disturbances. A protein that can serve this purpose is corn gluten (Williams and Hewitt, 1979) which has a very low lysine content and is readily digested.

In the present study the addition of lysine to a milk replacer deficient in lysine for growing ruminant calves significantly improved the animals performance as assessed by the average daily gain (ADG) and G/F ratio (Table 7). This was in agreement with other studies. Lysine increased N retention in growing lambs (Schelling and Hatfield, 1968), in growing steers (Burris et al., 1976; Richardson and Hatfield, 1978; Hill et al., 1980), and in growing young calves (Williams and Hewitt, 1979; Tzeng and Davis, 1980). Lysine also increased ADG (Williams and Hewitt, 1979; Tzeng and Davis, 1980).

In the present study, lysine supplementation to the milk replacer led to a marked increase in plasma concentrations of lysine at all hours after feeding (Figure 4) as well as weeks after feeding (Table 8). The increases in plasma lysine here indicated that the groove reflex worked and the rumen bypass occurred. This method to determine rumen bypass occurrence has been previously used by other workers (Huber et al., 1967; Robinson et al., 1976). Rapid rise in serum glucose after suckling showed that suckled glucose by-passed the rumen.

Burris et al. (1976) reported an accumulation of lysine in plasma when graded levels of lysine were abomasally infused in steers. In the first postfeeding plasma sample (Figure 4) the plasma lysine increased in concentration in both groups. However, increases were relatively larger in lysine-supplemented animals compared to increases when the unsupplemented lysine replacer was fed. This is in agreement with the observations of Williams and Hewitt (1979) and Tzeng and Davis (1980).

The increase in plasma lysine concentration with weeks after feeding (Table 8) indicates that either the lysine was supplied in excess (Nimrick

et al., 1970a) or that physiological demand for lysine decreased while the animals become older.

This study indicates that the proposed system is sensitive and could be easily applied to study amino acid requirements of young ruminants with functional rumen.

# RESULTS

## AMINO ACID METABOLISM STUDIES

## EXPERIMENT TWO

### I. Effect of Dietary Protein

The blood flow (BF), packed cell volume (PCV) and plasma flow (PF) of steers fed control, low-protein and high-protein diets are in Table 11. The BF and PF significantly decreased ($P<.01$) when the low protein diet was fed. When the high protein diet was fed, BF and PF increased above controls, but these increases were not significantly due to a high standard error. There were no significant differences between the three treatments for PCV, which had an overall mean value of 29% of the whole blood.

The arteriovenous (AV) concentration differences and the net uptake of amino acids by the hind limb of the control-fed steers immediately before feeding ($T_0$), at two hours ($T_2$) and at four hours ($T_4$) after feeding are presented in Tables 12, 13 and 14, respectively.

Data in Table 12 indicates that before feeding there was net release of almost all AAs, especially ser, ala, and BCAA. When the animals were fed, the arterial PAA significantly increased at $T_2$ and $T_4$ (Tables 13 and 14). Increases, were mainly in the concentrations of asp, thr, ser, glu, pro, val, cyst, met, ile, tyr, phe, orn, lys, and his. There was also a significant net uptake by the hind limb at $T_2$ and $T_4$ of asp, thr, ser, asn, pro, val, ile, tyr, orn, lys, his, arg, EAA, NEAA and BCAA. As shown in the same tables, ala was released by the hind limb at the same rate as before feeding while gln was released in significant

TABLE 11. Effect of Dietary Protein Level on the Plasma Flow Rate Across the Hind Limb

| Treatment | Criteria | | |
|---|---|---|---|
| | Blood flow liter/hr | Packed Cell Vol. % | Plasma flow liter/hr |
| Control diet[a] | 640 ± 22.8[c] | 28.3 ± 1.22[c] | 459 ± 15.9[c] |
| High protein diet[b] | 919 ± 238.0[c] | 29.1 ± 1.17[c] | 652 ± 168.9[c] |
| Low protein diet[b] | 372 ± 39.2[d] | 30.3 ± 0.62[c] | 259 ± 27.3[d] |

[a]Values are means and standard error of two steers.

[b]Values are means and standard error of four steers.

[c,d]Values not sharing common superscript in column were significantly different ($P<.01$).

TABLE 12. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet (Immediately before Feeding).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.66 ± .14 | 2.59 ± .32 | -0.93 ± .28 | -4.27 ± .83 |
| Thr | 6.22 ± .15 | 7.21 ± .24 | -0.99 ± .19 | -4.54 ± .41 |
| Ser | 9.45 ± .03 | 18.50 ± .58 | -9.05 ± .65 | -41.54 ± 2.52 |
| Asn | 1.63 ± .20 | 2.00 ± .18 | -0.37 ± .04 | -1.70 ± .09 |
| Glu | 11.94 ± .13 | 7.52 ± 1.41 | 4.42 ± 1.18 | 20.29 ± 5.88 |
| Gln | 18.14 ± .13 | 18.88 ± 1.01 | -.74 ± .98 | -3.40 ± 4.04 |
| Gly | 28.58 ± .94 | 24.51 ± 3.10 | 4.07 ± 2.16 | 18.68 ± 9.91 |
| Ala | 10.18 ± .18 | 19.23 ± .54 | -9.05 ± .86 | -41.54 ± 1.65 |
| Pro | 9.99 ± .56 | 6.83 ± .45 | 3.17 ± .16 | 14.52 ± .50 |
| Val | 15.30 ± .45 | 17.72 ± 1.44 | -2.42 ± .99 | -11.11 ± 4.54 |
| Cyst | 1.11 ± .06 | 0.94 ± .11 | 0.17 ± .05 | .78 ± .23 |
| Met | 0.26 ± .06 | 0.40 ± .07 | -0.14 ± .05 | -.64 ± .23 |
| Ile | 7.13 ± .09 | 8.53 ± .33 | -1.40 ± .14 | -6.43 ± .64 |
| Leu | 10.46 ± 2.58 | 12.78 ± .97 | -2.32 ± 1.61 | -10.65 ± 7.39 |
| Tyr | 2.83 ± .08 | 3.92 ± .73 | -1.09 ± .05 | -5.0 ± .23 |
| Phe | 4.57 ± .11 | 3.53 ± 1.88 | 1.04 ± 1.77 | 4.77 ± 8.12 |
| Orn | 12.12 ± .15 | 13.79 ± .57 | -1.67 ± .42 | -7.64 ± 1.93 |
| Lys | 8.08 ± .10 | 9.19 ± .38 | -1.11 ± .38 | -5.09 ± 1.29 |
| His | 6.66 ± .37 | 4.55 ± .30 | 2.11 ± .17 | 9.68 ± .32 |
| Arg | 7.37 ± .19 | 7.72 ± .77 | -0.35 ± .02 | -1.61 ± .09 |
| EAA[a] | 66.05 ± 4.10 | 71.63 ± 5.62 | -5.58 ± 1.52 | -25.61 ± 6.81 |
| NEAA[b] | 107.63 ±12.60 | 118.71 ± 8.40 | -11.08 ± 4.26 | -50.86 ±19.28 |
| BCAA[c] | 32.89 ± 3.12 | 39.03 ± 2.64 | -6.14 ± 0.48 | -28.18 ± 2.21 |

Values are means and standard error of two steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids

[c]BCAA = Branched-chain amino acids

TABLE 13. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet (Two Hours after Feeding).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 2.79 ± .19* | 2.07 ± .79 | .72 ± .42 | 3.30 ± 2.75 |
| Thr | 7.78 ± .05** | 6.84 ± .67 | .94 ± .02 | 4.31 ± 2.85 |
| Ser | 19.37 ± 1.77** | 9.16 ± .18 | 10.21 ± 1.59 | 46.86 ± 7.30** |
| Asn | 1.61 ± .06 | 2.06 ± .77 | -0.45 ± .11 | -2.07 ± 0.50** |
| Glu | 12.61 ± .72 | 9.75 ± .52 | 2.86 ± .26 | 13.13 ± .92 |
| Gln | 12.54 ± .45** | 20.70 ± 1.75 | -8.16 ± 1.36 | -37.45 ± 5.97* |
| Gly | 30.96 ± .73 | 24.20 ± 1.69 | 6.76 ± .36 | 31.03 ± 1.65 |
| Ala | 11.10 ± .16 | 19.63 ± 1.80 | -8.53 ± .64 | -39.15 ± 2.94 |
| Pro | 12.98 ± 1.02 | 8.91 ± .95 | 4.07 ± .67 | 18.65 ± .32** |
| Val | 17.02 ± .10 | 16.56 ± .96 | .46 ± .86 | 2.11 ± 3.95 |
| Cyst | 1.37 ± .08 | 1.32 ± .17 | .05 ± .03 | .23 ± .14 |
| Met | .37 ± .09 | .22 ± .08 | .15 ± .06 | .69 ± .05 |
| Ile | 9.57 ± .02** | 8.33 ± .48 | 1.24 ± .46 | 5.69 ± 2.11* |
| Leu | 14.37 ± .13 | 12.19 ± 1.68 | 2.18 ± .55 | 10.01 ± 2.52 |
| Tyr | 4.06 ± .11** | 3.32 ± .43 | .74 ± .32 | 3.40 ± 1.47** |
| Phe | 5.27 ± .20 | 4.80 ± .37 | .47 ± .17 | 2.16 ± .78 |
| Orn | 19.31 ± .47** | 13.28 ± .63 | 6.03 ± .76 | 27.68 ± .73** |
| Lys | 12.87 ± .31** | 8.85 ± .42 | 4.02 ± .17 | 18.45 ± .50** |
| His | 8.65 ± .68 | 5.94 ± .63 | 2.71 ± .05 | 12.43 ± .23** |
| Arg | 8.34 ± .40 | 7.76 ± 1.48 | .58 ± .08 | 2.66 ± .37** |
| EAA[a] | 84.24 ± 1.98* | 71.49 ± 4.69 | 12.75 ± 2.71 | 58.52 ±12.43* |
| NEAA[b] | 128.70 ± 5.76 | 114.40 ± 7.92 | 14.30 ± 1.66 | 65.64 ± 7.62* |
| BCAA[c] | 40.96 ± 0.25 | 37.08 ± 2.62 | 3.88 ± 1.87 | 17.81 ± 8.58* |

Values are means and standard error of two steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than before feeding (P<.05).

**Significantly different than before feeding (P<.01).

TABLE 14. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet (Four Hours after Feeding).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 2.65 ± .16* | 1.73 ± .62 | .92 ± .14 | 4.22 ± .64** |
| Thr | 7.30 ± .15* | 5.53 ± .79 | 1.77 ± .69 | 8.12 ± 2.94* |
| Ser | 25.43 ± .12** | 12.64 ± 1.66 | 12.79 ± 1.48 | 58.71 ± 6.79** |
| Asn | 1.80 ± .21 | 2.57 ± 1.32 | -.77 ± .11 | -3.53 ± .50** |
| Glu | 13.92 ± .14** | 8.30 ± 1.13 | 5.62 ± .01 | 25.80 ± .46 |
| Gln | 14.95 ± .13** | 24.72 ± 3.67 | -9.77 ± 3.54 | -44.84 ±16.25 |
| Gly | 31.11 ± .95 | 19.64 ± 2.32 | 11.47 ± 1.37 | 52.65 ± 6.29 |
| Ala | 9.76 ± .20 | 15.83 ± 1.98 | -6.07 ± 1.73 | -27.86 ± 7.94 |
| Pro | 13.34 ± .56* | 7.71 ± 1.14 | 5.63 ± .58 | 25.82 ± 2.66* |
| Val | 21.28 ± .46** | 14.35 ± 1.95 | 6.93 ± 1.99 | 31.81 ± 6.84* |
| Cyst | 1.57 ± .07* | 1.20 ± .73 | .37 ± .06 | 1.70 ± .28 |
| Met | .98 ± .06** | .85 ± .20 | .13 ± .14 | .60 ± .64 |
| Ile | 8.90 ± .10** | 6.79 ± 1.15 | 2.11 ± 1.05 | 9.68 ± 4.82 |
| Leu | 11.11 ± 2.60 | 10.13 ± 1.39 | .98 ± 1.27 | 4.50 ± 5.55 |
| Tyr | 4.15 ± .09** | 3.10 ± .38 | 1.05 ± .29 | 4.82 ± 1.33** |
| Phe | 5.44 ± .11* | 4.41 ± .37 | 1.03 ± .26 | 4.73 ± 1.19 |
| Orn | 13.25 ± .17* | 10.25 ± 2.21 | 3.00 ± 2.64 | 13.77 ± 9.36 |
| Lys | 8.83 ± .11* | 6.83 ± 1.47 | 2.00 ± 1.36 | 9.18 ± 6.25 |
| His | 8.89 ± .37* | 5.14 ± .76 | 3.75 ± .39 | 17.21 ± 1.79* |
| Arg | 8.11 ± .19 | 5.82 ± .80 | 2.29 ± .61 | 10.51 ± 2.80* |
| EAA[a] | 80.84 ± 4.15 | 59.85 ± 8.88 | 20.99 ± 4.78 | 96.34 ±21.71* |
| NEAA[b] | 131.93 ± 2.80 | 107.69 ±13.82 | 24.24 ±11.62 | 111.26 ±50.58 |
| BCAA[c] | 41.29 ± 3.16 | 31.27 ± 4.49 | 10.02 ± 1.38 | 45.99 ± 6.10** |

Values are means and standard error of two steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than before feeding (P<.05).

