AMINO ACID STUDIES IN RUMINANTS: INVESTIGATION OF A METHOD FOR DETERMINING THE LIMITING AMINO ACID

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ABSTRACT

AMINO ACID STUDIES IN RUMINANTS
INVESTIGATION OF A METHOD FOR DETERMINING THE
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by

Emerson Lucine Potter

This dissertation is concerned with developing a procedure which would identify the limiting amino acid of ruminants from plasma amino acid (PAA) concentrations. The limiting amino acid in monogastric animals is defined as the essential amino acid in the dietary protein which is least able to fulfill the animal's requirements. In comparison, the limiting amino acid of ruminants is the essential amino acid in the protein passing to the small intestine which is least able to fulfill the animal's requirements. Dietary protein degradation and protein synthesis by the microbes in the rumen account for this difference.

Glucose infusions into the carotid artery caused decreases in the PAA levels which suggested that protein synthesis occured (Potter et al. 1968). If glucose stimulates protein synthesis then the amino acid limiting protein synthesis should be the plasma essential amino acid whose post-glucose concentration is decreased the most, when expressed as a percent of the pre-glucose PAA level. Potter et al. (1968) expressed post-glucose PAA concentrations as a percent of pre-glucose PAA concentrations and termed these ratios plasma amino acid indices (PAAI).

$$PAAI = \frac{A}{B} \times 100$$

When A = post-glucose PAA concentration
B = pre-glucose PAA concentration

The lowest PAAI should correspond to the limiting amino acid since this corresponds to the PAA which was utilized in protein synthesis to the largest degree in respect to the pre-glucose level. Experiment one, a 3 x 3 latin square replicated 4 times, was designed to investigate PAA levels of sheep as they changed due to intravenous infusions of saline and glucose (two levels of glucose, low and high). The one hour post-glucose infusion PAA concentrations were significantly ($P < .05$) decreased below the pre-glucose PAA levels. The pattern of decrease in plasma essential amino acids was significantly correlated to the amino acid composition of striated muscle of lamb ($P < .05$). Isoleucine and leucine had the lowest PAAI and, based on the assumptions outlined above, were thus indicated as the limiting amino acids.

Experiment two, using 12 rats per dietary protein (egg albumin, casein, soy and zein), was designed to determine the accuracy of the PAAI procedure in predicting the limiting amino acid. The PAAI procedure did not identify the limiting amino acid in 3 of the 4 proteins.

Expressing PAA concentrations from rats (10/diet) fed test diets in experiment 3 as a percent of the average PAA concentration from rats fed whole egg, whole egg plus amino acids and casein diets (high quality proteins), correctly identified the limiting amino acid in 6 of the 8 diets (indicated by lowest percent value). The percent values calculated in this way were defined as "plasma amino acid reference indices" whereas the PAA concentrations in the rats fed whole egg, whole egg plus amino acid and casein were termed "reference concentrations."

$$\text{Plasma amino acid reference indices} = \frac{A}{B} \times 100$$

When A = PAA concentration from rats fed a test diet
B = Reference concentration from rats fed high quality proteins

Plasma amino acid concentrations were determined in sheep (surgically prepared with duodenal re-entrant cannulas) duodenally infused with proteins of "known" amino acid composition. PAAI did not identify the limiting amino acid of the infused proteins. Reference indices, calculated using the average PAA concentrations from sheep infused with whole egg and casein as reference concentrations, identified the limiting amino acid in 6 of the 8 infusions. When these reference concentrations were compared to PAA concentrations of sheep in other studies, methionine, phenylalanine, lysine and isoleucine were indicated as the limiting amino acids.

The final experiment showed that amino acid levels in blood collected from the jugular vein and carotid artery of sheep did not differ. This suggests that blood from either site can be used in PAA studies.

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ABBREVIATIONS

Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
Cas	Casein
CGM	Corn Gluten Meal
Cys	Cystine
EA	Egg Albumin
EAA	Essential Amino Acids
ft	feet
T_1	Glucose or 1 hr Post Infusion
Glu	Glutamic acid
Gly	Glycine
gm	gram
g	gravity
His	Histidine
hr	hour
Ile	Isoleucine
kg	kilogram
Leu	Leucine
l	liter
Lys	Lysine
Met	Methionine

ABBREVIATIONS (cont'd)

ml	milliliter
N	Nitrogen
Orn	Ornithine
Phe	Phenylalanine
PAA	Plasma Amino Acids
PAAI	Plasma Amino Acid Indices
$P < .05$	Probability less than 5%
Pro	Proline
T_0	Saline or Pre-Infusion
Ser	Serine
NaCl	Sodium Chloride
Soy	Soybean Meal in Experiment 3 Promosoy in Experiment 4
Soy + AA	Soybean Meal plus Amino Acids
Soy + Met	Soybean plus Methionine
Thr	Threonine
TEAA	Total Essential Amino Acids
TNEAA	Total Non-essential Amino Acids
Tyr	Tyrosine
Val	Valine
W/V	Weight per Volume
WE	Whole Egg
WE + AA	Whole Egg plus Amino Acids

INTRODUCTION

Ruminants are important to man because they produce meat, milk and fiber from forages and non-protein nitrogen. Increased productive efficiency may result from studies defining the amino acid which limits the synthesis of milk or muscle proteins. Since the amino acid supply to the ruminant consists of a mixture of undegraded dietary and ruminally synthesized microbial proteins, the limiting amino acids of these protein mixtures will determine productive functions. The limiting amino acid is that acid whose availability is such that it is least able to fulfill the animals requirements (Almquist, 1954). Since the amino acid composition of the dietary protein differs from that supplied to the small intestine, growth responses to changes in the dietary amino acid supply will not define the limiting amino acid in ruminants. Plasma amino acids (PAA) concentrations should reflect the supply of amino acids to tissues of ruminants and may be useful for identification of the limiting amino acid.

Purser et al. (1966) infused a starch-glucose mixture into the rumen of sheep and reported subsequent decreases in PAA levels. When compared to the pre-infusion PAA levels, lysine was decreased the most by this starch-glucose infusion in defaunated sheep whereas no single amino acid was decreased more than any of the others in faunated sheep fed the same diet. Lysine was expected as the limiting amino acid since these sheep were fed a corn diet which is limiting in lysine. It appeared that the starch-glucose induced changes in PAA levels might

be useful in determining the limiting amino acid in ruminants. The infusions of glucose into the carotid artery of sheep decreased the essential PAA levels (Potter et al., 1968). Decreases in the absolute concentrations of each essential amino acid in the plasma were significantly ($P < .05$) correlated with the essential amino acid composition of striated muscle of lamb, suggesting that glucose induced protein synthesis. Theoretically, the limiting amino acid is that acid which decreased the most in respect to the amount present in the plasma before the glucose infusion; by this definition isoleucine was the limiting amino acid.

Research presented in this dissertation deals with the effect of intravenous glucose infusions on PAA concentrations and the determination of the limiting amino acid in ruminants from PAA concentrations.

LITERATURE REVIEW

Amino Acid Supply to the Ruminant

Nitrogen metabolism in the ruminant has been the subject of several reviews (Blackburn, 1965; Hungate, 1966; Waldo, 1968; Chalupa, 1968; Conrad and Hibbs, 1968; Smith, 1969; Kay, 1969; McDonald, 1968 and Purser, 1970a,b). The ability of the ruminant to use non-protein nitrogen is related to its digestive tract which includes large feed reservoirs known as the rumen and reticulum. Forage digestion and non-protein nitrogen utilization occur in the rumen as the result of bacterial and protozoal metabolism.

Rumen bacteria and protozoa influence the amino acid supply to the animal by degrading dietary protein and by synthesizing microbial proteins (Bryant and Robinson, 1961; Hungate, 1966 and Allison, 1969). Loosli et al. (1949) reported that the essential amino acids (EAA) could be synthesized in the rumen of sheep fed only urea as a nitrogen source. Black et al. (1957) and Downes (1961) reported that threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine and histidine were essential amino acids for ruminants since they were not synthesized in the ruminant's tissue.

The quantity and quality of protein reaching the duodenum of the ruminant depends upon the degradation of dietary protein and the quantity of microbial protein synthesized in the rumen (Smith, 1969). Clark et al. (1966) using sheep compared the dietary nitrogen (N) intake to the quantity of N reaching the duodenum. When diets containing

5.1, 7.3, 16.4 and 24.8 gm N/day were fed, 9.0, 12.2, 17.6 and 19.2 gm N/day, respectively reached the duodenum. The quantity of N reaching the duodenum increased on the two lowest N diets and decreased on the highest N diet in respect to the dietary N level. The amino acid composition of abomasal contents differs from the dietary amino acid composition (Clark et al., 1966; Bigwood, 1964 and Little et al., 1968). Clark et al. (1966) and Bigwood (1964) reported that lysine, threonine and isoleucine formed a larger fraction whereas proline, arginine and leucine formed a smaller fraction of the total amino acids in duodenal digesta than they did in the diet. Little et al. (1968) reported the amino acid composition of abomasal digesta to be similar in sheep fed soybean meal, gelatin and casein but different in zein-fed sheep.

The quantity of undegraded dietary protein which passes to the duodenum was 10 and 60%, respectively, when casein and zein were the dietary proteins fed to sheep (McDonald and Hall, 1957; McDonald, 1954 and Ely et al., 1967). Solubility determines whether a protein will be degraded by microbes in the rumen. Annison (1956) investigated ruminal degradation of casein, soy, ground-nut protein, zein, bovine serum albumin and wheat gluten and found rapid hydrolysis of the first three proteins. This was substantiated by Hendricks and Martin (1963) who reported a correlation between protein solubility in a salt solution and rate of degradation in the rumen. Treating protein with heat, tannic acid and formalin will decrease the solubility and increase the quantity of dietary protein passing to the rumen (Chalmers et al., 1964; Driedger and Hatfield, 1970; Ferguson et al., 1967; Tagari et al., 1962 and Whitelaw et al., 1967).

Hungate (1966) reviewed data for protein production in the rumen

and estimated production as 15 gm of microbial cells for each 100 gm of substrate fermented by a mixed microbial population. A cell yield of 21.3% was reported (Hungate, 1963) for Ruminococcus albus grown on cellobiose media in continuous culture while, a 46.8% cell yield was observed for Bacteroides amylophylus when grown on maltose media (Hobson and Somers, 1967). Walker and Nader (1968) measured microbial growth rates using radioactive ^{35}S sulfide and found a 14% cell yield for a mixed rumen population in vitro. Conrad et al. (1967) published estimates of ruminal methionine synthesis based upon the incorporation of labelled ^{35}S into methionine. Between 31 and 59 mg of methionine were synthesized per kg of body weight per day by the dairy cow. This corresponds to the synthesis of 1.4-2.6 gm of bacterial protein per kg/day. El-Shazly and Hungate (1966) used the diaminopimelic acid concentration of bacteria to estimate that 69-80% of the total N in the rumen was of microbial origin.

The quality of both microbial and undegraded dietary protein which reaches the small intestine will determine the amino acid supply to the ruminant. Purser (1970b) and Waldo (1968) reviewed data regarding quality of rumen microbial proteins. McNaught et al. (1954) and Bergen et al. (1968a) agree in their estimates of the quality of rumen protozoal and bacterial proteins fed to rats. McNaught et al. (1954) reported the "true digestibility" (TD), biological value (BV) and net protein utilization (NPU) as 74, 81 and 60; respectively, for rumen bacterial protein and 90, 80 and 73; respectively, for rumen protozoal protein. Since the digestibility of protozoal protein is higher than that of rumen bacterial protein the quality of protein passing to the lower gut may increase as increased proportions of protozoal protein

pass to the lower digestive tract. Bergen et al. (1968b) reported no differences in the TD, BV or NPU of microbial proteins taken from the rumen of sheep fed different rations.

The amino acid composition of rumen microbial proteins has been investigated; Purser and Buechler (1966) reported 22 strains of rumen bacteria to have the same amino acid composition. The amino acid composition of different rumen protozoal preparations are similar (Weller, 1957 and Purser and Buechler, 1966). In comparison to rumen bacterial protein, rumen protozoal protein contains a larger proportion of lysine, leucine, phenylalanine and tyrosine (Purser and Buechler, 1966). Therefore the amino acid composition of microbial protein which passes out of the rumen is quite constant.

The digestibility and release of amino acids from individual strains of rumen bacteria were studied with a pepsin-pancreatin in vitro digest system (Bergen et al., 1967). Since digestibility of the protein in these strains of bacteria varied between 45 and 85%, the supply of amino acids available for absorption by the animal may depend upon the population of rumen bacteria.

The quantity of microbial protein which passes to the abomasum is dependent upon the quantities of bacterial and protozoal protein synthesized in the rumen. The amount of bacterial protein in the rumen of faunated sheep is only half of that in defaunated sheep (Purser et al., 1966). Since protozoa use bacteria as a protein source (Coleman, 1967a,b and Wallis and Coleman, 1967), the decrease in bacterial counts of faunated sheep may be the result of protozoal feeding or competition for energy substrates in the feed.

Bergen et al. (1968) determined the limiting amino acid of rumen

protozoal and bacterial proteins by feeding rats diets which contained these proteins as the sole N source. Histidine and cystine were the limiting amino acids in the rats fed protozoa and bacteria, respectively; leucine, arginine and lysine were also in short supply. These conclusions were based upon determination of the limiting amino acid as described in the PAA score procedure (McLaughlan, 1964).

Since the dietary supply of amino acids is not the same as that reaching the abomasum, the amino acid composition of abomasal digesta has been measured. The sulfur amino acids were limiting when abomasal amino acid levels were compared to the amino acid requirements of swine (Poley et al., 1963). Schelling et al. (1967) and Duncan et al. (1953) reported the amino acid composition of rumen digesta after sheep were fed a non-protein nitrogen diet. Upon expressing the amino acid composition of rumen ingesta as a ratio of the amino acid composition of whole egg, histidine and methionine were suggested as being in short supply (Schelling et al., 1967).

The N passing to the small intestine of the ruminant is mainly protein and protein degradation products produced in the abomasum. The N passing to the duodenum is composed of 65-75% amino acid nitrogen, 5-10% ammonia nitrogen (Clark et al., 1966 and Hogan et al., 1969) and 15% nucleic acid nitrogen (Smith et al., 1969). Based on differences between the quantities of amino N appearing at the duodenum and iluem, Clark et al. (1966) found 4-10 gm/day of amino N absorbed in sheep. Amino acid disappearance does not indicate net absorption since there are endogenous secretions of protein in the gut (Kay, 1969).

Hogan (1957) reported abomasal N secretion of 1.2 gm/day and small intestinal N secretion (8-12 gm/day) was found by Campbell et al. (1961) using ^{131}I labelled albumin in sheep. Nasset (1964) concludes

from rat and dog studies, that endogenous gut secretions may dilute the dietary protein 1-4 fold and serve as a homeostatic mechanism to prevent an amino acid deficiency after a single meal. However, this will not prevent amino acid deficiencies when feeding an imbalanced diet for a longer period (Rogers and Harper, 1965). Olmstead et al. (1966) fed 4 different protein diets to men and found the dietary protein had some influence on the amino acid composition of duodenal digesta.

Whereas the major supply of amino acids to ruminant tissue is from absorption through the small intestine, other absorption sites have been investigated. Leibholtz (1965 and 1969) reported rumen fluid amino acid concentrations to be less than plasma levels, except in the case of lysine, histidine, cystine and alanine which rose above the plasma levels for short periods of time after high protein diets were fed. Cook, Brown and Davis (1965) inserted a catheter into the right ruminal vein of a goat and a steer before adding 15 gm of glycine to the rumen. The ruminal glycine concentration in this study was higher than the normal glycine levels in rumen fluid reported by Wright and Hungate (1968). There is passive amino acid absorption from the rumen, but it is not a major contributor to the free amino acid pool (Kay, 1969). Hogan and Phillipson (1960) reported the disappearance of 0.5-2.0 gm of N/day from the large intestine of sheep, but amino acid absorption from the large intestine has not been established. The N which disappeared from the large intestine was probably absorbed as ammonia, transported to the liver, incorporated into urea and the urea excreted in urine or recycled to the rumen via saliva or by diffusion across the rumen wall (Somers, 1961; Weston and Hogan, 1967 and Houpt and Houpt, 1968). Kay (1969) postulates back-flow of ingesta from the caecum

to the ileum, however a significant contribution to the amino acid pool in this manner is questionable and unsubstantiated.