**Significantly different than before feeding (P<.01).

higher amount than before feeding.

The net uptake of ala, BCAA, glu, gln, EAA and NEAA at different times after feeding by the hind limb of the steers fed the control diet is graphically displayed in Figure 5.

Tables 15, 16 and 17 present the AV difference and the net uptake of AA by the hind limb of steers fed the low protein diet at different times after feeding. These results (Tables 15, 16, 17) when compared to their respective results obtained with the control animals (Table 12, 13, 14) indicate that there were no significant differences in the arterial PAA concentration between the two diets except for ser, pro, and his which were significantly ($P<.05$) decreased at $T_4$.

Data in Table 15 ($T_0$) shows a net release of almost all AAs, mainly ala which accounts for 89% of the total NEAA released. This is in agreement with the data in Table 12 ($T_0$-control) in which ala accounted for 82% of the total NEAA released by the hind limb of the steers fed the control diet.

At $T_2$ (Table 16) there were significant net uptake of only pro, cyst and his. However, the net uptake of those AAs was small when compared to the respective control-fed-group. At $T_4$, further uptake of asp, ser, glu, gly, pro, cyst and his by the hind limb was observed, but this net uptake of amino acids was also small when compared to the results obtained with the control-fed steers. This small net uptake in steers fed the low protein diet was not only due to a small AV difference but also to the low PF rate across the hind quarter (Table 11).

Figure 6 shows that ala and gln behaved similarly in the low protein and the control steers (Figure 5). The ala was released in almost equal amount before and after feeding, while gln was released

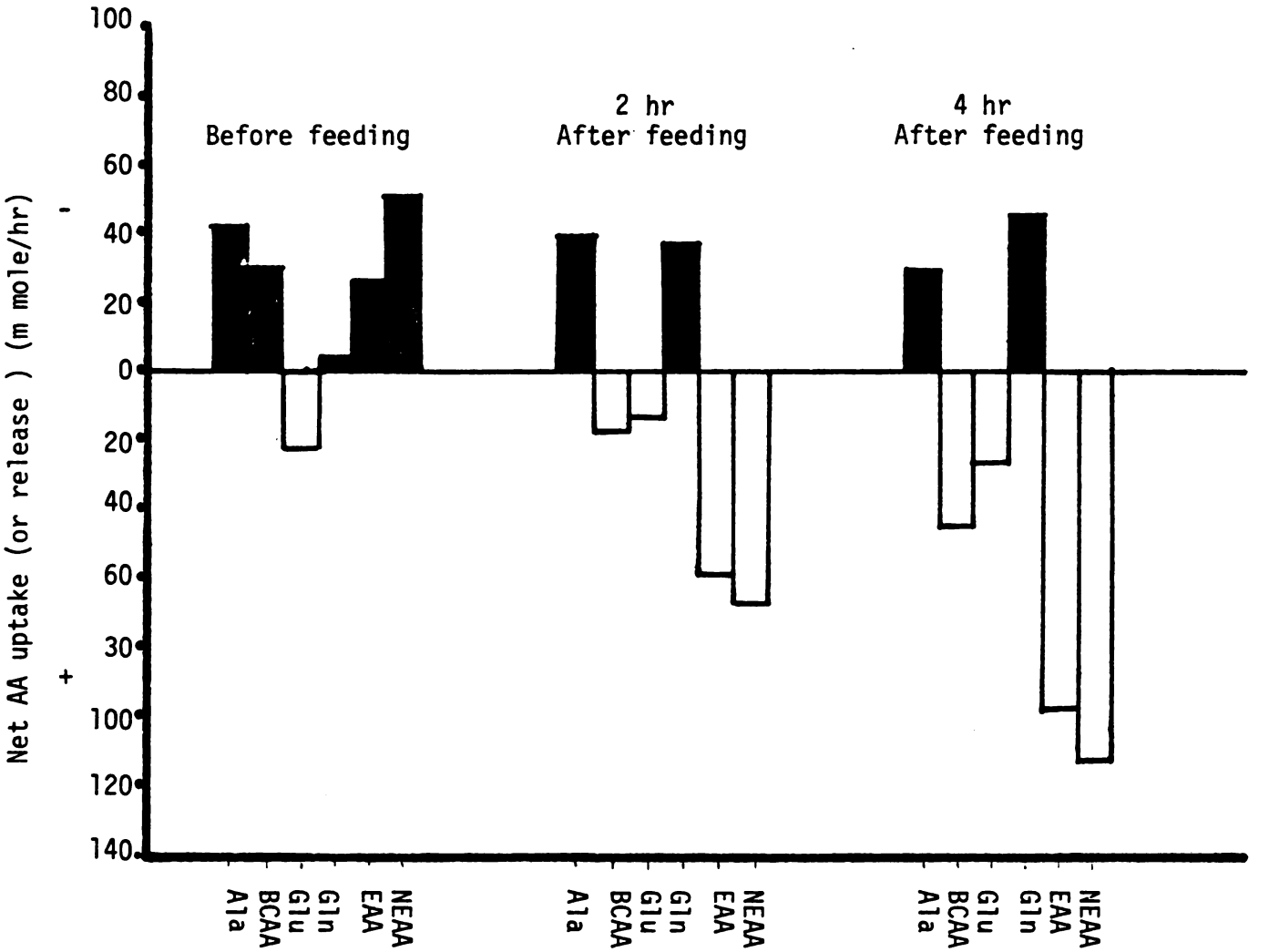


Figure 5. The net amino acid uptake (or release) by the hind limb of steers fed the control diet (I).

TABLE 15. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Low Protein Diet (Immediately Before Feeding)

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.96 ± .23 | 2.24 ± 1.29 | - .28 ± .06 | - .73 ± .16 |
| Thr | 6.01 ± .24 | 6.54 ± .66 | - .53 ± .36 | -1.37 ± .93 |
| Ser | 15.65 ± 3.13 | 14.45 ± 1.76 | 1.20 ± 1.87 | 3.11 ± 3.55 |
| Asn | 1.40 ± .15 | 1.83 ± .16 | - .43 ± .05 | -1.11 ± .13 |
| Glu | 11.81 ± .53 | 11.47 ± 1.38 | .33 ± .85 | .85 ± 2.20 |
| Gln | 14.99 ± 1.18 | 18.18 ± 1.76 | -3.19 ± .53 | -8.26 ± 1.37 |
| Gly | 22.79 ± 3.56 | 22.81 ± 3.80 | -0.02 ± .24 | - .05 ± .62 |
| Ala | 12.21 ± 1.74 | 26.05 ± 1.56 | -13.84 ± .78 | -39.95 ± .47 |
| Pro | 9.27 ± 1.01 | 9.12 ± .42 | .15 ± .58 | .39 ± 1.53 |
| Val | 16.12 ± 1.63 | 16.22 ± 1.43 | - .10 ± .26 | - .26 ± .52 |
| Cyst | 1.21 ± .09 | 1.37 ± .08 | - .16 ± .07 | - .41 ± .03 |
| Met | .73 ± .11 | .35 ± .14 | .38 ± .03 | .98 ± .08 |
| Ile | 7.14 ± .55 | 7.45 ± .82 | - .31 ± .27 | - .80 ± .70 |
| Leu | 10.56 ± .34 | 11.47 ± 1.91 | - .91 ± .67 | -2.36 ± 1.74 |
| Tyr | 2.81 ± .10 | 3.40 ± 1.45 | - .59 ± .35 | -1.53 ± .91 |
| Phe | 4.05 ± .14 | 4.61 ± 1.42 | - .56 ± .28 | -1.45 ± .73 |
| Orn | 11.45 ± .69 | 11.79 ± 1.32 | - .35 ± .63 | - .90 ± 1.63 |
| Lys | 7.63 ± .46 | 7.86 ± .83 | - .23 ± .42 | - .60 ± 1.09 |
| His | 6.18 ± .67 | 6.08 ± .28 | .10 ± .39 | .26 ± 1.01 |
| Arg | 5.23 ± .90 | 6.33 ± .99 | -1.10 ± .69 | -2.85 ± .23 |
| EAA[a] | 63.65 ± 5.04 | 66.91 ± 6.57 | -3.26 ± 1.58 | -8.44 ± 3.96 |
| NEAA[b] | 105.55 ±12.41 | 122.71 ±12.86 | -17.16 ± 3.45 | -44.44 ± 1.66 |
| BCAA[c] | 33.82 ± 2.52 | 35.14 ± 8.26 | -1.32 ± .74 | -3.42 ± 1.92 |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

TABLE 16. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids By the Hind Limb of Steers Fed the Low Protein Diet (Two Hours After Feeding)

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 2.74 ± 1.05 | 1.94 ± .85 | .80 ± .7 | 2.07 ± 1.81 |
| Thr | 6.59 ± .50 | 5.55 ± .69 | 1.04 ± .79 | 2.69 ± .49 |
| Ser | 19.17 ± 1.30 | 10.29 ± 1.63 | 8.88 ± .27 | 23.00 ± .70 |
| Asn | 1.64 ± .05 | 1.71 ± .25 | - .07 ± .25 | - .18 ± .57 |
| Glu | 12.46 ± 1.85 | 9.82 ± 1.66 | 2.64 ± .79 | 6.84 ± 2.05 |
| Gln | 14.36 ± .48 | 25.85 ± 3.99 | -11.49 ± 2.61 | -29.26 ± 6.76 |
| Gly | 24.61 ± 2.16 | 21.03 ± 1.76 | 3.58 ± 1.40 | 9.27 ± 1.04 |
| Ala | 11.48 ± 1.66 | 26.13 ± 1.44 | -14.65 ± 1.22 | -37.94 ± .57 |
| Pro | 9.47 ± .99 | 6.45 ± 1.41 | 3.02 ± .58 | 7.82 ± 1.50** |
| Val | 16.60 ± 1.27 | 15.08 ± 1.06 | 1.52 ± .27 | 3.94 ± .70 |
| Cyst | 1.34 ± .09 | 1.05 ± .68 | .29 ± .01 | .75 ± .03** |
| Met | 1.28 ± .74 | .53 ± .22 | .75 ± .32 | 1.95 ± 1.35 |
| Ile | 8.54 ± 1.08 | 6.85 ± 1.61 | 1.69 ± .47 | 4.38 ± 1.22 |
| Leu | 12.86 ± 1.99 | 10.60 ± 2.56 | 2.26 ± 1.43 | 5.85 ± 3.70 |
| Tyr | 3.72 ± .58 | 3.04 ± .88 | .68 ± .20 | 1.76 ± .52 |
| Phe | 5.53 ± .65 | 4.02 ± 1.30 | 1.51 ± .35 | 3.91 ± .91 |
| Orn | 16.52 ± 3.05 | 10.46 ± 2.17 | 6.06 ± 1.88 | 15.69 ± 4.87 |
| Lys | 11.01 ± 2.03 | 6.97 ± 1.78 | 4.04 ± 1.25 | 10.46 ± 3.30 |
| His | 6.31 ± .66 | 4.30 ± 1.27 | 2.01 ± .39 | 5.21 ± 1.01** |
| Arg | 6.70 ± .81 | 6.35 ± 1.49 | - .35 ± .32 | .91 ± .83 |
| EAA[a] | 75.42 ± 9.73 | 60.25 ± 7.92 | 15.17 ± 4.81 | 39.29 ±12.46 |
| NEAA[b] | 117.51 ±13.24 | 117.77 ±11.62 | -0.26 ± 2.22 | 0.67 ± 5.75** |
| BCAA[c] | 38.00 ± 4.34 | 32.53 ± 2.77 | 5.47 ± 2.17 | 14.17 ± 5.62 |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