Although protein digestion and amino acid absorption in the small intestine of the ruminant is not known to differ from that in the monogastric there are several physiological differences. While flow through the small intestine of the monogastric subsides as the stomach empties, flow through the small intestine of the ruminant is continuous throughout the day as ingesta continually passes from the rumen (Balch and Campling, 1965). Therefore there should always be some protein available for digestion in the small intestine of the ruminant. The volume of ingesta which passes into the duodenum of sheep is about 10 liters/day. The major contributors to this large flow are 6-16 liters of salivary and 4-6 liters of abomasal secretions (Phillipson, 1964). Monogastrics absorb most of their metabolizable energy from the small intestine as carbohydrate, and ruminants acquire theirs from the rumen in the form of acetic, propionic and butyric acids. These acids are end products of the rumen fermentation and serve along with small quantities of intestinally absorbed carbohydrate to fulfill the energy requirements of the ruminant. The amino acids absorbed from the small intestine may be more efficiently used for productive purposes by the ruminant if metabolizable energy substrates are absorbed at the same time (Purser 1970a).

Dietary protein utilization in monogastrics depends upon several integrated factors: amino acid composition of the protein, rate of movement through the intestinal tract, intestinal digestion, amino acid absorption and availability of energy sources (Munro, 1964). The amino acid composition of infusions into the small intestine of man influences amino acid absorption (Orton, 1963 and Abibi et al., 1967). The pattern

of amino acids released from protein during enzymatic digestion in the abomasum and small intestine may influence subsequent amino acid absorption. Bergen et al. (1967) reported that arginine was not released from either protozoal or bacterial proteins during a 2 hour pepsin digest in vitro. The consequence of this upon amino acid absorption is unknown.

Methods of Determining the Limiting Amino Acid

Determination of the limiting amino acid is an integral part of protein quality evaluation because this identifies the amino acid which limits the utilization of the other amino acids for protein synthesis. The limiting amino acid is defined as that which, by quantity, is least able to meet the animal requirements. It is the acid that will give the greatest growth response when supplemented into the diet. The limiting amino acids of dietary proteins were first determined by measuring growth response when different amino acids were supplemented into the diet (Mitchell and Smuts, 1932).

Knowledge about both the dietary amino acid composition and the amino acid requirement of the animal should allow determination of the limiting amino acid by direct comparison (Black and Mitchell, 1946-47). The limiting amino acid should give the smallest value when expressing the dietary amino acid levels as a percent of their requirements. The studies of Pecora and Hundley (1951) and Sauberlich et al. (1953) showed that the limiting amino acid was not always so identified. Carrol et al. (1953) used the amino acids available from digestion in the intestine, as determined from chromic oxide-amino acid ratio in feed and small intestinal ingesta, in conjunction with the amino acid requirements to predict the limiting amino acid. This procedure is lengthy and has not been accepted.

Microbiological assays used to access protein quality (Ford, 1962; Miller et al., 1964, 1965b and 1965c) are dependent upon the use of specific micro-organisms which require the presence of one specific amino acid for growth. The amount of that amino acid in the protein is directly proportional to the growth of the micro-organism. This measures the utilization of an amino acid in a protein by the micro-organism, which may differ from utilization in mammalian systems. These investigators concluded that this method was not sensitive enough to determine the limiting amino acids of dietary proteins.

Plasma amino acid concentrations may (McLaughlan and Morrison, 1968; Richardson et al., 1953; Charkey et al., 1950 and Guggenheim et al., 1960) or may not (Denton and Elvehjem, 1954; Goldberg and Guggenheim, 1962; McLaughlan, 1963 and Frame, 1968) reflect the dietary amino acid levels. Differences in PAA patterns after feeding protein diets can be attributed to the digestibility of the protein fed (Denton and Elvehjem, 1954 and Goldberg and Guggenheim, 1962). PAA concentrations can reflect the amino acid composition of dietary proteins, but a direct relationship is not always observed.

The metabolic state of the animal will influence the PAA levels independently of dietary protein. Charkey et al. (1953) reported elevated levels of lysine and threonine during periods of catabolism and postulated that these amino acids are resistant to deamination. Munro (1964) reviewed data showing increases in PAA levels during fasting periods. The presence of readily metabolizable energy, fed to rats (Bergen and Purser, 1968 and Knipfel et al., 1969), chickens (Hill and Olsen, 1963) and humans (Harris and Harris, 1947; Munro and Thompson, 1953; Swendseid et al., 1967 and Crofford et al., 1964) or infused into

the rumen (Purser et al., 1966) or carotid artery of sheep (Potter et al., 1968), resulted in decreases in the PAA concentrations.

The plasma concentration of the limiting dietary amino acid remained low until the total dietary level of that amino acid was increased above the animals requirement after which the plasma concentration increased linearly with supplementation levels (Zimmerman and Scott, 1965). Similar responses were reported for pigs fed a test diet for one week but not for pigs fed the diet for only one day (Mitchell et al., 1968b). These data suggest that a period of metabolic adjustment is necessary before PAA level will reflect the dietary amino acid composition.

Interrelationships between the blood levels of different amino acids have been observed (Kumta and Harper, 1962). Removal of one amino acid from the diet, causing a severe amino acid deficiency, will result in a low plasma level of that amino acid whereas the blood levels of the other acids will increase. The increase in the blood levels of the other amino acids is the result of a lower utilization rate when protein synthesis is impeded by an amino acid deficiency. Thus, high blood amino acid concentrations do not always indicate that the animal is absorbing large quantities of amino acids. The relative distribution between PAA levels or changes in levels resulting from the diet may be useful in determining the limiting amino acid from blood.

Plasma amino acid concentrations are the measured parameter in several procedures which determine the limiting dietary amino acid. Longenecker and Hause (1959) used "plasma amino acid ratios" to predict the limiting amino acid of several dietary proteins. The plasma amino acid ratio procedure predicted the same amino acid to be limiting as had been previously determined in rat growth response trials.

These plasma amino acid ratios were calculated according to the following formula.

$$\text{Plasma amino acid ratio} = \frac{A-B}{C}$$

Where A = PAA concentration after consumption of a meal
B = PAA concentration during fast
C = amino acid requirement of the animal

Theoretically the difference between "fasting" and "after feeding" PAA concentrations should reflect the amino acid composition of the dietary protein. When these differences are expressed as a percent of the amino acid requirements, the lowest value should correspond to the limiting amino acid or the amino acid least able to fulfill the requirement. Hill and Olsen (1963) used a similar method for predicting the limiting amino acid of protein fed to chickens. PAA concentrations after feeding a non-protein-calorically adequate diet were used in place of the fasting PAA concentrations. This procedure predicted the same amino acids as limiting in zein, gelatin and casein diets as did growth trials.

The "plasma amino acid score" procedure has identified the limiting amino acid of many proteins (McLaughlan, 1964; McLaughlan, 1967; and Rao et al., 1968). Plasma amino acid scores are defined as:

$$\text{Plasma amino acid score} = \frac{A}{B} \times 100$$

Where A = PAA concentration of fed animal
B = PAA concentration of fasted animal

Smith and Scott (1965) compared PAA concentrations from chickens fed a reference crystalline amino acid diet, shown to support optimal growth, to PAA concentrations from chicks fed test protein diets. The limiting amino acids in sesame meal and soybean meal were identified by:

$$\frac{A-B}{B}$$

When A = PAA concentration for animals fed test diet
B = PAA concentration for animals fed crystalline amino acid diet

While three of these procedures have never been extended beyond the developmental stage, the plasma amino acid score procedure (McLaughlan, 1964) has been easy and extensively used in determination of the limiting amino acid of proteins fed to rats. While its use has not been extended to other animals, it has been very accurate in identifying the limiting amino acid as determined in growth trials.

Plasma Amino Acids in Ruminants

The PAA methods (Longenecker and Hause, 1959; Hill and Olsen, 1963; McLaughlan, 1964; and Zimmerman and Scott, 1965) used to determine the limiting dietary amino acids in monogastrics cannot be used in ruminants. The methods of Longenecker and Hause (1959) and Hill and Olsen (1963) require both knowledge of the amino acid requirements of the animal and, either fasting PAA concentrations or PAA concentrations after a non-protein meal. The PAA score method (McLaughlan, 1964) requires fasting PAA concentrations whereas the method of Smith and Scott (1965) requires a reference diet composed of crystalline amino acids (shown to promote optimal growth of the chick). These data are not available for the ruminant.

Purser et al. (1966) suggested using a modification of the Hill and Olsen (1963) method to determine the limiting amino acid in ruminants. Plasma amino acid concentrations increased after feeding a low caloric diet whereas a high caloric diet decreased the PAA levels (Purser et al., 1966). Decreases in PAA levels resulted from intra-arterial infusion of glucose and propionate (Potter et al., 1968).