**Significantly different than the control group (P<.01).

TABLE 17. The Arteriovenous Concentration Differences and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Low Protein Diet (Four Hours After Feeding).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.81 ± .17 | 1.45 ± .16 | .36 ± .07 | .93 ± .19* |
| Thr | 6.24 ± .36 | 5.77 ± .63 | .47 ± .26 | 1.22 ± .67 |
| Ser | 18.97 ± 1.40* | 8.14 ± 1.64 | 10.83 ± .36 | 28.05 ± .93* |
| Asn | 1.78 ± 0.15 | 1.96 ± .67 | - .18 ± .08 | - .47 ± .21* |
| Glu | 9.23 ± 2.52 | 8.96 ± 1.13 | .27 ± .39 | .70 ± 3.60** |
| Gln | 16.88 ± 1.91 | 28.13 ± 2.46 | -11.25 ± 1.45 | -29.14 ± 3.76 |
| Gly | 28.87 ± .56 | 21.75 ± 1.66 | 7.12 ± .50 | 18.44 ± 1.30 |
| Ala | 8.96 ± 1.20 | 26.55 ± 1.78 | -17.59 ± .53 | -45.56 ± 1.37* |
| Pro | 9.62 ± .65* | 7.68 ± .71 | 1.94 ± .66 | 5.01 ± .16** |
| Val | 18.17 ± .75 | 15.85 ± 3.47 | 2.32 ± 2.72 | 6.01 ± 7.04 |
| Cyst | 1.34 ± .13 | 1.20 ± .17 | .14 ± .02 | .36 ± .05* |
| Met | .61 ± .31 | .55 ± .18 | .06 ± .18 | .16 ± .34 |
| Ile | 7.82 ± .46 | 6.60 ± 1.53 | 1.22 ± .67 | 3.16 ± .18 |
| Leu | 11.37 ± .53 | 10.42 ± 2.68 | .95 ± .15 | 2.46 ± .39 |
| Tyr | 3.17 ± .21 | 2.57 ± .88 | .60 ± .17 | 1.55 ± .44 |
| Phe | 4.69 ± .26 | 3.96 ± .91 | .73 ± .15 | 1.89 ± .39 |
| Orn | 12.54 ± .75 | 9.36 ± .87 | 3.18 ± .12 | 8.24 ± .32 |
| Lys | 8.36 ± .50 | 6.24 ± .58 | 2.12 ± .68 | 5.49 ± .21 |
| His | 6.41 ± .43* | 5.12 ± .47 | 1.29 ± .64 | 3.34 ± .10** |
| Arg | 7.53 ± .41 | 5.65 ± .36 | 1.88 ± .05 | 4.87 ± .13 |
| EAA[a] | 71.20 ± 4.01 | 60.16 ± 7.30 | 11.04 ± 3.29 | 28.59 ± 8.52 |
| NEAA[b] | 113.17 ± 9.65 | 117.75 ± 7.65 | -4.58 ± 2.66 | -11.86 ± 5.18 |
| BCAA[c] | 37.36 ± 1.75 | 32.87 ± 4.68 | 4.49 ± 2.94 | 11.63 ± 7.62 |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than the control group ($P<.05$).

**Significantly different than the control group ($P<.01$).

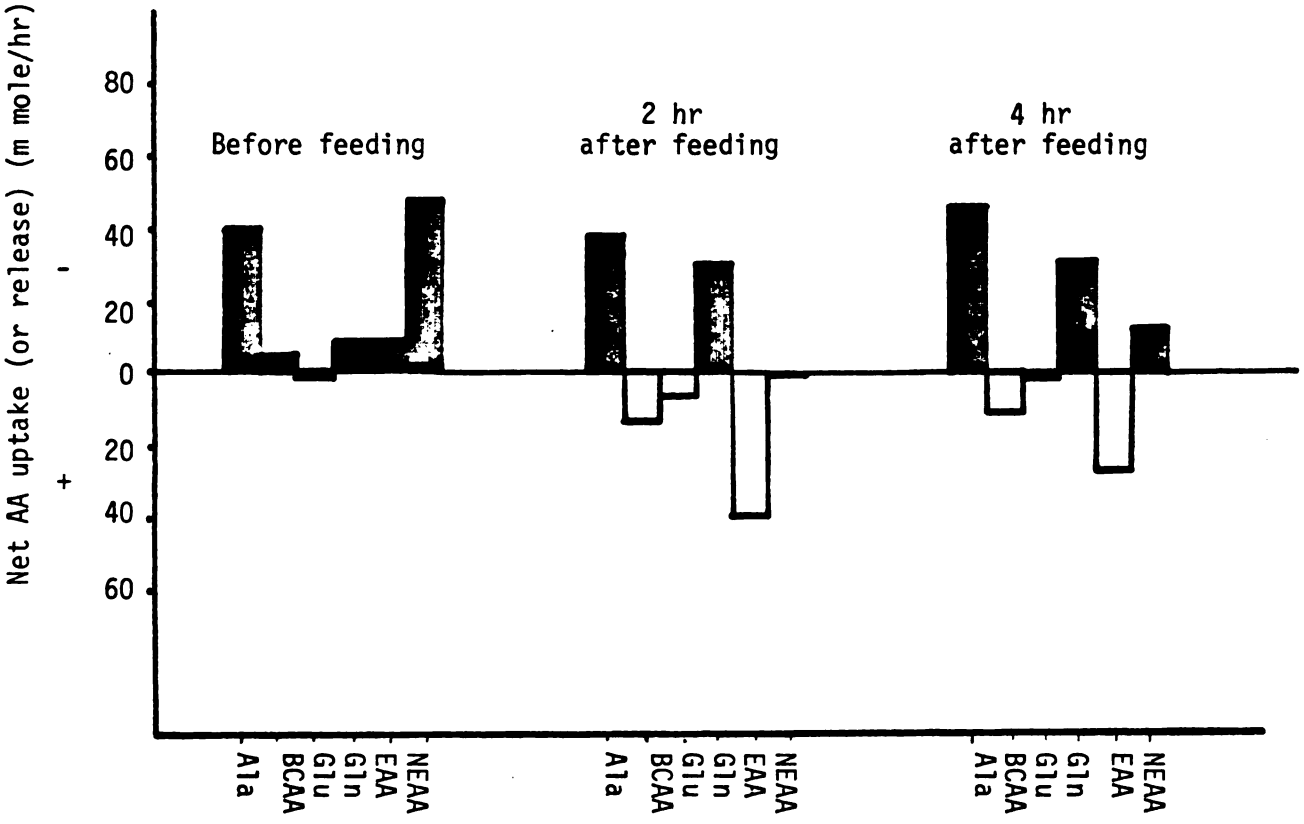


Figure 6. The net amino acid uptake (or release) by the hind limb of steers fed the low protein diet.

mainly after feeding.

When the high protein diet was fed, a complete change in the behavior of all AAs was observed. Even before feeding (Table 18 and Figure 7) there was a significant net uptake of thr, ser, ile, tyr, arg, EAA and BCAA. This observation might be due, in part, to a higher net availability of amino acids to the steers during the experimental period (two weeks).

At $T_2$ as well as $T_4$, the arterial blood had higher AAs concentrations as shown in Tables 19 and 20. Almost all AAs (except asp, asn, gln) concentrations were significantly increased in the arterial blood at either $T_2$ or $T_4$. Total BCAA concentrations significantly increased (85%) from 40 μ mole/dl to reach a maximum level of 76 μ mole/dl at two hours after feeding.

Tables 19 and 20 also show the significantly high net uptake of all AAs (except ala, gln, asn which were released) at all times after feeding. The AV differences were also larger.

The high net amino acid uptake generally noted in the high protein fed steers was due to high arterial PAA concentrations (Tables 19 and 20), as well as the high PF (Table 11) through the hind limbs.

The release of ala increased in a linear pattern with time after feeding to reach a maximum of 88 m mole/hour which is more than double the amount released before feeding. Glutamine was also released. However, it was released in lesser quantities than in either control- or low protein-fed steers.

Figure 7 shows that the high EAA uptake continued to be at the same high level at $T_4$ (last time interval measured) as at $T_2$, while the net uptake of NEAA at $T_4$ decreased to almost one half of that noted at $T_2$.

TABLE 18. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the High Protein Diet (Immediately Before Feeding)

| AA | Plasma AA level (μ moel/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.36 ± .07 | 1.39 ± .67 | - .03 ± .04 | - .20 ± .26* |
| Thr | 6.44 ± .24 | 4.20 ± .16 | 2.24 ± .14 | 14.60 ± .91** |
| Ser | 8.33 ± .51 | 4.30 ± .24 | 4.03 ± .27 | 26.28 ± 1.76** |
| Asn | 1.40 ± .08 | 1.61 ± .65 | - .21 ± .03 | -1.37 ± .20 |
| Glu | 11.81 ± .50 | 11.31 ± .82 | .50 ± .18 | 3.26 ± 1.17 |
| Gln | 15.42 ± 1.88 | 17.31 ± 1.54 | -1.89 ± 1.34 | -12.32 ± 8.71 |
| Gly | 16.05 ± .60** | 17.57 ± 2.26 | -1.52 ± .34 | -9.91 ± 2.21 |
| Ala | 8.89 ± .18 | 14.38 ± 1.37 | -5.49 ± .20 | -35.79 ± 1.30 |
| Pro | 8.01 ± .21 | 8.07 ± 1.21 | - .06 ± .02 | - .39 ± .13** |
| Val | 18.49 ± .80 | 15.59 ± 2.16 | 2.90 ± .64 | 18.91 ± 4.16 |
| Cyst | 2.21 ± .07** | 2.33 ± .66 | - .12 ± .01 | - .78 ± .07** |
| Met | .53 ± .23 | .64 ± .17 | - .11 ± .06 | - .72 ± .40 |
| Ile | 8.30 ± .31 | 6.92 ± 1.12 | 1.38 ± .19 | 9.00 ± 1.24** |
| Leu | 13.67 ± .36 | 10.86 ± 1.12 | 2.81 ± .24 | 18.32 ± 1.56 |
| Tyr | 3.04 ± .11 | 2.56 ± .64 | .48 ± .07 | 3.13 ± .46** |
| Phe | 4.70 ± .16 | 4.64 ± .67 | .06 ± .09 | .39 ± .60 |
| Orn | 10.43 ± .78 | 10.28 ± 1.71 | .15 ± .93 | .98 ± 6.24 |
| Lys | 6.95 ± .52 | 6.85 ± 1.14 | .10 ± .62 | .65 ± 4.03 |
| His | 5.34 ± .14 | 5.38 ± 1.14 | - .04 ± .02 | - .26 ± .13** |
| Arg | 6.24 ± .51 | 4.73 ± 1.06 | 1.51 ± .45 | 9.85 ± 2.93* |
| EAA[a] | 70.66 ± 3.27 | 62.81 ± 2.68 | 8.25 ± 1.19 | 53.79 ± 7.76** |
| NEAA[b] | 86.95 ± 4.99 | 91.11 ± 8.87 | -4.16 ± 1.12 | -27.12 ± 7.30 |
| BCAA[c] | 40.46 ± 1.47 | 33.37 ± 3.40 | 7.09 ± 1.07 | 46.23 ± 6.98** |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than the control group (P<.05).

**Significantly different than the control group (P<.01).

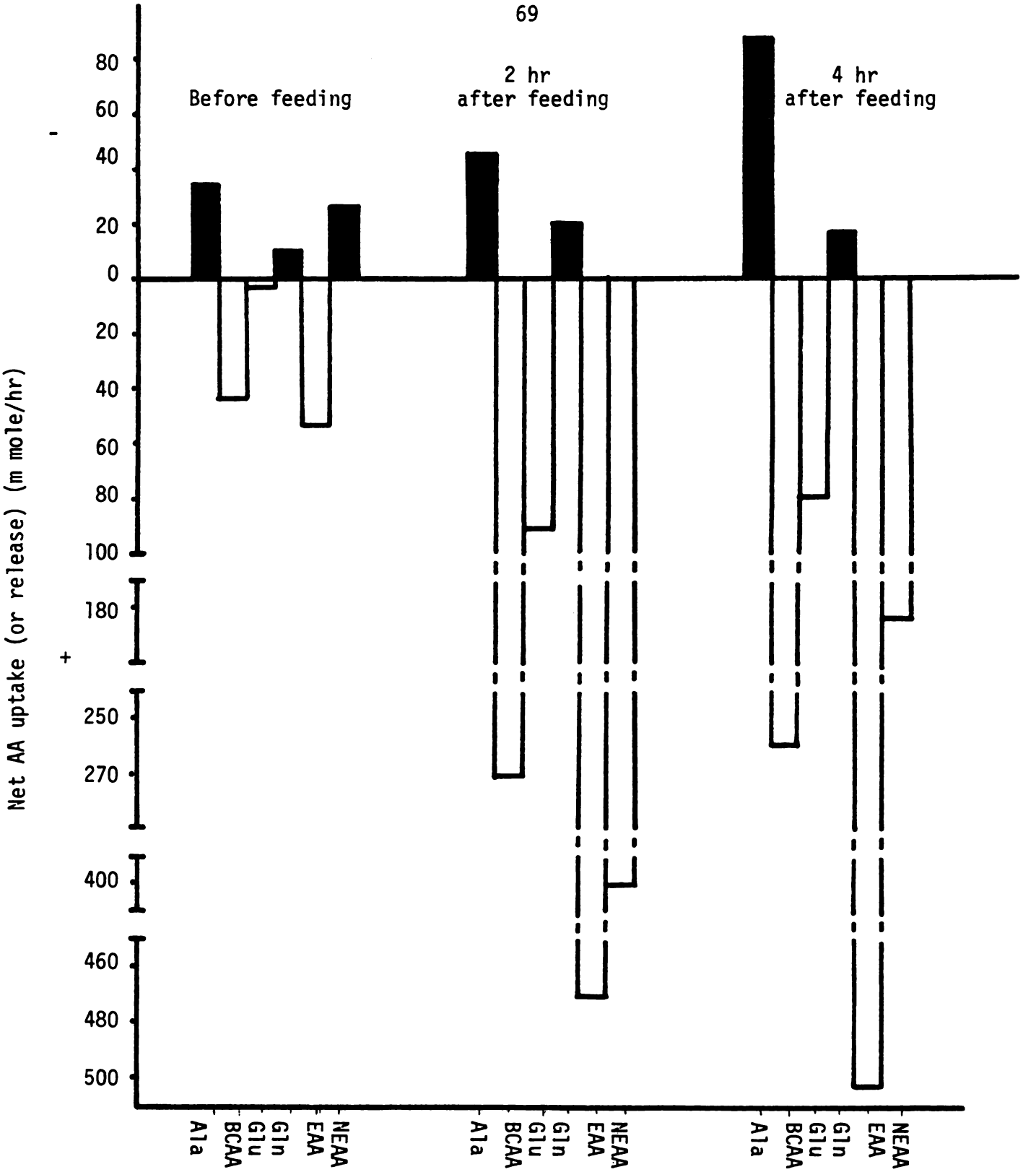


Figure 7. The net amino acid uptake (or release) by the hind limb of steers fed the high protein diet.