Purser et al. (1966) expressed the PAA levels after a starch-glucose infusion as a percent of the pre-infusion level, and Potter et al. (1968) expressed the post-glucose PAA levels as a percent of the pre-glucose levels. These infusions of glucose or starch and glucose appeared to result in protein synthesis since the decreases in the essential PAA levels correlated to the essential amino acid composition of striated muscle of lamb. The amino acid which decreased the most during protein synthesis (relative to the original levels) should be the limiting amino acid. The limiting amino acid so determined was lysine for defaunated sheep whereas no single amino acid was limiting in faunated sheep (Purser et al., 1966) and isoleucine was limiting in glucose infused sheep (Potter et al., 1968).

The effect of a metabolic energy shortage upon the PAA levels that occur in the fasting state, was reported by Leibholtz and Cook (1967) in sheep and by Brown et al. (1965) in cattle. Three weeks of starvation decreased the plasma levels of threonine, glutamic acid, alanine, valine, isoleucine and leucine whereas lysine levels increased (Leibholtz and Cook, 1967). The plasma levels of phenylalanine, tyrosine, citrulline, ornithine, lysine, valine, threonine, leucine and isoleucine increased gradually over an 88 hour fast (Brown et al., 1965). The differences between these studies may be due to length of fast as the source of energy to the animal may change with length of fast.

The amino acid composition of abomasal contents, which indicates the amino acid supply to the small intestine of the ruminant was reflected in the PAA concentration pattern of these sheep (Poley and Trenkle, 1963 and Little et al., 1968). Hogan et al. (1968) reported that plasma essential amino acid levels increased as increasing quantities of casein were infused into the duodenum.

Increases in wool growth (Reis and Schinckel, 1964; Reis, 1969; and Colebrook and Reis, 1969) and nitrogen retention (Egan, 1965 and Schelling et al., 1968) has resulted from the abomasal supplementation of protein and/or amino acids to sheep. Results from these studies suggest that either protein or an amino acid is limiting productive performance of the unsupplemented animals.

Study of changes in the plasma amino acid levels of ruminants abomasally infused with protein and/or amino acids may be a useful guide in future limiting amino acid determinations. Schelling (1970) supplemented methionine or casein into the abomasum of growing lambs fed a soybean meal diet (methionine is limiting in rats fed soybean protein) and measured N retention and PAA concentrations. Lambs fed a 11% crude protein diet and abomasally supplemented with 0, 1.0, 2.0 and 3.0 gm of dL-methionine or 30 gm of casein/day had positive N balances of 3.74, 3.94, 4.12, 4.25 and 5.12 gm/day respectively, whereas lambs fed a 14% crude protein diet and receiving either 0, 1.0 and 2.0 gm of methionine had positive N balances of only 1.46, 2.07 and 3.11 gm/day respectively. These results suggest that methionine was the limiting amino acid in sheep fed the 14% protein diet and protein was limiting the 11% group. Plasma methionine levels of 3.7, 6.9, 11.9 and 39.3 micrograms/ml were reported for lambs abomasally infused with 0.0, 1.0, 2.0 and 4.0 gm of dL-methionine/day, respectively (Schelling, 1970). The increase in plasma methionine levels, which resulted from the supplementation of 4.0 gm of methionine, suggest that the methionine supply had exceeded the requirement. Thus, methionine was the limiting amino acid in unsupplemented animals. Ely et al. (1969) reported an increase in the plasma lysine and a decrease in the other PAA levels when lysine was intravenously infused into sheep fed a zein diet

(lysine is limiting in zein fed rats). Thus lysine was suggested as limiting in the lysine unsupplemented sheep. This approach, of titratation with the expected limiting amino acid until there is an increase in the plasma level of the supplemented amino acid may prove useful in determining the limiting amino acid in ruminants. In this system an increase in the plasma concentration indicates the amino acid is no longer limiting.

Ørskov and Frazer (1969) reported N balance data for lambs bottle-fed liquid protein diets. When fed through a nipple, the diet will pass directly to the abomasum and avoid ruminal modification (Ørskov and Frazer, 1969). The use of liquid protein diets may aid in the development of a method for determining the limiting amino acid in ruminants as this would eliminate the modifications of the dietary protein in the rumen.

The present research was undertaken to investigate PAA concentrations and changes in PAA levels which result from glucose infusion as these may be useful in determining the limiting amino acid of ruminants. Theoretically, PAA concentrations reflect the "tissue pool" of free amino acids (Rogers and Harper, 1968) which, in turn, are influenced by the supply from absorption and by utilization for protein synthesis (Munro, 1968). The rate of incorporation of amino acids into protein is influenced by energy availability for protein synthesis (Munro et al., 1962). Assuming that the PAA concentrations reflect the amino acid supply in ruminants, and that glucose infusion will stimulate protein synthesis should show the largest relative decrease in the plasma immediately after glucose is infused. This relative decrease has been expressed as "plasma amino acid index" (PAAI), which are calculated

according to the following formula.

$$PAAI = \frac{\text{PAA concentration post-glucose}}{\text{PAA concentration pre-glucose}} \times 100$$

The limiting amino acid for the ruminant should be indicated by the lowest index value.

METHODS AND MATERIALS

Blood Samples for Amino Acid Analysis

Blood samples were collected in heparinized syringes and the plasma separated by centrifugation in a refrigerated centrifuge at 10,000 x g for 10 minutes. To each ml of plasma, .1 micro mole of norleucine was added as an internal standard. After mixing, .1 ml of 50% (w/v) sulfosalycylic acid, per ml of plasma, was added to precipitate plasma proteins. After centrifugation at 25,000 x g, the protein-free filtrate was frozen and stored until analyzed. Plasma amino acid concentrations were determined with a dual column TSM-1 Technicon Auto Analyzer using a procedure developed in this laboratory and reported by Makdani et al. (1970).

Experiment One

This experiment was designed to substantiate the report by Potter et al. (1968) that PAA levels of sheep decreased following an intra-arterial glucose infusion. Two groups of three, growing wether lambs were selected on the basis of equal body weight and surgically prepared with exteriorized carotid arterial loops, according to the method of Bone et al. (1962). The lambs in one group (replicate 1) each weighed 14.9 kg whereas the lambs in the other group each weighed 12.3 kg (replicate 2). Within each group, the lambs were randomly assigned to a 3 x 3 latin square; treatments were saline, low glucose and high glucose. Replicates 1 and 2 were

repeated and these are referred to as "replicates 3 and 4", respectively. There were 15 days between replicates and 5 days between collection days (periods) within replicates. The experimental design is shown in Table 1.

Lambs were housed indoors, singly, in 4 ft x 6 ft, metal stalls and had free access to water. They were fed daily (8 a.m.) a portion of diet 1 (composition shown in Table 2) equal to 7% of the metabolic body weight ($BW^{.75}$) (Kleiber, 1961). On treatment days, feeding was delayed until after the second blood sample was collected. At 8 a.m. on treatment days, 10 ml of blood were withdrawn from the carotid artery into a heparinized syringe and then treatments (saline or glucose) were infused into the jugular vein via catheter. A second blood sample was collected one hour after the infusion. The 8 a.m. blood sample is referred to as the "T_0" sample and the post infusion sample as "T_1".

The treatment infusion mixtures were; .9% NaCl (W/V), low glucose (25% W/V) and high glucose (50% W/V); these glucose infusions provided glucose at .05% and .1% of the metabolic body weight ($BW^{.75}$), respectively. Infusion volumes were 29.8 ml and 24.6 ml for the heavy and light groups of sheep, respectively.

Experiment Two

The purpose of this experiment was to determine if the PAAI method (Potter et al., 1968) would identify the limiting amino acid of dietary proteins fed to rats. Four diets (Table 3) were prepared with egg albumin, casein, soya or zein as the protein sources. Forty-eight, 160 gm white male rats were randomly assigned to four diets. Two rats were housed in each cage. Rats were fed between 8 a.m. and 5 p.m. for a period of 2 weeks.

Table 1

Experimental Design for Experiment One

Period	Replicate	Sheep no. 2	4	6	Replicate	Sheep no. 8	9	11
1	1	X[a]	Y	Z	2	X	Y	Z
2[b]	1	Z	X	Y	2	Y	Z	X
3	1	Y	Z	X	2	Z	X	Y
1	3[c]	X	Y	Z	4	X	Y	Z
2	3	Z	X	Y	4	Y	Z	X
3	3	Y	Z	X	4	Z	X	Y

[a]Indicates the treatment: X = saline; Y = low glucose
Z = high glucose

[b]Five days between periods

[c]Fifteen days between replicates

Table 2

Ration[a], Experiment One

Ingredient	Percent
Corn cobs[b]	45.0
Alfalfa meal, 17% CP[c]	35.0
Whole rolled oats	12.6
Cane molasses	5.0
Urea, 262% CP	0.4
Dicalcium phosphate	0.94
Trace mineral salt, high Zn	0.94
Sodium sulfate, anhydrous	0.09
Vitamin A premix, 10,000 IU/gm	0.006
Vitamin D premix, 9,000 IU/gm	0.002

[a]Ration contained 1.90% nitrogen

[b]Andersons' No. 4 Fines, The Andersons, Maumee, Ohio. Remainder of cob after hard cylinder has been removed for production of industrial abrasives. Consists of bracts and pith (soft parenchyma without vascular bundles). Cell wall constituents, 81.2%; acid detergent fiber, 37.5% lignin, 6.5%.

[c]Crude protein

Table 3

Diets, Experiment Two

Ingredient	Protein source: Egg albumin %	Casein %	Soya %	Zein %
Egg albumin[a]	25			
Casein, high protein[a]		20		
Soya[a]			20	
Zein[a]				25
Corn starch	31	36	36	31
Cerelose	34	34	34	34
Corn oil	5	5	5	5
Salt[b]	4	4	4	4
Vitamin[c]	1	1	1	1
Percent crude protein[d]	18.1	17.8	18.5	23.0

[a]Protein sources were obtained from General Biochemical Inc., Chargin Falls, Ohio
[b]USP XIV plus 20 ppm Zn
[c]Vitamin Fortification Mix 40060, General Biochemical Inc., Chargin Falls, Ohio
[d]Determined by Kjeldahl (N x 6.25)

Following a 16 hour fast on the 15th day, one rat in each cage was given glucose (50% W/V) and the other (.9% NaCl W/V) by stomach tube. Infusion volumes were equal to .002 ml/gm of the metabolic body weight ($BW^{.75}$). One hour after the infusion, each rat was anesthetized with ether and blood was collected by heart puncture with a heparinized syringe.

Experiment Three

This experiment was designed to evaluate limiting amino acid determination by expressing PAAI from rats fed different proteins as a percent of PAAI of rats fed a high quality protein. Eight different protein diets (Table 4) were prepared using whole proteins and crystalline amino acids. Eighty, 150 gm rats were assigned to treatments and housed as described for experiment two.

Experiment 3 differed from experiment 2 in the following ways: (a.) 10 rats were used per dietary treatment instead of 12; (b.) rats were fed for only one week instead of two; (c.) rats were fasted 8 hours instead of 16 hours; and (d.) 4 ml of saline or 50% glucose were administered regardless of weight.

Experiment Four

This experiment was designed to study PAA concentrations and glucose induced changes in the PAA levels of sheep duodenally infused with proteins of "known" amino acid composition. Eight sheep, weighing between 35 and 45 kg, were surgically prepared with both rumen (Jarrett, 1948) and re-entrant duodenal cannulae (modified from procedure described by Markowitz et al., 1964). A 15 cm long skin incision was made 3 cm below, and parallel to, the abdominal ends of the

Table 4

Experimental Diets, Experiment Three

Ingredient	Nitrogen source 1 Whole egg WE[a] %	2 Whole egg amino acid WE + AA %	3 Casein Cas %	4 Corn gluten meal CGM %	5 Soybean meal Soy %	6 Soybean meal amino acid Soy + AA %	7 Soybean meal amino acid Soy + AA %	8 Soybean meal amino acid Soy + AA %
Whole egg protein 65% CP[b]	22	22						
Casein high protein 80% CP[b]			22					
Corn gluten meal 61% CP[c]				48				
Soybean meal 50% CP					40	40	40	40
Corn starch	43	41	43	17	25	23	23	23
Cerelose	25	25	25	25	25	25	25	25
Corn oil	5	5	5	5	5	5	5	5
Salt mixture[d]	4	4	4	4	4	4	4	4
Vitamins[e]	1	1	1	1	1	1	1	1
L-Histidine		0.13				0.04	0.04	0.04
L-Lysine		0.44					0.28	0.28
L-Phenylalanine		0.60				0.56	0.56	0.56
L-Methionine		0.44				0.72	0.72	0.72
L-Threonine		0.16				0.12	0.12	0.12
L-Valine						0.16		0.16

[a]Abbreviations will be used in subsequent tables

[b]Whole egg protein and high protein casein were obtained from General Biochemical Inc., Chargin Falls, Ohio

[c]Corn gluten meal was obtained from A.E. Staley Manufacturing Co., Decatur, Ill.

[d]Mineral Mix: Same as used by Miller, et al., J. Nutr. 85:347. 1965.

[e]Vitamin Fortification mix, 40060, General Biochemical Inc., Chargin Falls, Ohio

last three stationary ribs on the right side of the sheep. Access to the abomasal-duodenal junction was gained by either cutting through the muscles and peritoneum or by spreading the muscles and then cutting the peritoneum. The small blood vessels, located around the duodenum and 5-7 cm beyond the abomasal-duodenal junction, were ligated with 00 chromic gut. Two Kocher clamps were placed adjacent to each other and the small intestine sectioned between these clamps. The ends of the transected small intestine were closed by "oversewing" each clamp and then adding a second suture row for reinforcement.

The anterior cannula was located in the duodenum midway between the abomasal-duodenal junction and the closed end of the duodenum. The posterior cannula was located in the duodenum 12 cm beyond the point where the common bile duct enters the duodenum. The posterior cannula was inserted after making a 6 cm long incision through the skin, muscles and peritoneum.

Both cannulas were placed in the small intestine through 2 cm long incisions. Chromic gut purse-string sutures were aligned around the parameter of the incision, and then tightened to hold the cannula. A second set of purse-string sutures were used for reinforcement. The head of each cannula was exteriorized through a stab wound 1 inch lateral to the incision. The incisions were closed and the cannulas connected.

Cannulae were made from .040 inch thick silastic sheets, reinforced with dacron mesh, and silastic medical adhesive type A (both obtained from Dow Corning Corp, Midland, Michigan). The cannula mold was made from two pieces of copper tubing 3/8" O.D. and 3½" long. The end of one of the pieces was cut so that it would fit 1/3 of the way around the other piece when they were at a 45° angle

and flat on the table top. The two pieces were soddered together so that the end of the cut piece was attached to the mid point of the other piece. The mold assumed its final shape when the free end of the neck piece was bent downward toward the base piece, a distance of one inch.

After the sheep were on full feed for one week, the connection between the two duodenal cannulas was removed and a protein-mineral infusion was pumped into the posterior cannula whereas ingesta coming from the abomasum passed to the floor through the anterior cannula. The compositions of the protein-mineral infusions are shown in Table 5. The infusion mixtures were prepared by ball-milling the protein (not necessary for egg), homogenizing the protein-mineral-vitamin-fat mixture, adjusting the pH to below 3.0 with 6N HCl, adding 1 gm of pepsin and diluting to 6 liters with tap water. Each infusion was pumped continuously over a seven day period so that 6 liters were infused each day. The number of sheep per protein infusion is shown in Table 5. Glucose treatment and blood collections were on days 4 and 6 of each period. Twelve ml of blood were withdrawn with a heparinized syringe from the jugular vein at 8 a.m. This was followed immediately by the infusion of 30 ml of 35% (W/V) glucose into the jugular vein via a catheter. Twelve ml of blood were collected one hour after the glucose infusion. These blood samples were designated as "T_0" and "T_1" for pre- and post-glucose infusion, respectively.