TABLE 19. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the High Protein Diet (Two Hours After Feeding).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 2.76 ± .20 | 1.35 ± .19 | 1.41 ± .64 | 9.19 ± .26* |
| Thr | 11.42 ± .27** | 4.80 ± .44 | 6.62 ± 1.19 | 43.16 ± 1.24** |
| Ser | 15.54 ± .20 | 5.65 ± 1.31 | 9.89 ± 1.71 | 64.48 ± 7.22* |
| Asn | 1.65 ± .02 | 1.79 ± .76 | - .14 ± .14 | - .91 ± .91 |
| Glu | 25.88 ± 0.41** | 11.64 ± 1.41 | 14.24 ± 1.98 | 92.84 ± 6.37** |
| Gln | 14.47 ± .66 | 17.59 ± 2.58 | -3.12 ± 1.18 | -20.34 ± 1.17 |
| Gly | 35.75 ± .93** | 14.65 ± 1.13 | 21.10 ± 2.23 | 137.57 ± 7.99** |
| Ala | 18.30 ± .30** | 25.50 ± 3.45 | -7.20 ± 1.15 | -46.94 ± 7.48 |
| Pro | 16.08 ± .38** | 7.41 ± 1.81 | 8.67 ± .43 | 56.52 ± 2.80** |
| Val | 34.32 ± .86** | 16.68 ± 1.35 | 17.64 ± .51 | 115.01 ± 3.32** |
| Cyst | 4.98 ± .20** | 2.09 ± .63 | 2.89 ± .17 | 18.84 ± 1.11** |
| Met | 1.80 ± .16** | .71 ± .28 | 1.10 ± .12 | 7.17 ± .78** |
| Ile | 15.78 ± .52** | 6.88 ± 1.15 | 8.90 ± .37 | 58.03 ± 2.41** |
| Leu | 26.44 ± .62** | 11.46 ± .38 | 14.98 ± 2.24 | 97.66 ± 1.56** |
| Try | 6.12 ± .10* | 2.62 ± .24 | 3.50 ± 1.14 | 22.82 ± .91** |
| Phe | 10.08 ± .34** | 4.82 ± .49 | 5.26 ± 1.15 | 34.30 ± .98** |
| Orn | 19.20 ± .57 | 8.88 ± 2.69 | 10.32 ± 1.14 | 67.29 ± 7.41* |
| Lys | 12.80 ± .38 | 5.92 ± .46 | 6.88 ± 1.28 | 44.86 ± 1.82** |
| His | 10.72 ± .25 | 4.94 ± .54 | 5.78 ± 1.25 | 37.68 ± 1.63** |
| Arg | 14.12 ± .23** | 5.44 ± .87 | 8.56 ± 1.62 | 55.81 ± 4.03** |
| EAA[a] | 134.48 ± 8.63* | 61.65 ± 8.96 | 72.83 ± 4.67 | 474.85 ±30.45** |
| NEAA[b] | 160.73 ± 3.97* | 99.17 ± 8.60 | 61.56 ± 4.03 | 401.37 ±26.27** |
| BCAA[c] | 76.54 ± 2.00** | 35.02 ± 3.88 | 41.52 ± 1.12 | 270.71 ± 7.30** |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than the control group (P<.05).

**Significantly different than the control group (P<.01).

TABLE 20. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the High Protein Diet (Four Hours After Feeding).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 2.78 ± .08 | .94 ± .18 | 1.84 ± .11 | 11.99 ± .72** |
| Thr | 11.80 ± .14** | 4.28 ± .27 | 7.52 ± 1.14 | 49.03 ± .91** |
| Ser | 15.14 ± .06** | 4.82 ± .53 | 10.32 ± 1.76 | 67.29 ± 4.94* |
| Asn | 1.58 ± .14 | 1.69 ± .65 | - .11 ± .10 | - .72 ± .65 |
| Glu | 23.16 ± 1.36 | 11.06 ± 1.36 | 12.10 ± 2.17 | 78.89 ±13.65* |
| Gln | 16.82 ± .59 | 19.26 ± 2.26 | -2.44 ± .33 | -15.91 ± 2.15 |
| Gly | 36.64 ± 3.43 | 15.35 ± 1.46 | 21.29 ± 2.02 | 138.81 ±13.13* |
| Ala | 13.66 ± .15** | 27.26 ± 4.58 | -13.51 ± 4.43 | -88.09 ± 8.80* |
| Pro | 16.32 ± 1.20 | 6.36 ± .36 | 9.96 ± 1.84 | 64.94 ± 5.46** |
| Val | 29.58 ± .92** | 15.32 ± 2.35 | 14.26 ± .57 | 92.98 ± 3.71** |
| Cyst | 4.32 ± .08** | 1.88 ± .04 | 2.44 ± .64 | 15.91 ± .26** |
| Met | 1.66 ± .14* | .39 ± .06 | 1.27 ± .68 | 8.28 ± .52** |
| Ile | 16.20 ± 1.22** | 6.60 ± 1.21 | 9.60 ± 1.01 | 62.59 ± 6.56** |
| Leu | 26.20 ± 1.27** | 10.73 ± 2.10 | 15.47 ± 1.17 | 100.86 ± 7.61** |
| Tyr | 6.06 ± 1.09** | 2.08 ± .67 | 3.98 ± .02 | 25.95 ± .13** |
| Phe | 9.54 ± 1.13* | 4.10 ± .62 | 5.44 ± 1.01 | 35.47 ± 6.57* |
| Orn | 20.40 ± 1.50* | 8.19 ± .36 | 12.21 ± 1.20 | 79.61 ± 7.80* |
| Lys | 13.60 ± .33** | 5.46 ± .26 | 8.14 ± .13 | 53.07 ± .85** |
| His | 10.88 ± .13* | 4.24 ± .24 | 6.64 ± 1.11 | 43.29 ± .72** |
| Arg | 13.96 ± 1.20* | 4.47 ± 1.16 | 9.49 ± 1.04 | 61.87 ± 6.76** |
| EAA[a] | 133.42 ± 2.48** | 55.59 ± 1.71 | 77.83 ± 2.77 | 507.45 ± 5.02** |
| NEAA[b] | 126.77 ± 2.68 | 98.89 ± 7.74 | 27.88 ± 4.46 | 181.78 ±29.08 |
| BCAA[c] | 71.98 ± 1.41** | 32.65 ± 1.66 | 39.33 ± 2.75 | 256.43 ± 4.89** |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than the control group (P<.05).

**Significantly different than the control group (P<.01).

II. Effect of Insulin and Starvation

Table 21 summarizes the data of BF, PCV and PF of another group of steers fed the same control diet (Table 4), insulin-treated, starved for 24 hours or starved for 48 hours. As observed in the first study of this series, there were no significant differences in PCV between all treatments with an overall mean of 29%. Insulin had no effect on either BF or PF, while starvation (for either 24 or 48 hours) led to a marked decrease in both.

Tables 22, 23 and 24 present the AV difference and the net uptake of AAs by the hind limb of steers fed the control diet (at $T_0$, $T_2$ and $T_4$ respectively). Before feeding the primary AA released were ala and to some extent, gln (Figure 8). However, at $T_2$ as well as $T_4$ net release was significantly ($P<.01$) decreased, especially for ala.

Tables 23 and 24 show that there were no significant increases in the arterial PAA concentrations (except for gly at $T_2$) over the time interval sampled. However, the same tables also show a significant net uptake of asp, ser, asn, gly, val, and leu, with highest net uptakes observed at $T_2$ (Figure 8).

The AA concentrations and net uptake of this control group behaved similarly to the first control group. However, AV differences and the net uptake (Figure 8) were lower than those of the first controls (Figure 5). These differences may be due to: 1) in the first study there were only two steers whose cannulas stayed patent (see Materials and Methods for details), which were used as control for the first study. These steers had been fed the high-protein diet previously and hence, there may have been a carryover effect from that treatment (high-protein diet) on the control. 2) The small number of animals used

TABLE 21. Effect of Insulin Injection and Starvation on the Plasma Flow Rate Across the Hind Limb.

| Treatment | Criteria | | |
|---|---|---|---|
| | Blood flow liter/hr | Packed Cell Vol. % | Plasma flow liter/hr |
| Control | 530 ± 41.9[b] | 28.45 ± 0.95[b] | 414 ± 30.0[b] |
| Insulin Injection | 605 ± 37.7[b] | 29.05 ± 1.32[b] | 429 ± 26.8[b] |
| 24 hr Starvation | 335 ± 23.9[c] | 28.90 ± 0.50[b] | 238 ± 17.0[c] |
| 48 hr Starvation | 315 ± 29.9[c] | 29.60 ± 0.67[b] | 221 ± 21.1[c] |

[a]Values are means and standard error of four steers.

[b,c]Values not sharing common superscript in column were significantly different ($P<.01$).

TABLE 22. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet (Immediately Before Feeding).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | .98 ± .10 | 1.46 ± .73 | -0.48 ± .03 | -1.99 ± .13 |
| Thr | 2.72 ± .20 | 2.67 ± .37 | .05 ± .07 | .21 ± .29 |
| Ser | 6.07 ± 1.32 | 6.04 ± 1.87 | .03 ± .04 | .12 ± .17 |
| Asn | .89 ± .10 | 1.09 ± .65 | - .18 ± .05 | - .75 ± .21 |
| Glu | 6.26 ± .31 | 5.86 ± .46 | .40 ± .15 | 1.66 ± .62 |
| Gln | 8.58 ± .70 | 12.31 ± 1.78 | -3.73 ± .68 | -15.44 ± .33 |
| Gly | 10.46 ± .50 | 12.88 ± 1.75 | -2.42 ± .25 | -10.02 ± 1.04 |
| Ala | 8.64 ± .08 | 19.97 ± 2.60 | -11.33 ± 1.52 | -45.51 ± 2.15 |
| Pro | 4.97 ± .30 | 3.92 ± .29 | 1.05 ± .06 | 4.35 ± .25 |
| Val | 9.45 ± .78 | 8.45 ± 1.15 | 1.00 ± .37 | 4.14 ± 1.53 |
| Cyst | .28 ± .14 | .61 ± .04 | .17 ± .10 | .70 ± .41 |
| Met | .61 ± .14 | 1.21 ± 1.07 | - .60 ± .93 | -2.48 ± 3.85 |
| Ile | 4.92 ± .31 | 3.92 ± 1.25 | 1.00 ± .06 | 4.14 ± .25 |
| Leu | 7.26 ± .37 | 5.65 ± 1.83 | 1.61 ± .46 | 6.67 ± 1.90 |
| Tyr | 1.95 ± .20 | 1.50 ± .68 | .45 ± .12 | 1.86 ± .50 |
| Phe | 3.68 ± .38 | 3.42 ± .43 | .26 ± .05 | 1.08 ± .21 |
| Orn | 6.39 ± .32 | 5.49 ± 1.67 | .90 ± .29 | 3.72 ± 1.20 |
| Lys | 4.26 ± .21 | 3.66 ± .41 | .60 ± .26 | 2.48 ± .82 |
| His | 3.31 ± .20 | 2.61 ± .76 | .70 ± .04 | 2.90 ± .17 |
| Arg | 3.57 ± .37 | 4.27 ± .74 | - .70 ± .23 | -2.90 ± .95 |
| EAA[a] | 39.78 ± 2.96 | 35.86 ± 4.71 | 3.92 ± 1.75 | 16.23 ± 7.25 |
| NEAA[b] | 55.97 ± 4.07 | 71.11 ± 6.11 | -15.14 ± 1.04 | -62.70 ± 4.32 |
| BCAA[c] | 21.63 ± 1.46 | 18.02 ± 2.23 | 3.61 ± 1.77 | 14.95 ± 3.19 |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