Sheep were offered 800 gm of either ration 1 (Table 2) or a ration of cracked corn and rolled oats. If they consumed less than 200 gm of their diet, 400 gm of the diet was infused into the rumen through the rumen cannula. These animals were fed at 8 a.m., except on

Table 5

Protein Mineral Infusion Mixtures, Experiment Four

Ingredient	Infusion							
	Whole Egg WE[a]	Casein Cas	Promosoy Soy	Corn gluten meal CGM	Promosoy + methionine Soy + Met	Egg albumin EA	Whole egg 2X WE 2X	Whole egg 3X WE 3X
Whole egg[b]	70[c]						140	210
Casein[d]		70						
Promosoy[e]			70		70			
Egg albumin[d]						70		
Corn gluten meal[f]				85				
dL-Methionine					2.4			
Vitamins[g]	2	2	2	2	2	2	2	2
Fat[h]	8	8	8	8	8	8	8	8
KCl	22	22	22	22	22	22	22	22
NaCl	14	14	14	14	14	14	14	14
$CaCl_2$	11	11	11	11	11	11	11	11
$NaH_2PO_4H_2O$	30	30	30	30	30	30	30	30
$MgCl_2 \cdot 6H_2O$	10	10	10	10	10	10	10	10
Trace mineral mix[i]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Number of sheep/infusion	5	4	7	4	1	3	1	1

[a]Abbreviations used in subsequent tables

[b]All values are listed as gm of infusion per day

[c]Standard edible whole egg obtained from Henninnsen Food, Springfield, Missouri

[d]Casein and egg albumin were obtained from General Biochemical Inc., Chargin Falls, Ohio

[e]Soy protein concentrate obtained from Central Soya Inc., Chicago Ill.

[f]Corn gluten meal was 61% CP, obtained from A.E. Staley Manufacturing Co. Decatur, Ill.

[g]Vitamin fortification mix 40060, General Biochemical Inc., Chargin Falls, Ohio

[h]Mixture of corn oil and emulsified lard (1:1 w/w)

[i]Composition is listed in Table 6

Table 6

Trace Mineral Mixture, Experiment Four

Ingredient	Percent
KCl	10.0
KI	0.002
$FeSO_4 \cdot 2H_2O$	0.7
$CuSO_4$	0.1
$CoCO_3$	0.1
$MnSO_4 \cdot H_2O$	0.1
$ZnSO_4 \cdot 7H_2O$	0.4
$MgCO_3$	2.0
$NaHCO_3$	25.0
$CaHPO_4 \cdot 2H_2O$	36.0
$CaCO_3$	12.5
Cerelose	13.098
	100.000

sample days when feeding was delayed one hour until after both blood samples were collected.

Experiment Five

This experiment was disigned to determine if blood collected from the jugular vein differed in PAA levels from blood collected from the carotid artery of sheep. Three, two-year old wethers, which had been surgically prepared with exteriorized carotid arterial loops (Bone et al., 1962), were used in a 3 x 3 latin square experiment. The sheep were fed 800 gm of maintenance ration (composition shown in Table 7) daily at 8 a.m., for 2 months preceeding and through out the experiment. The second and third collection days followed the first by 7 and 14 days, respectively. On collection days feeding was delayed until after all blood samples were collected.

At 8 am. on collection days, 10 ml of blood was withdrawn simultaneously from the carotid artery and jugular vein. Thirty ml of either saline (.9% NaCl, W/V), glucose (30% W/V) or acetate (30% W/V) solutions were then infused via a catheter into the jugular vein. The pre-infusion and post-infusion blood samples were disignated as "T_0" and "T_1", respectively.

Statistical Analysis

All data reported in this dissertation were analyzed on an IBM 3600 computer at the Michigan State University Computer Laboratory. Least squares analysis of variance (Harvey, 1960) were used in experiments 1, 4 and 5 while analysis of variance procedures were used in experiments 2 and 3. Duncan's new multiple range test was used to determine mean differences (Steel and Torrie, 1960). An example of

the analysis of variance and Duncan's new multiple range test procedures is shown in Appendix I.

Table 7

Ration[a], Experiment Five

Ingredient	Percent
Ground corn	32.0
Ground hay[b]	30.0
Cane molasses	5.0
Ground corn cobs[c]	30.0
Dicalcium phosphate	0.94
Trace mineral salt, high Zn	0.94
Sodium sulfate, anhydrous	0.09
Vitamin A premix 10,000 IU/gm	0.006
Vitamin D premix 9,000 IU/gm	0.002

[a]Contained 1.36% nitrogen

[b]Mixture of grass and legume hay

[c]Anderson's No. 4 Fines, The Anderson, Maumee, Ohio. Remainder of cob after hard cylinder has been removed for production of industrial abrasives. Consists of bracts and pith (soft parenchyma without vascular bundles). Cell wall constituents, 81.2%; acid detergent fiber, 37.5% lignin, 6.5%.

RESULTS

Experiment One

Mean pre-treatment (T_0) and one hour post-treatment (T_1) PAA concentrations are shown in Table 8. There were no differences among the individual PAA levels of the saline, low glucose and high glucose groups at T_0. The low glucose infusion significantly ($P < .05$) decreased the T_1 plasma levels of all essential amino acids (EAA) except threonine, methionine and histidine whereas the high glucose infusion significantly ($P < .05$) depressed all EAA except threonine. Total T_0 plasma EAA levels were 14.77, 14.44 and 15.12 mg/100 ml, whereas total T_1 concentrations were 13.01, 10.70 and 9.64 mg/100 ml respectively for the saline, low glucose and high glucose treated sheep. These PAA concentration are similar to those reported in sheep fasted for 24 hours (Potter *et al.*, 1968).

Potter *et al.* (1968) expressed T_1 PAA concentrations as a percent of T_0 PAA concentrations, and referred to these as plasma amino acid indices (PAAI). PAAI, which express the change in PAA due to treatment are shown for this experiment in Table 9. The average PAAI for the EAA were 88, 74 and 64 whereas the average non-essential PAAI were 85, 85 and 81 for the saline, low glucose and high glucose treatments, respectively. Thus the decreases in the plasma EAA levels were 12, 26 and 36 percent for the saline, low glucose and high glucose, respectively. This decrease after saline infusion must

Table 8

Mean Plasma Amino Acid Concentrations[a] by Treatment and Sample Time for Experiment One

Amino acid	Sample time: Pre-treatment (T_0) Saline	Low glucose	High glucose	SEM	Post-treatment (T_1) Saline	Low glucose	High glucose	SEM
Essential amino acids								
Thr	1.86	1.69	1.77	±.16	1.62	1.32	1.33	±.22
Val	3.02	3.01	3.12	±.22	2.71[ad]	2.29[b]	2.19[b]	±.25**[c]
Met	.35	.39	.37	±.06	.29[a]	.29[a]	.22[b]	±.05*
Ile	1.33	1.30	1.39	±.13	1.16[a]	.86[b]	.74[b]	±.12**
Leu	1.73	1.72	1.86	±.21	1.51[a]	1.14[b]	.94[c]	±.17**
Tyr	1.04	1.03	1.05	±.06	.95[a]	.75[b]	.67[b]	±.12**
Phe	.70	.74	.72	±.05	.62[a]	.56[b]	.49[c]	±.06**
Lys	1.90	1.82	1.95	±.28	1.66[a]	1.43[b]	1.08[c]	±.15**
His[b]	.78	.78	.75	±.07	.66[a]	.63[ab]	.59[b]	±.04**
Arg	2.06	1.96	2.14	±.32	1.84[a]	1.43[b]	1.39[b]	±.25*
TEAA[e]	14.77	14.44	15.12		13.01	10.70	9.64	
Change	-	-	-		-1.76	-3.74	-5.48	
Non-essential amino acids								
Asp	.54	.82	.56	±.45	.46	.46	.38	±.08
Ser	1.55	1.47	1.64	±.20	1.24	1.25	1.30	±.24
Asn	.43	.43	.54	±.12	.27	.27	.41	±.07
Glu	2.47	2.47	2.61	±.33	2.13	2.15	1.86	±.30
Pro	1.56	1.33	1.47	±.31	1.15	1.32	.89	±.42
Gly	7.54	7.78	7.42	±.86	6.82	6.60	6.44	±.51
Ala	2.20	2.24	2.04	±.27	1.94	1.78	1.76	±.26
Cys	.22	.24	.23	±.05	.21	.21	.21	±.03
Orn	1.30	1.12	.67	±.58	.94	1.19	.68	±.26
TNEAA[f]	17.81	17.90	17.18		15.16	15.23	13.90	
Change	-	-	-		-2.65	-2.67	-3.28	
TAA[g]	32.58	32.34	32.30		28.17	25.93	23.54	

[a] All values are expressed as mg/100 ml

[b] The histidine values are from replicates 3 and 4 only

[c] *Differences between the means were significant ($P < .05$)
**Differences between the means were significant ($P < .01$)

[d] Values with similar superscript form a statistically homeogenous grouping ($P < .05$)

[e] Total essential amino acids

[f] Total non-essential amino acids

[g] Total amino acids

Table 9

Mean Plasma Amino Acid Indices[a] by Treatment
Experiment One

Amino acid	Treatment: Saline	Low glucose	High glucose
Thr	87	78	75
Val	90	76	70
Met	83[b]	74[b]	59
Ile	87	66[b]	53[b]
Leu	87	66[b]	51[b]
Tyr	91	73	64
Phe	89	76	68
Lys	87	79	55
His	85	81	79
Arg	89	73	65
Ave EAAI[c]	88	74	64
Asp	85	56	68
Ser	80	85	79
Asn	63	63	76
Glu	86	87	71
Pro	74	99	61
Gly	91	85	87
Ala	88	79	86
Cys	95	88	78
Orn	72	106	101
Ave NEAAI[d]	85	85	81
Ave TAAI[e]	86	80	73

[a] $\text{Index} = \frac{T_1 \text{ Plasma amino acid concentration}}{T_0 \text{ Plasma amino acid concentration}} \times 100$

[b] Lowest EAA index for each treatment

[c] Average essential amino acid index

[d] Average non-essential amino acid index

[e] Average total amino acid index

represent the normal change since the volume of infusion (30 ml) would account for less than a 2 percent dilution in the blood. In comparison to the average decrease in plasma non-essential amino acids, the average plasma EAA were decreased more by glucose. These results confirm the results of Potter et al. (1968) who suggested that glucose caused decreases in the plasma levels of all amino acids, but that synthesis of non-essential amino acids accounted for the smaller depression in plasma non-essential amino acid levels.

The amino acids predicted as limiting (lowest PAAI) were isoleucine and leucine for the low glucose group and leucine for the high glucose group. In theory, these amino acids should be the limiting amino acids of the protein in the small intestine of the ruminant. These results agree with previous PAAI predictions which show isoleucine and leucine as the limiting amino acid after glucose infusion in sheep (Potter et al., 1968).

The actual change in the concentration of the plasma EAA from T_0 to T_1 were compared to the EAA composition of striated lamb muscle (Block and Weiss, 1956) by linear regression (line of best fit). The resulting correlation coefficients (r) were; .73 ($P < .05$), .67 ($P < .05$) and .82 ($P < .01$) for the saline, low glucose and high glucose treatments, respectively. The correlation coefficients obtained for the low and high glucose treatments may have been influenced by physiological changes in the animal attributable to factors other than glucose. Subtracting the T_0-T_1 saline (control) concentration differences from the T_0-T_1 glucose concentration differences should give concentration changes due to just glucose. Linear correlation analysis between the saline corrected glucose differences and the EAA composition of lamb showed coefficients (r) of .54 (N.S.) and .80 ($P < .01$)

Table 10

Differences[d] Between the Pre-Treatment (T_0) and Post-Treatment (T_1) PAA Concentrations

Amino acid	Treatment group X Saline	Y Low glucose	Z High glucose	Corrected differences[e] Y-X	Z-X
Thr	.25	.37	.46	.12	.21
Val	.31[a,f]	.72[ab]	.93[b]	.41	.62
Met	.06	.10	.15	.04	.09
Ile	.17[a]	.44[ab]	.65[b]	.27	.48
Leu	.22[a]	.58[ab]	.92[b]	.36	.70
Tyr	.09[a]	.28[ab]	.38[b]	.19	.29
Phe	.08[a]	.18[ab]	.23[b]	.10	.15
Lys	.24[a]	.39[ab]	.87[b]	.15	.63
His	.12	.15	.16	.03	.04
Arg	.22[a]	.53[ab]	.75[b]	.31	.53
r[g]	.73	.67	.82	.54	.80
	P < .05	P < .05	P < .01	N.S.	P < .01

[d] Values are expressed as mg/100 ml

[e] Corrected differences were obtained by subtracting the differences for the saline treatment (column X) from the glucose groups (columns Y and Z)

[f] Values with similar superscript form a statistically homeogenous grouping (P < .05)

[g] Correlation between plasma essential amino acid depression pattern and EAA composition of striated lamb muscle.

for the low and high glucose groups, respectively. The results of the high glucose treatment confirms observations by Munro and Thompson (1953), Crofford et al. (1964), Swendseid et al. (1967) and Potter et al. (1968) who reported that glucose ingestion or infusion caused a decrease in plasma EAA levels which was similar in composition to the EAA composition of striated muscle. This relationship between changes in plasma EAA levels and the EAA composition of muscle suggests that protein synthesis occurred after the high glucose infusion.

Despite the suggestion that glucose caused protein synthesis, the accuracy of the PAAI method in predicting the limiting amino acid was questioned for the following reasons. First, PAAI calculated from data reported by Munro and Thompson (1953) and Crofford et al. (1964) showed isoleucine had the lowest index when subjects were fed proteins not limiting in isoleucine. Second, methionine is suspected as limiting in sheep since sheep have a high sulfur amino acid requirement for wool growth and since the supply of methionine in bacterial protein is low.

Experiment Two

This experiment was designed to test the accuracy of the PAAI method (Potter et al. 1968) in predicting the limiting amino acid in dietary proteins fed to rats. Mean PAA concentrations for 16 hour fasted rats are shown for each dietary group in Table 11. Except for aspartic acid, the plasma levels of all amino acids differed significantly ($P < .05$) with dietary protein sources. The relationship between dietary and plasma amino acid levels was examined by determining correlation coefficients. No significant correlations were found when the plasma EAA concentration pattern was correlated to the EAA

Table 11

Mean[d] Plasma Amino Acid Concentrations of Rats Fed Different Protein Diets, Experiment Two

Amino acid	Diets: Egg albumin	Casein	Soy	Zein	SEM	Significance of F value
			Essential amino acids			
Thr	1.44[a e]	4.27[c]	2.86[b]	4.15[c]	±.20	P < .001[f]
Val	1.21[a]	1.18[a]	.87[a]	.77[b]	±.06	P < .001
Met	.21[a]	.20[a]	.16[b]	.32[b]	±.03	P < .001
Ile	.55[a]	.55[a]	.42[b]	.38[c]	±.03	P < .001
Leu	.98[a]	.93[ab]	.71[a]	1.17[b]	±.07	P < .001
Tyr	.45[a]	.59[ab]	.45[a]	.51[b]	±.04	P < .05
Phe	.48[a]	.49[a]	.37[a]	.59[b]	±.03	P < .001
Lys	4.36[a]	4.84[b]	4.41[b]	3.03[c]	±.40	P < .05
His	.40[a]	.53[a]	.60[b]	.88[c]	±.04	P < .001
Arg	1.08[a]	1.03[a]	1.28[b]	1.58[c]	±.08	P < .001
TEAA[g]	11.16	14.61	12.13	13.38		
			Non-essential amino acids			
Asp	.87	.69[ab]	.71[b]	.73[c]	±.06	N.S.
Ser	3.20[a]	3.67[ab]	4.02[b]	5.97[a]	±.19	P < .001
Glu	3.16[a]	2.79[ab]	2.34[a]	3.06[b]	±.15	P < .005
Pro	1.51[a]	1.62[b]	1.36[b]	1.91[a]	±.10	P < .005
Gly	4.03[a]	3.21[ab]	3.13[b]	3.83[ab]	±.19	P < .005
Ala	4.18[a]	3.75[a]	3.11[b]	3.72[ab]	±.21	P < .01
Cys	.37[a]	.39[a]	.28[a]	.34[b]	±.02	P < .05
Orn	.48[a]	.47	.47	.76	±.04	P < .001
TNEAA[h]	16.93	15.90	14.71	19.59		
TAA[i]	28.09	30.51	26.84	32.97		

[d]Means of 12 rats and are expressed as mg/100 ml

[e]Values with similar superscript form a statistically homeogenous grouping (P < .05)

[f]Determined by analysis of variance

[g]Total essential amino acids

[h]Total non-essential amino acids

[i]Total amino acids

composition (distribution pattern) of the dietary protein. While there was no significant correlations between the blood and dietary levels of EAA, the limiting amino acid of each dietary protein was reflected by a low plasma level of the limiting amino acid. The plasma threonine level in rats fed egg albumin was significantly ($P < .05$) lower than the plasma threonine levels in rats fed the other diets. This relationship was also true for lysine in zein-fed rats, for methionine in soy-fed rats and for arginine in casein-fed rats, however the lysine and methionine levels were not significantly lower then their levels in rats fed the other diets. Methionine and arginine have been suggested as the limiting amino acids in casein; the plasma methionine level, in the casein-fed rats was lower than its level in egg albumin- and zein-fed rats. Thus to a limited extent PAA levels alone reflected deficiencies in dietary amino acid intake. However, this relationship is not such that the limiting amino acid can be predicted on this basis alone.