TABLE 23. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet (Two Hours After Feeding).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.34 ± .66 | 1.12 ± .02 | .22 ± .04 | .91 ± .17** |
| Thr | 3.09 ± .38 | 2.56 ± .16 | .53 ± .22 | 2.19 ± .91 |
| Ser | 6.79 ± 1.27 | 5.04 ± 2.14 | 1.75 ± .13 | 7.25 ± .54** |
| Asn | 1.27 ± .09 | 1.09 ± .64 | .18 ± .05 | .75 ± .21* |
| Glu | 6.93 ± .19 | 5.90 ± 1.51 | 1.03 ± .32 | 4.26 ± 1.32 |
| Gln | 11.44 ± 1.03 | 14.02 ± 2.08 | -2.63 ± .95 | -10.89 ± 3.93 |
| Gly | 13.27 ± .39* | 11.52 ± 2.29 | 1.75 ± .10 | 7.25 ± .41** |
| Ala | 8.92 ± .23 | 10.02 ± 1.39 | -1.05 ± .16 | -4.35 ± .66** |
| Pro | 5.54 ± 1.78 | 3.95 ± .17 | 1.59 ± .61 | 6.59 ± 2.52 |
| Val | 11.09 ± 1.16 | 8.32 ± 1.20 | 2.77 ± .64 | 11.47 ± .17* |
| Cyst | .90 ± .15 | .65 ± .04 | .25 ± .11 | 1.04 ± .46 |
| Met | .71 ± .11 | .16 ± .06 | .55 ± .05 | 2.28 ± .21 |
| Ile | 5.39 ± .61 | 3.74 ± .28 | 1.65 ± .83 | 6.83 ± 1.37 |
| Leu | 7.82 ± .37 | 3.30 ± .67 | 4.52 ± .80 | 18.71 ± 1.24** |
| Tyr | 2.05 ± .31 | 1.43 ± .11 | .62 ± .21 | 2.57 ± .87 |
| Phe | 3.66 ± .38 | 3.27 ± .89 | .39 ± .01 | 1.61 ± .04 |
| Orn | 6.86 ± 1.39 | 5.30 ± .68 | 1.56 ± .29 | 6.47 ± 1.20 |
| Lys | 4.57 ± 1.26 | 3.53 ± .45 | 1.04 ± .19 | 4.31 ± .79 |
| His | 3.69 ± .52 | 2.63 ± .71 | 1.06 ± .41 | 4.39 ± 1.70 |
| Arg | 4.83 ± .36 | 4.28 ± .68 | .55 ± .28 | 2.28 ± 1.16 |
| EAA[a] | 44.85 ± 4.15 | 31.79 ± 3.40 | 13.06 ± 1.75 | 54.07 ± 3.11* |
| NEAA[b] | 65.36 ± 4.89 | 60.09 ± 8.47 | 5.27 ± 1.42 | 21.82 ± 5.88** |
| BCAA[c] | 24.30 ± 2.14 | 15.36 ± 2.75 | 8.94 ± 1.70 | 37.01 ± 7.04 |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than before feeding (P<.05).

**Significantly different than before feeding (P<.01).

TABLE 24. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet (Four Hours After Feeding)

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.56 ± .63 | 1.29 ± .08 | .27 ± .05 | 1.12 ± .21** |
| Thr | 3.32 ± .46 | 2.67 ± .21 | .65 ± .20 | 2.69 ± .82 |
| Ser | 6.64 ± 1.26 | 5.63 ± 1.18 | 1.01 ± .68 | 4.18 ± .33** |
| Asn | 1.32 ± .16 | 1.19 ± .67 | .13 ± .09 | .54 ± .37 |
| Glu | 7.72 ± .83 | 6.70 ± .48 | 1.02 ± .15 | 4.22 ± .62 |
| Gln | 9.30 ± .73 | 12.03 ± 1.49 | -2.73 ± .24 | -11.30 ± .99 |
| Gly | 11.57 ± .29 | 11.48 ± 1.06 | .09 ± .23 | .37 ± .94** |
| Ala | 9.49 ± .22 | 10.48 ± 2.39 | - .99 ± .17 | -4.10 ± .69** |
| Pro | 4.56 ± 1.33 | 4.10 ± .26 | .47 ± .07 | 1.92 ± .29 |
| Val | 9.49 ± .34 | 7.42 ± .59 | 2.07 ± .25 | 8.57 ± 1.03 |
| Cyst | .67 ± .02 | .59 ± .05 | .08 ± .08 | .33 ± .13 |
| Met | .46 ± .03 | .31 ± .06 | .15 ± .03 | .62 ± .12 |
| Ile | 4.89 ± 1.29 | 3.88 ± .63 | 1.01 ± .26 | 4.18 ± 1.07 |
| Leu | 7.74 ± 1.21 | 6.16 ± .82 | 1.58 ± .61 | 6.54 ± 2.50 |
| Tyr | 1.98 ± .29 | 1.51 ± .69 | .47 ± .20 | 1.95 ± .82 |
| Phe | 3.54 ± .26 | 3.43 ± .88 | .11 ± .11 | .46 ± .45 |
| Orn | 6.44 ± .18 | 5.43 ± 1.65 | 1.01 ± .47 | 4.17 ± 1.93 |
| Lys | 4.29 ± .12 | 3.62 ± .48 | .67 ± .31 | 2.78 ± 1.27 |
| His | 3.04 ± .22 | 2.73 ± .17 | .31 ± .05 | 1.28 ± .21 |
| Arg | 4.60 ± .29 | 4.50 ± .09 | .10 ± .20 | .41 ± .82 |
| EAA[a] | 41.37 ± 2.16 | 34.72 ± 2.78 | 6.65 ± .62 | 27.53 ± 2.57 |
| NEAA[b] | 61.25 ± 3.84 | 60.43 ± 8.80 | .82 ± .04 | 3.39 ± .17** |
| BCAA | 22.12 ± .84 | 17.46 ± 1.44 | 4.66 ± 1.60 | 19.29 ± 2.48 |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

**Significantly different than before feeding (P<.01).

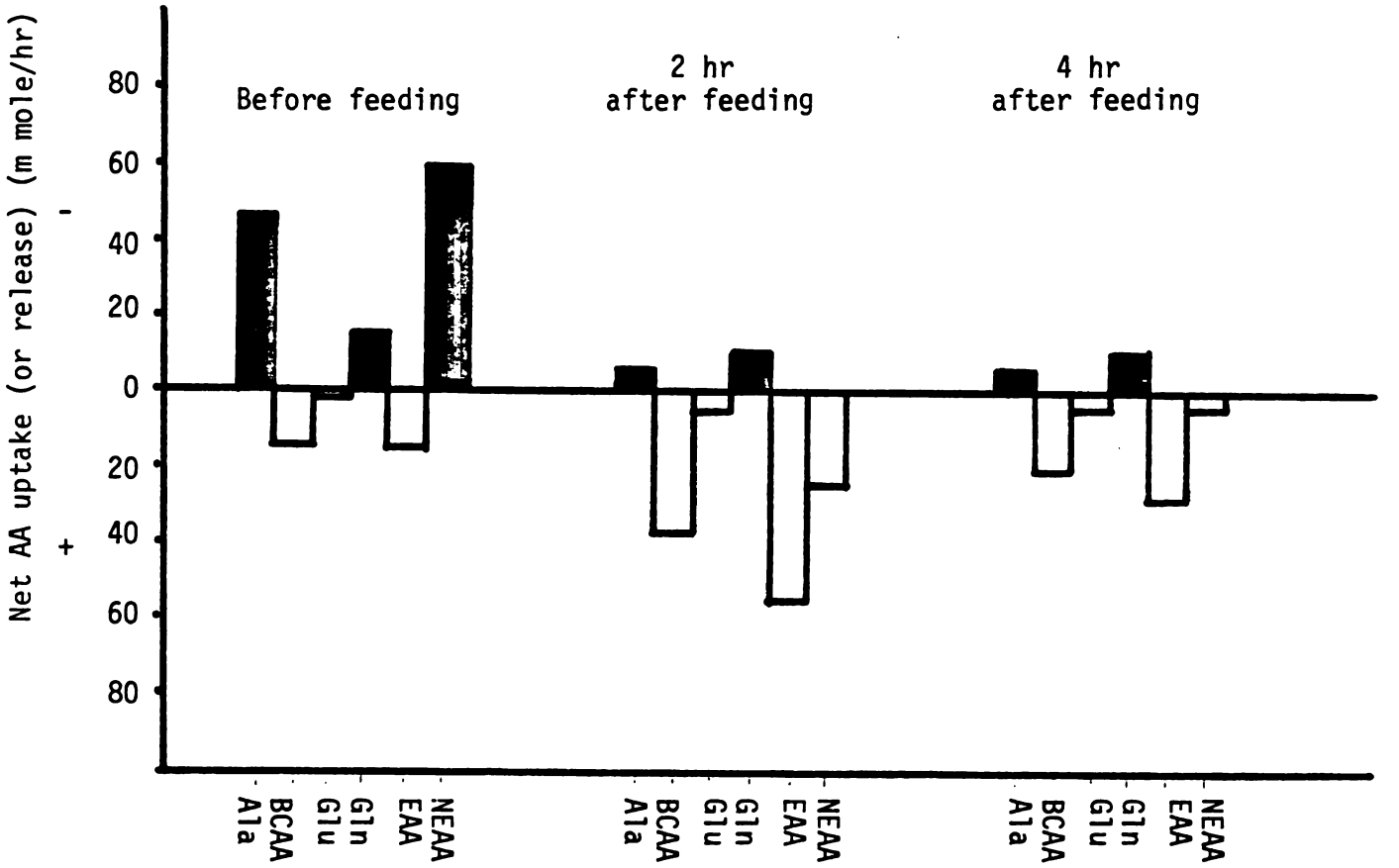


Figure 8. The net amino acid uptake (or release) by the hind limb of steers fed the control diet (II).

(two steers in the first control vs. four in the second) along with differences in their body weight may account for part of the differences observed.

A. Effect of Insulin

When insulin was injected into the jugular vein of the steers fed the control diet, the data in Tables 25, 26, 27 and 28 were obtained.

Data in Table 25 and Figure 9 show that the AAs behaved similarly at $T_4$ for the control-fed and $T_0$ (before injection) of insulin-injected steers. Therefore, statistical comparisons were made with $T_0$ (before injection) and each animal served as his own control.

Insulin injection had no effect on the AA concentration in the arterial blood (Tables 26, 27 and 28) but it affected net hind limb uptake of amino acids. As shown in Figure 10 there was an overall net uptake of BCAA and EAA at $T_1$ and the uptake due to insulin at $T_2$ of gly, phe, arg, BCAA, EAA and NEAA was significant ($P<.05$) (Table 27). Such an effect on AA uptake by insulin should have been observable at $T_1$ (Table 26), but, the high standard error of those data masked differences. Four hour post-injection the net uptake of both EAA and NEAA started to diminish as shown in Table 28 and Figure 9; and ala release was significant. The net release of ala, gln and NEAA at $T_4$ was similar to those at $T_0$ of the first control group (Figure 5).

B. Effect of Starvation

When the steers were starved for either 24 or 48 hour a net release of almost all AAs was observed.

Table 29 shows that 24 hour starvation has a slight effect on the arterial PAA concentrations. Glutamate and leu significantly decreased

TABLE 25. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet and Treated with Insulin (Immediately Before Insulin Injections).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.33 ± .22 | .94 ± .04 | .39 ± .18 | 1.67 ± .77 |
| Thr | 4.79 ± 1.69 | 3.97 ± .49 | .82 ± .20 | 3.52 ± .86 |
| Ser | 4.53 ± 1.33 | 4.74 ± .90 | - .21 ± .57 | - .90 ± 2.45 |
| Asn | 1.37 ± .62 | 1.45 ± .06 | - .08 ± .04 | - .34 ± .17 |
| Glu | 5.94 ± .90 | 4.95 ± 1.70 | .99 ± .20 | 4.25 ± .86 |
| Gln | 12.48 ± .84 | 14.43 ± 2.12 | -1.95 ± .72 | -8.37 ± 3.09 |
| Gly | 13.96 ± 1.51 | 13.25 ± 1.28 | .71 ± .77 | 3.05 ± 3.30 |
| Ala | 9.85 ± .54 | 11.52 ± 1.91 | -1.67 ± .37 | -7.16 ± 1.59 |
| Pro | 6.39 ± 1.21 | 6.17 ± .12 | .23 ± .09 | .96 ± .39 |
| Val | 11.57 ± .43 | 10.95 ± 1.63 | .62 ± .20 | 2.66 ± .86 |
| Cyst | 1.08 ± .04 | 1.06 ± .67 | .02 ± .03 | .09 ± .13 |
| Met | 1.49 ± .27 | .86 ± .27 | .63 ± .06 | 2.70 ± .26 |
| Ile | 6.71 ± .25 | 6.48 ± 1.62 | .23 ± .37 | .99 ± 1.59 |
| Leu | 8.89 ± 1.66 | 7.74 ± .45 | 1.15 ± .30 | 4.93 ± 1.29 |
| Tyr | 3.32 ± .47 | 2.53 ± .86 | .79 ± .11 | 3.39 ± .47 |
| Phe | 3.31 ± .15 | 3.07 ± .75 | .24 ± .05 | 1.03 ± .21 |
| Orn | 10.07 ± 1.28 | 7.95 ± 1.40 | 2.12 ± .72 | 9.08 ± .51 |
| Lys | 6.71 ± .85 | 5.30 ± .93 | 1.41 ± .68 | 6.05 ± .34 |
| His | 4.26 ± .74 | 4.11 ± .08 | .15 ± .09 | .64 ± .39 |
| Arg | 4.97 ± .13 | 4.69 ± .20 | .28 ± .07 | 1.20 ± .30 |
| EAA[a] | 52.7 ± 3.51 | 47.17 ± 8.82 | 5.53 ± 0.31 | 23.72 ± 1.33 |
| NEAA[b] | 70.32 ± 5.36 | 68.99 ± 5.96 | 1.33 ± .66 | 5.71 ± 2.57 |
| BCAA[c] | 27.17 ± 1.37 | 25.17 ± 2.20 | 2.00 ± 0.36 | 8.58 ± 1.54 |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids

TABLE 26. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet and Treated With Insulin (One Hour After Insulin Injection).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp. | 1.12 ± .27 | .87 ± .12 | .25 ± .15 | 1.07 ± .63 |
| Thr | 4.17 ± .96 | 3.87 ± 1.32 | .30 ± .64 | 1.29 ± 2.69 |
| Ser | 3.69 ± .76 | 2.08 ± .85 | 1.61 ± .41 | 6.91 ± 1.72 |
| Asn | 1.38 ± .34 | 1.89 ± .74 | - .51 ± .20 | -2.19 ± .84 |
| Glu | 5.08 ± 1.24 | 3.96 ± .78 | 1.12 ± 1.06 | 4.80 ± 4.45 |
| Gln | 11.10 ± 2.01 | 11.88 ± 1.74 | - .78 ± .87 | -3.35 ± 3.65 |
| Gly | 11.36 ± 2.11 | 6.99 ± 1.96 | 4.37 ± 1.15 | 18.75 ± 4.83 |
| Ala | 9.71 ± 1.97 | 10.16 ± 1.61 | - .45 ± 1.01 | -1.93 ± 4.24 |
| Pro | 5.96 ± 1.38 | 3.19 ± .29 | 2.77 ± 1.69 | 11.84 ± 4.58 |
| Val. | 9.50 ± 1.73 | 5.67 ± 1.85 | 3.83 ± 1.12 | 16.43 ± .50** |
| Cyst | 1.12 ± .28 | .71 ± .06 | .41 ± .22 | 1.76 ± .92 |
| Met | 1.13 ± .29 | .72 ± .15 | .41 ± .14 | 1.76 ± .59 |
| Ile | 6.17 ± 1.32 | 4.05 ± 1.59 | 2.12 ± .73 | 9.09 ± 3.07 |
| Leu. | 7.66 ± 1.57 | 4.52 ± 1.70 | 3.14 ± .87 | 13.47 ± 3.65 |
| Tyr | 2.92 ± .65 | 1.49 ± .27 | 1.43 ± .38 | 6.13 ± 1.60 |
| Phe | 2.84 ± 1.55 | 1.76 ± .27 | 1.09 ± .28 | 4.68 ± 1.18 |
| Orn | 8.67 ± 1.86 | 4.65 ± 1.65 | 4.02 ± 1.20 | 17.25 ± 5.04 |
| Lys | 5.78 ± 1.24 | 3.10 ± .48 | 2.68 ± .81 | 11.50 ± 3.40 |
| His | 3.97 ± .92 | 2.13 ± .79 | 1.84 ± .73 | 7.89 ± 3.07 |
| Arg. | 5.01 ± 1.22 | 3.38 ± .42 | 1.63 ± .80 | 6.99 ± 3.36 |
| EAA[a] | 46.23 ± 9.80 | 29.20 ± 4.92 | 17.03 ± 4.88 | 73.06 ±20.94 |
| NEAA[b] | 62.11 ±12.87 | 47.87 ± 4.77 | 14.24 ± 3.10 | 61.09 ±34.75 |
| BCAA | 23.33 ± 4.62 | 14.24 ± 3.67 | 9.09 ± 1.48 | 39.00 ± 6.35 |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids

**Significantly different than before injection (P<.01).

TABLE 27. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Eight Steers Fed the Control Diet and Treated with Insulin (Two Hours After Insulin Injection)

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.20 ± .68 | .81 ± .06 | .39 ± .02 | 1.67 ± .08 |
| Thr | 4.49 ± 1.15 | 3.02 ± .15 | 1.47 ± .05 | 6.31 ± .21 |
| Ser | 3.67 ± .77 | 3.42 ± .14 | .25 ± .03 | 1.07 ± .13 |
| Asn | 1.44 ± .06 | 1.46 ± .67 | - .02 ± .01 | - .09 ± .04 |
| Glu | 4.62 ± 1.26 | 3.59 ± .27 | 1.03 ± .61 | 4.42 ± .04 |
| Gln | 13.21 ± 1.40 | 13.91 ± 1.63 | - .70 ± .77 | -3.00 ± 3.24 |
| Gly | 12.13 ± .95 | 6.26 ± .58 | 5.87 ± .42 | 25.18 ± 1.76* |
| Ala | 9.99 ± .44 | 10.08 ± 1.41 | - .09 ± .03 | - .39 ± .13 |
| Pro | 7.82 ± 1.94 | 5.25 ± .65 | 2.57 ± 1.29 | 11.01 ± 5.42 |
| Val | 9.84 ± .79 | 4.80 ± .87 | 5.04 ± .68 | 21.62 ± .34** |
| Cyst | .90 ± .09 | .52 ± .76 | .38 ± .07 | 1.63 ± .29 |
| Met | .94 ± .16 | .46 ± .11 | .48 ± .01 | 2.06 ± .04 |
| Ile | 5.14 ± .36 | 2.84 ± .65 | 2.30 ± .35 | 9.87 ± 1.47* |
| Leu | 6.42 ± 1.25 | 3.51 ± .38 | 2.91 ± .13 | 12.48 ± .55* |
| Tyr | 2.71 ± .66 | 2.11 ± .16 | .60 ± .10 | 2.57 ± .42 |
| Phe | 2.62 ± .69 | 1.39 ± .14 | 1.23 ± .06 | 5.28 ± .25** |
| Orn | 7.91 ± 1.26 | 6.78 ± .51 | 1.13 ± .25 | 4.83 ± 1.05 |
| Lys | 5.27 ± 1.17 | 4.52 ± .34 | .75 ± .17 | 3.22 ± .71 |
| His | 5.21 ± 1.29 | 3.50 ± .48 | 1.71 ± .86 | 7.34 ± 3.61 |
| Arg | 5.35 ± .21 | 2.78 ± .31 | 2.57 ± .16 | 11.03 ± .42** |
| EAA[a] | 45.28 ± 3.35 | 26.82 ± 3.38 | 18.46 ± 1.03 | 79.19 ± .13** |
| NEAA[b] | 65.60 ± 5.71 | 54.19 ± 8.59 | 11.41 ± 2.12 | 48.95 ± 9.09* |
| BCAA[c] | 21.40 ± 1.34 | 11.15 ± 1.90 | 10.25 ± .56 | 43.97 ± 2.40** |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than before injection (P<.05).

**Significantly different than before injection (P<.01).

TABLE 28. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of Steers Fed the Control Diet and Treated with Insulin (Four Hours After Insulin Injection).

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.93 ± .81 | 1.58 ± .05 | .35 ± .26 | 1.50 ± 1.09 |
| Thr | 3.25 ± .12 | 2.64 ± .67 | .61 ± .05 | 2.62 ± .21 |
| Ser | 3.51 ± 1.14 | 3.15 ± .14 | .36 ± .05 | 1.54 ± .21 |
| Asn | 1.42 ± .69 | 1.26 ± .02 | .16 ± .07 | .69 ± .29 |
| Glu | 6.83 ± .27 | 6.14 ± 1.02 | .69 ± .25 | 2.96 ± 1.05 |
| Gln | 11.40 ± 1.42 | 12.79 ± 1.07 | -1.39 ± .65 | -5.96 ± 2.73 |
| Gly | 11.40 ± 1.43 | 10.84 ± .21 | .56 ± .22 | 2.40 ± .93 |
| Ala | 9.36 ± .34 | 19.71 ± 2.25 | -10.37 ± .09 | -44.49 ± .38** |
| Pro | 4.34 ± 1.18 | 4.02 ± .69 | .32 ± .09 | 1.35 ± .38 |
| Val | 7.71 ± .38** | 7.52 ± 1.30 | .19 ± .08 | .82 ± .34 |
| Cyst | .78 ± .06 | .67 ± .08 | .11 ± .03 | .47 ± .13 |
| Met | .74 ± 1.06 | .66 ± .06 | .08 ± .94 | .34 ± 3.95 |
| Ile | 5.06 ± .14* | 4.37 ± 1.09 | .69 ± .05 | 2.96 ± .21 |
| Leu | 7.33 ± .69 | 5.76 ± 1.15 | 1.57 ± .54 | 6.74 ± 2.27 |
| Tyr | 2.22 ± .10 | 1.76 ± 1.03 | .46 ± .07 | 1.97 ± .29 |
| Phe | 2.74 ± .12 | 2.40 ± .67 | .34 ± .05 | 1.46 ± .21 |
| Orn | 5.99 ± .18 | 5.30 ± .68 | .69 ± .10 | 2.96 ± .42** |
| Lys | 3.99 ± .12 | 3.53 ± .65 | .46 ± .07 | 1.97 ± .29 |
| His | 2.89 ± .12 | 2.68 ± .96 | .21 ± .06 | .90 ± .25 |
| Arg | 5.09 ± .14 | 4.66 ± 1.09 | .43 ± .05 | 1.84 ± .21 |
| EAA[a] | 38.80 ± 2.83 | 34.22 ± 2.94 | 4.58 ± 1.89 | 19.65 ± 8.11 |
| NEAA[b] | 59.18 ± 2.52 | 67.22 ± 2.99 | -8.04 ± .53 | -34.49 ± 2.27** |
| BCAA[c] | 20.10 ± 1.21* | 17.65 ± 1.54 | 2.45 ± 0.67 | 10.51 ± 2.87 |

Values are means and standard error of four steers
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than before injection ($P<.05$).

**Significantly different than before injection ($P<.01$).

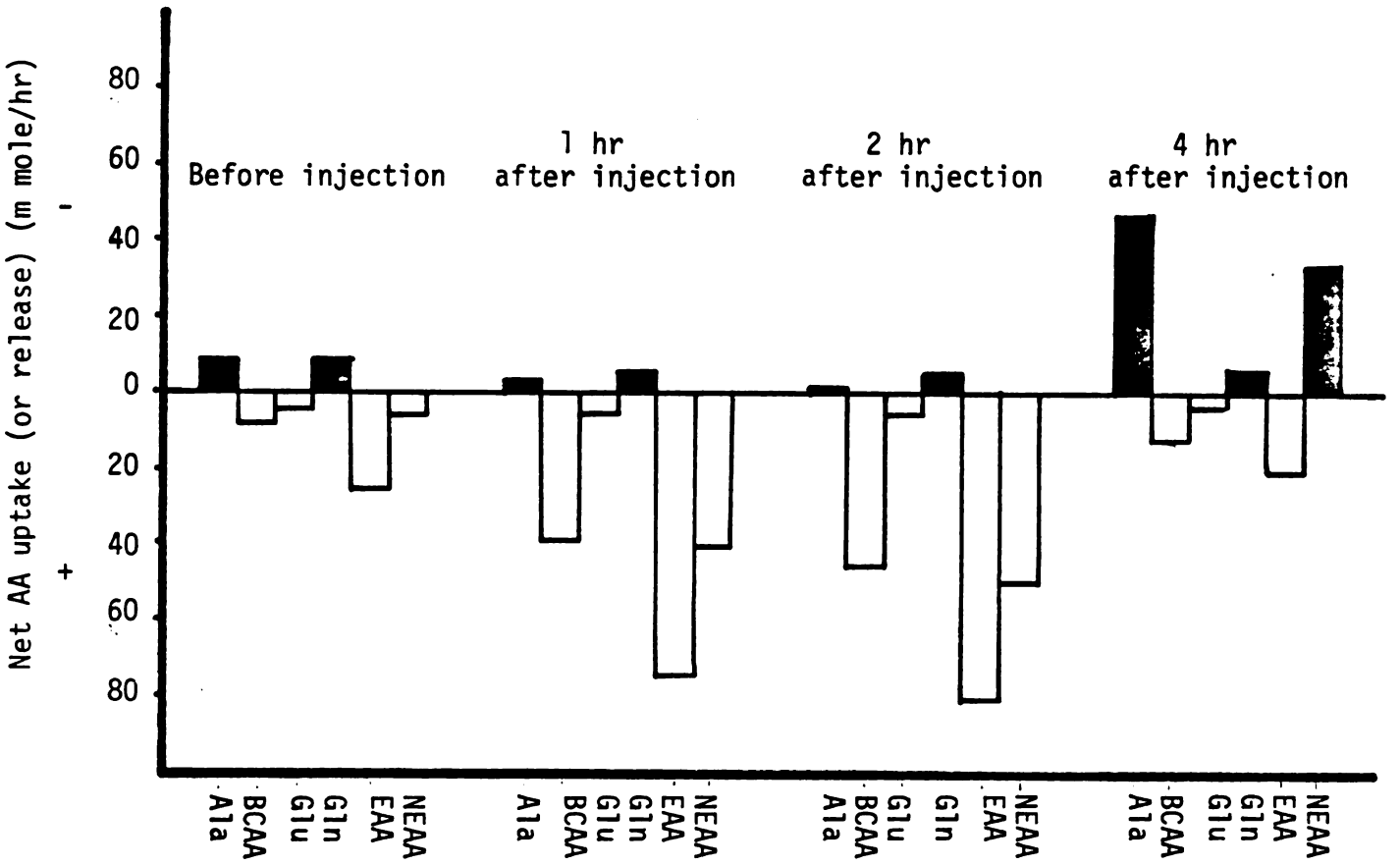


Figure 9. The net amino acid uptake (or release) by the hind limb of steers fed the control diet and injected with insulin.

TABLE 29. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of 24 Hour-starved Steers.

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | 1.05 ± .73 | .81 ± .05 | .24 ± .08 | .57 ± .19 |
| Thr | 3.53 ± .39 | 3.99 ± 1.25 | - .46 ± .14 | -1.09 ± .33 |
| Ser | 6.46 ± 1.45 | 6.59 ± 1.97 | - .13 ± .48 | - .31 ± 1.14* |
| Asn | 1.13 ± .69 | 1.87 ± .07 | - .74 ± .02 | -1.76 ± .05** |
| Glu | 4.06 ± .45* | 3.77 ± 1.29 | .29 ± .16 | .69 ± .38 |
| Gln | 12.06 ± 2.31 | 16.05 ± 2.09 | -3.99 ± 1.22 | -9.50 ± .52 |
| Gly | 11.41 ± 1.60 | 11.33 ± 1.69 | .08 ± .51 | .19 ± 1.21* |
| Ala | 13.25 ± .86* | 24.83 ± 1.14 | -11.58 ± 1.28 | -27.56 ± .67** |
| Pro | 5.39 ± .51 | 5.03 ± .46 | .36 ± .10 | .86 ± .24 |
| Val | 7.97 ± .49 | 8.49 ± .82 | - .52 ± .38 | -1.24 ± .78** |
| Cyst | .98 ± .03 | .88 ± .64 | - .10 ± .01 | - .24 ± .03 |
| Met | .61 ± .21 | .54 ± .16 | .07 ± .05 | .17 ± .12 |
| Ile | 3.69 ± .27 | 3.77 ± .37 | - .08 ± .16 | - .19 ± .24* |
| Leu | 5.09 ± .31* | 6.06 ± 1.22 | - .97 ± .09 | -2.31 ± .21** |
| Tyr | 1.71 ± .08 | 1.86 ± .67 | - .15 ± .01 | - .36 ± .03* |
| Phe | 3.13 ± .13 | 3.50 ± 1.25 | - .37 ± .12 | - .88 ± .29 |
| Orn | 6.36 ± .17 | 5.34 ± 1.56 | 1.02 ± .39 | 2.43 ± .93 |
| Lys | 4.24 ± .11 | 3.56 ± .87 | .68 ± .26 | 1.62 ± .62 |
| His | 3.59 ± .34 | 3.35 ± 1.27 | .24 ± .07 | .57 ± .17 |
| Arg | 4.92 ± 1.79 | 4.47 ± 1.29 | .45 ± 1.50 | 1.07 ± 3.56 |
| EAA[a] | 36.77 ± 4.04 | 37.93 ± 3.00 | -1.16 ± 1.64 | -2.76 ± 2.48** |
| NEAA[b] | 63.86 ± 7.68 | 78.36 ± 6.78 | -14.50 ± 0.96 | -34.51 ± 2.14** |
| BCAA[c] | 16.75 ± 1.07 | 18.32 ± 1.41 | -1.57 ± .04 | -3.74 ± .88** |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than fed steers (P<.05).

**Significantly different than fed steers (P<.01).

while ala concentration increased ($P<.05$). When the animals continued starvation to 48 hour asp also decreased (Table 30).

At 24 hour starvation there was a significant net release of ser, asn, gly, ala, val, ile, leu and tyr. In addition to those, the hind quarter started to release even more AAs after 48 hour starvation. These AAs were thr, phe, orn, lys, his, arg and met.

As shown in Figure 10, after 24 hour starvation more NEAA were released, while after 48 hour starvation the hind limb started to release the EAA in higher rate. The BCAA released accounted for 100% of the total EAA released at 24 hour starvation, while it accounted for only 42% after 48 hour starvation.

TABLE 30. The Arteriovenous Concentration Difference and the Net Uptake of Amino Acids by the Hind Limb of 48 Hour-Starved Steers.

| AA | Plasma AA level (μ mole/dl) Arterial | Venous | Difference | Net uptake m mole/hr |
|---|---|---|---|---|
| Asp | .97 ± .03* | .80 ± .63 | .17 ± .10 | .38 ± .22 |
| Thr | 3.45 ± .36 | 4.42 ± 1.47 | - .97 ± .11 | -2.14 ± .25* |
| Ser | 5.15 ± .28 | 5.67 ± .73 | - .52 ± .15 | -1.15 ± .34** |
| Asn | 1.15 ± .07 | 1.94 ± .63 | - .79 ± .04 | -1.75 ± .09** |
| Glu | 4.15 ± .21** | 3.17 ± .54 | .98 ± .38 | 2.17 ± .74 |
| Gln | 9.19 ± 1.06 | 10.84 ± 1.64 | -1.65 ± .42 | -3.65 ± .94 |
| Gly | 10.79 ± .92 | 12.28 ± 2.70 | -1.49 ± .22 | -3.29 ± .48** |
| Ala | 12.76 ± .30** | 23.45 ± 3.35 | -10.69 ± 1.05 | -23.62 ± 1.11** |
| Pro | 4.77 ± .51 | 5.19 ± 1.36 | - .42 ± .15 | - .93 ± .34 |
| Val | 8.21 ± .54 | 9.59 ± 1.62 | -1.38 ± .08 | -3.05 ± .18** |
| Cyst | .91 ± .04 | 1.15 ± .65 | - .25 ± .01 | - .53 ± .03 |
| Met | .55 ± .03 | .77 ± .63 | - .22 ± .03 | - .49 ± .07** |
| Ile | 4.11 ± .40 | 5.14 ± .23 | -1.03 ± .77 | -2.28 ± .38* |
| Leu | 5.53 ± .45* | 6.35 ± .79 | - .82 ± .26 | -1.81 ± .59** |
| Tyr | 1.72 ± .08 | 2.13 ± .69 | - .41 ± .01 | - .91 ± .03 |
| Phe | 3.19 ± .24 | 3.90 ± .68 | - .71 ± .16 | -1.57 ± .36** |
| Orn | 6.36 ± .56 | 8.10 ± 1.48 | -1.74 ± .08 | -3.84 ± .18* |
| Lys | 4.24 ± .37 | 5.40 ± 1.32 | -1.16 ± .05 | -2.56 ± .11** |
| His | 3.18 ± .34 | 3.46 ± .24 | - .28 ± .16 | - .62 ± .22* |
| Arg | 4.82 ± .24 | 5.95 ± .26 | -1.13 ± .62 | -2.50 ± .05* |
| EAA[a] | 37.28 ± 2.97 | 44.98 ± 2.44 | -7.70 ± 1.53 | -17.02 ± 1.17** |
| NEAA[b] | 57.92 ± 4.06 | 74.72 ± 3.50 | -16.80 ± 2.56 | -37.13 ± 1.24** |
| BCAA[c] | 17.85 ± 1.39 | 21.08 ± 1.64 | -3.23 ± .35 | -7.14 ± .77** |

Values are means and standard error of four steers.
The (-) indicates the net release of AA.

[a]EAA = Essential amino acids.

[b]NEAA = Non-essential amino acids.

[c]BCAA = Branched-chain amino acids.

*Significantly different than fed steers ($P<.05$).

**Significantly different than fed steers ($P<.01$).

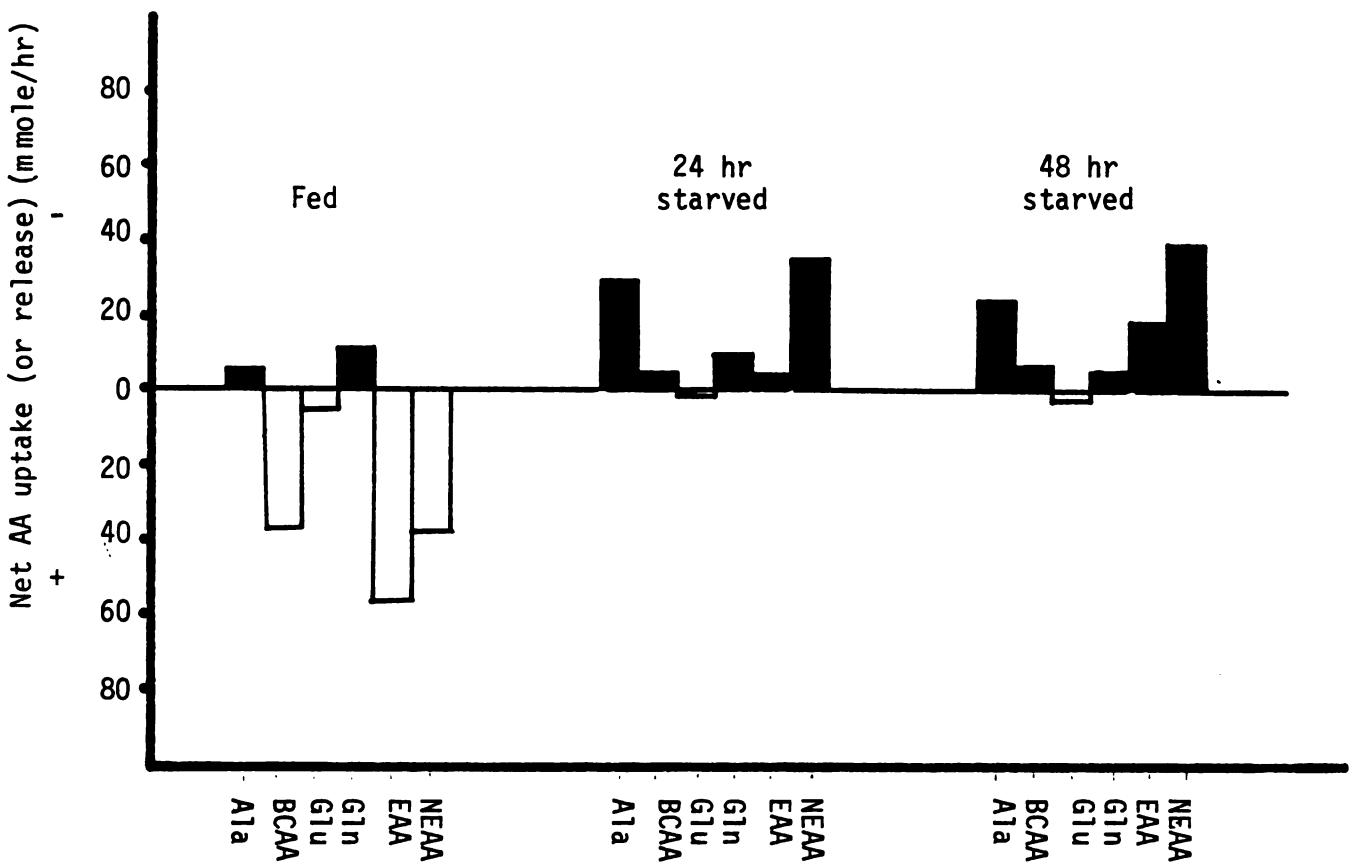


Figure 10. The net amino acid uptake (or release) by the hind limb of starved steers.

# DISCUSSION

## AMINO ACID METABOLISM STUDIES

## EXPERIMENT TWO

### I. Plasma Flow

During the blood flow measurement, all animals (except the starved ones) were on feed. The overall average amount eaten was 64% (of the total feed) within the three hours of blood flow measurement.

The literature contains very little information on the plasma flow across the hind limb of steers under different physiological states. Data in Tables 11 and 21 shows that blood flow (BF) and plasma flow (PF) were affected by the different treatments used in this study. Blood flow significantly decreased on low protein diet and starvation, while insulin injection had no effect. The high protein diet increased the BF and PF. However, due to the high standard error, a statistical difference could not be ascertained. Bell et al. (1975) reported that cold stress increased hind leg plasma flow in steers three- to five-fold. However, their PF values were two to three times lower than PF obtained in this study. Differences may be due to the markers (Para amino hippuric acid vs indocyanine green dye), methods used, breed, age, body weight or diets.

Unlike the data in Table 21, Heitmann and Bergman (1978) found no significant difference in PF across the hind quarter between fed and

fasted sheep. They also reported that the blood flow had a high measurement error (about 4%). When the AV differences are coupled with the high rates of blood flow (about 4% error), the overall error in the calculation could be 30-40% (Heitmann and Bergman, 1978). The PF rate across the hind quarter observed by Heitmann and Bergman (1978) was equal to the portal PF rate obtained by Prior et al. (1981). Since Prior et al. (1981) used the same marker and methods as in this study, they obtained portal PF values of fed steers equal to those in Table 11. They also reported that the PF of concentrate-fed steers and sheep was higher than of hay-fed animals.

The PCV value obtained in the present study averaged 29% and is in agreement with that obtained by Bell et al. (1975) who reported range of 28 to 31%.

## II. Effect of Dietary Protein

The maintenance of body protein economy is the integrated result of various interactive process affecting each of the individual AAs. Because the metabolism of each AA is, in turn, regulated by a specific control mechanism, understanding whole-body protein dynamics requires knowledge of the individual AA kinetic changes induced by physiological and pathological events.

Examination of AA-exchange across the deep tissues of the human forearm demonstrated that in normal man in the postabsorptive state (i.e. following a 12 to 14 hour overnight fast) there is a net release of AAs from muscle tissue, as reflected by consistantly negative AV differences (London et al., 1965; Pozefsky et al., 1969; Felig et al., 1970). This also exists in steers as shown in Tables 12, 15, 18 and 22.

The pattern of this release is quite distinctive with output of ala and gln exceeding that of all other AAs and accounting for over 50% of the total AAs released (Figures 5,6). This agreed with the work of Felig et al. (1970) in man and Ballard et al. (1976) in sheep.

As shown in Tables 13, 14, and Figure 5 the arterial plasma and net uptake of most individual AAs were significantly ($P<.05$) increased after feeding. This was mainly due to the energy availability and the increased production of volatile fatty acid in rumen. Propionate and butyrate stimulate the release of insulin (Forbes, 1980), which in turn stimualte the net AAs uptake and incorporation into muscle protein. This would cause a decrease in the venous PAA concentrations. Bergen (1978) reported that the jugular PAA concentrations after feeding remain unchanged or actually tend to decline.

Bell et al. (1975) reported that after feeding most individual AA concentrations increased in the arterial plasma, these being significant for arg, thr, gln, tyr, phe, ala and met. Bell et al. (1975) further reported that net exchanges of individual AAs across the leg were variable, and, with the exception of arg and glu, feeding caused no significant changes.

The second control group (Figure 8), however, showed lower AV differences and net uptake than those obtained for the first group (Figure 5). The differences may be due in part to the small number of animals used along with the differences in body weight. Also there may have been a carryover effect from the previous adjustment diet (high protein) for control one, but the steers were adjusted to the control diet for 14 days.

The various responses in AA metabolism to alterations in dietary

protein intake of the fed steers are graphically displayed in Figures 5, 6 and 7. For this purpose, the 12% CP (Figure 5) diet was used to represent, more or less, recommended protein requirements (NRC 1976) as the reference level for comparison of the results. At this level there was a net uptake of both NEAA and EAA. The net uptake of BCAA accounted for 30% of the EAA at $T_2$ to 50% at $T_4$. The AA release was due to ala and gln. These observations are similar to the results obtained by Motil et al. (1981) with man. Arteriovenous AA concentration difference and net uptake by the hind limb of steers fed the low protein diet behaved almost the same way as the starved animals (Figure 10). The only net uptake was of EAA. The BCAA accounted for 35 to 42% of the total EAA uptake. The net release was of ala and gln which accounted for 100% of NEAA released in this case.

Working with young men Motil et al. (1981) concluded that at submaintenance level of dietary protein the reduction in leu flux during the postabsorptive phase was due to a reduction in both the rate of leu incorporation into protein as well as leu oxidation.

The protein ingestion resulted in increased level of AAs in the circulation of nonruminant (Felig, 1975). However, in ruminant the plasma amino acid (jugular samples) concentrations after feeding tend to remain unchanged or slightly decline (Bergen, 1978). Tables 19, 20 showed that the AA concentration in the arterial plasma increased about two folds reached its peak at two hours after feeding. There were no changes in the venous PAA concentrations. When the high CP diet was fed the EAA (Mainly BCAA) remained elevated for four hours, while the NEAA started to decline within the four fours after feeding.

With respect to the effect of protein intake on muscle AA exchange,

studies in the rat have demonstrated a net uptake by peripheral tissues of the BCAA in the absorptive period (Yamamoto et al., 1974). Studies in normal human subjects show the same trend (Felig, 1975). Figure 7 shows the net AA uptake of hind limb of high protein-fed steers observed in the present study. Compared to control-fed steers, the net uptake of EAA increased eight fold at two hours after feeding with a 15 fold increase in BCAA. At two hours after feeding NEAA increased six fold. At four hours after feeding the net uptake decreased to five fold for EAA as well as for BCAA, while it decreased to 1.6 fold for NEAA.

In contrast, ala output from muscle continued to reach a peak of 88 m mole/hr at four hours after feeding. Bergman and Heitmann (1978) reported that ala was continuously released from the peripheral tissues in fed as well as fasted sheep to meet the liver requirement of the main gluconeogenesis precursor, ala. Aoki et al. (1973) and Yamamoto et al. (1974) reported that ala release continued unchanged or is reduced for only one hour after protein diet has been given to young man.

From the conclusion of Felig (1975) and the data observed here, it is clear that ala playsa major role in moving the alpha amino nitrogen from muscle in the fed as well as the fasted state.

## III. Effect of Insulin

Insulin is essential for the normal metabolism of carbohydrate, lipids and protein in the monogastric animals (Tepperman, 1968), however, ts role in the normal metabolism of ruminants is poorly understood. Since alloxan-induced diabetes produced a similar response in ruminant and nonruminant animals (Reid et al., 1963) insulin is apparently important in carbohydrate metabolism by ruminants. Additional evidence

supporting an important role of insulin in ruminant animals was obtained when insulin release from the pancreas was shown to occur following an elevation of the blood levels of glucose and fructose (Manns and Boda, 1967) and of propionic and butyric acids (Manns et al., 1967 and Forbes, 1980). There were also some evidence of the role of insulin in AAs metabolism.

The present data (Tables 25 to 28) show that arterial PAA was slightly (but not significantly) lower after insulin injection. The total AA reduced to 86% of the initial level. Similar results were obtained with sheep by Call et al. (1972). Their average reductions were 83% for NEAA and 66% for the EAA.

Insulin is believed to increase protein synthesis in muscle (Bergen, 1974; Trenkle, 1974). Following a stimulation of protein synthesis by substrate availability (energy and AAs) and/or endocrine influences, the increase in net AA uptake is most likely. Figure 9 shows that insulin does have a significant effect on the net AA uptake by the hind limb in steers. The net uptake increased three fold for EAA, eight fold for NEAA and five fold for BCAA. These increases remained for two hours. At four hours post-injection the net uptake of EAA decrease, while the NEAA released in net amounts. Ala and gln accounted for all the NEAA released. Insulin has the same effect on the net AA uptake in the non-ruminant animals (Felig et al., 1975; Hutson et al., 1980). Bergman and Heitmann (1978) concluded that insulin had no effect on net hepatic removal and concentrations of AAs. Insulin did, however, decrease the concentrations of the BCAA indicating increased protein synthesis in muscle.

Addition of insulin to the perfused rat hind limb (Grubb, 1976) resulted in a significant increase in the rate of glucose uptake and de

novo synthesis of ala in ruminants vs nonruminants.

The proposed mechanism of insulin in stimulating the net AA uptake may be due to stimulation of AA transport (Felig, 1975; Etherton, 1982), due to a slowing of the rate of AA oxidative decarboxylation (Hutson et al., 1980), or protein degradation (Young, 1980).

IV. Effect of Starvation

A comparison between the levels of arterial PAA in fed and starved steers showed only slight changes for most AA (Tables 29, 30). However, glu, ala and leu were significantly reduced. The arterial concentration and the changes upon starvation are in accord with other data on blood AAs in sheep (Wolff et al., 1972; Bergman et al., 1974; Ballard et al., 1976). However, Bergman and Pell (1982), indicated that leu concentrations increased in the arterial plasma of starved sheep. They concluded that this rise in blood leu concentration in starved sheep occurred because net leu production by peripheral tissues overcompensated for the negligible leu absorption by the portal-drained viscera.

In comparison to sheep blood (Ballard et al., 1976), steer blood has much lower concentrations of all AAs. Bergen (1979) reported the same results in comparison between sheep and steers. Ser and ala exist in higher concentration in our studies. Phe level appears to be in similar amount in both species.

There were marked differences in the net release of AAs by the hind limb when starved steers were compared to fed steers (Figure 10). In fed steers, there was an approximate balance across the hind limb with respect to total AA, while asp, ser, asn, gly, val, and leu are taken up by the hind limb in significant amounts and there was a net release of ala and gln. With starvation (for 24 and 48 hours), however, there was a large

overall negative AV difference and net release (Figure 10) for all AAs across the hind limb as expected.

Comparison with data on other species is complicated by the general use of plasma rather than blood for AA measurement (Ballard et al., 1976) as well as the difficulty of defining equivalent conditions in monogastric to the fed and starved steers. Nevertheless, the ala AV difference of about 106 n mole/ml in starved steers is much higher than the value reported for sheep (26 n mole/ml) (Ballard et al., 1976) and is equal to the value of approximately 100 that have been reported for the human forearm or leg (Pozefsky et al., 1969; Felig et al., 1970). However if those values are corrected to the metabolic body weight (kg .75) it would be 1.4 n mole/kg .75 for steers, 1.38 n mole/kg .75 for sheep and 4.1 n mole/kg .75. With this correction one can see that human blood has higher ala AV difference than steers or sheep. Does this negate the generality of the proposed ala cycle or is it perhaps an adaptation in steers (the present study) or sheep (Ballard et al., 1976) to account for the restricted supply of glucose? This question needs further work to be answered. It seems that the ala cycle concept must be somehow modified to encompass the ruminant data. It has been proposed that the amino group of ala is derived not only from ala per se, but also from the BCAA (see the literature review for details). The residual α-keto acids corresponding to these AAs were presumed to be degraded in muscle (Krebs, 1972; Odessy et al., 1974). However, Hutson and Harper (1981) clearly showed that the BCKA were released from skeletal muscle and accumulate in the perfusion medium and not catabolized in the muscle.

The comparison between the present data plus Ballard et al. (1976) to the results of Pozefsky et al. (1969) and Felig et al. (1970); who

worked with man, suggests that either the restricted availability of glucose carbon reduces the amount of pyruvate available to be transaminated to ala, or, the oxidation of lipid substrates spares AA oxidation (Ballard et al., 1976).

Further studies are required to clarify these possibilities.

# CONCLUSION

Based on the data obtained in these studies, the following conclusions can be made:

1. Plasma amino acid response curves are a short term procedure to study AA requirements in growing cattle when long term studies are impossible or too expensive.
2. The total sulfur amino acid requirement was 17.82 g/d; at least 42% of the TSAA needs must be supplied by methionine.
3. Cysteine can supply part of the TSAA needs and can spare methionine in growing steers.
4. The esophageal groove reflex is useful for rumen bypass of liquid diets containing protein and supplemental amino acids and could be applied to sutdy AA requirements of young ruminants with developed rumens.
5. A low protein diet as well as starvation reduced the plasma flow across the hind limb in steers.
6. High protein diet as well as insulin injection had no effect on plasma flow across the hind limb in steers.
7. Arterial concentrations of almost all AA increased after feeding in growing steers.
8. Alanine and glutamine were continously released for all treatments before as well as after feeding in growing steers.
9. The arterial AA concentrations of the low protein - and control-steers were the same, while they were increased in the high protein-fed steers.

10. The net AA uptake in the hind limb was decreased in the low protein-fed steers, while it was increased in the high protein-fed steers comparing to the control-fed steers.
11. Insulin had no effect on the AA concentration in the arterial blood, but the hormone significantly increased the net uptake of AA in the steers hind limb.
12. Starvation for 24 hours or 48 hours caused a net release of almost all AAs from the steers hind limb.

# APPENDIX A

# APPENDIX A

## DETERMINATION OF PARA-AMINO HIPPURIC ACID

One vol. of whole blood was added to 12 vol. of trichloroacetic acid (10% wv) and filtered through No. 42 Whatman paper. 10 ml filtrate is heated in a boiling water bath for one half hour. A known quantity of PAH was added to appropriate volume of whole blood and used for recovery calculation.

For the colorimetric determination four reagents were prepared. (a) 1.2 N HCI; (b) 100 mg % Na $NO_2$; (c) 500 mg % ammonium sulfamate; and (d) 100 mg % N-(1-naphyl) ethylenediamine dihydrochloride.

To 10 ml of the filtrate, 2 ml of (a) and 1 ml of (b) were added and the solutions were mixed. Between three to five minutes, 1 ml of (c) was added and mixed. Another three to five minutes later 1 ml of (d) was added and mixed. A blank of 10 ml water was treated in the same manner. All the solutions were read at any time after 10 min, using 540 nm.

A standard curve was prepared from serial dilutions in distilled water range .02 - .25 mg of purified PAH.

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