Mean PAA concentrations, from rats fed all four diets, after either glucose or saline infusions are shown in Table 12. Glucose infusion resulted in a significant ($P < .05$) decrease in the plasma levels of valine, isoleucine, leucine, phenylalanine, serine and glycine and increases in plasma alanine and cystine. Mean PAA concentrations for the saline- and glucose-infused rats on each dietary protein are shown in Appendix II.

PAAI for each dietary protein treatment were calculated by expressing the PAA concentrations from glucose treated rats as a percent of the PAA concentrations from saline treated rats (Table 13). The amino acids predicted as limiting (lowest index) by the PAAI were isoleucine in egg albumin, leucine in casein, methionine in soy and

Table 12

Mean[a] Concentrations of PAA According to Saline or Glucose Treatment and Overall PAAI, Experiment Two

Amino acid	Treatment: Saline	Treatment: Glucose	SEM	Significance of F value	PAAI[c]
			Essential amino acids		
Thr	3.31	3.05	±.14	N.S.[b]	92
Val	1.07	.94	±.04	P < .05	88
Met	.22	.22	±.02	N.S.	100
Ile	.52	.43	±.02	P < .005	83
Leu	1.10	.79	±.05	P < .001	72
Tyr	.52	.49	±.03	N.S.	94
Phe	.53	.44	±.02	P < .005	83
Lys	4.03	4.30	±.28	N.S.	107
His	.59	.61	±.03	N.S.	103
Arg	1.19	1.29	±.06	N.S.	108
TEAA[d]	13.08	12.56		Ave EAAI	96
			Non-essential amino acids		
Asp	.81	.69	±.04	N.S.	85
Ser	4.56	3.89	±.13	P < .001	85
Glu	2.99	2.69	±.11	N.S.	90
Pro	1.66	1.54	±.07	N.S.	93
Gly	3.92	3.18	±.14	P < .001	81
Ala	3.20	4.19	±.15	P < .001	131
Cys	.31	.38	±.01	P < .005	123
Orn	.54	.55	±.03	N.S.	102
TNEAA[e]	17.99	17.11		Ave NEAAI	96
TAA[f]	31.07	29.67		Ave TAAI	96

[a]Mean of 24 rats and are expressed in mg/100 ml

[b]Determined by analysis of variance

[c]PAAI are glucose group values divided by saline group values x 100

[d]Total essential amino acids

[e]Total non-essential amino acids

[f]Total amino acids

Table 13

PAAI[a] for Treatments in Experiment Two

Amino acid	Dietary treatment			
	Egg albumin	Casein	Soy	Zein
Thr	101	97	98	83
Val	85	81	99	106
Met	115[c]	70	67[b]	106
Ile	72[b]	77	98	88
Leu	76	69[b]	88	77[b]
Tyr	86	105	109	87
Phe	80	76	95	82
Lys	96	112	133	99
His	105	106	122	93
Arg	110	99	129	103
TEAA[d]	93	97	114	91
TNEAA[e]	98	96	98	90
TAA[f]	96	96	105	90

[a] $\text{Index} = \frac{\text{PAA concentration from glucose treated rats}}{\text{PAA concentration from saline treated rats}} \times 100$

[b] Lowest index indicates the predicted limiting amino acid

[c] The limiting amino acid as determined by rat growth response or in theoretical calculation is underlined (Rao et al., 1964)

[d] Total essential amino acids

[e] Total non-essential amino acids

[f] Total amino acids

leucine in zein. The PAAI method correctly identified the "known" limiting amino acid in only the soy diet.

The PAAI method appeared to give low index values for isoleucine, leucine and phenylalanine regardless of the protein fed. This observation is supported by data recalculated from other studies (Munro and Thompson, 1953; Crofford et al., 1964 and Potter et al., 1968 and the first experiment). An attempt to correct this "bias", which the PAAI had toward certain amino acids, was made by expressing the PAAI from each of the casein, soy and zein fed rats as a percent of the PAAI from egg albumin fed-rats. Egg albumin PAAI were used for reference because egg is the protein which supports optimal growth in rats and since whole egg PAAI were not available. Values expressed in this manner are shown in Table 14. Methionine was the limiting amino acid in both the soy and casein fed rats. While these were correct identifications (agreed with growth studies by Russel et al., 1946), this procedure did not identify lysine as the limiting amino acid in zein. The failure of this procedure to identify lysine as the limiting amino acid in zein may have been the result of the catabolic state of these rats since they lost 50 gm (body weight) in the 2 week feeding period.

When PAAI from sheep in experiment one were expressed as a percent of the egg albumin PAAI (calculated in this experiment), methionine was the first limiting amino acid whereas lysine and arginine were the next limiting amino acids on the low and high glucose treatments, respectively. This prediction agrees with other reports (Bergen et al., 1968; Reis et al., 1969 and Conrad et al., 1968) which suggested methionine as the limiting amino acid of ruminants. Since dividing PAAI

Table 14

PAAI Divided by Egg Albumin PAAI
Experiment Two

Amino acid	Treatment group: Experiment two			Experiment one		
	Casein	Soy	Zein	Saline	Low glucose	High glucose
Thr	96[a]	97	82[b]	86	77	74
Val	95	116	124	106	89	82
Met	61[b,c]	58[b]	92	72[b]	64[b]	51[b]
Ile	107	136	122	121	92	74
Leu	91	116	101	114	87	67
Tyr	122	127	101	106	85	74
Phe	95	119	103	111	95	85
Lys	117	139	103	91	82	57
His	101	116	89	81	77	75
Arg	90	117	94	81	66	59

[a] $\text{Index} = \frac{\text{PAAI for other protein-fed rats}}{\text{PAAI for egg-albumin-fed rats}} \times 100$

[b] Lowest index indicates the predicted limiting amino acid

[c] The limiting amino acid as determined by rat growth responses or in theoretical calculation is underlined (Rao et al., 1964)

from test proteins by PAAI from egg albumin resulted in a higher percentage of correct predictions of the limiting amino acid (compared to the PAAI method by Potter et al., 1968) it seemed worthwhile to further investigate this procedure. There was also the possibility that using whole egg PAAI, instead of egg albumin PAAI as a reference might increase the accuracy of this procedure since whole egg is of higher quality.

Experiment Three

This experiment was designed to determine the accuracy of limiting amino acid predictions which express PAAI from rats fed low quality proteins as a percent of PAAI from animals fed high quality protein (whole egg). Mean PAA concentrations of rats fed each of the eight protein diets are shown in Table 15. The plasma levels of all amino acids except aspartic acid and glutamic acid differed significantly ($P < .05$) with diet. No significant correlations were found between the EAA composition of the diet and the plasma EAA level pattern of rats fed that diet. A second set of correlations were calculated between the level of each amino acid in the plasma of rats fed the eight diets (acrossed diets) and the levels of that amino acid in those diets. Significant ($P < .05$) correlation coefficients were found for only methionine ($r = .81$) and lysine ($r = .71$). These significant correlations may be attributed to two things: first, methionine and lysine were the limiting amino acids in several of the diets and second, the dietary supplementation of methionine and lysine increased the plasma levels of these amino acids.

The rats in this experiment had higher absolute PAA levels than the rats in experiment two. The average total plasma EAA level for

Table 15

Mean[g] Concentration of Plasma Amino Acids of Rats Fed Different Protein Diets, Experiment Three

Amino acid	Dietary treatments[h] WE 1	WE + AA 2	Cas 3	CGM 4	Soy 5	Soy + AA 6	Soy + AA 7	Soy + AA 8	SEM	Significance of F value
Essential amino acids										
Thr	4.08[ai]	6.47[b]	6.89[b]	6.64[b]	6.37[b]	5.40[ab]	4.13[a]	5.50[ab]	± .46	P < .001[j]
Val	2.06[bcde]	1.56[a]	2.47[f]	1.72[abc]	1.69[abcd]	2.36[f]	1.68[ab]	2.34[def]	± .15	P < .001
Met	.56[ab]	.91[c]	.86[c]	.46[a]	.44[a]	.76[d]	1.00[cd]	1.29[d]	± .08	P < .001
Ile	.83[ab]	.78[a]	1.37[d]	.86[ab]	1.13[c]	1.24[cd]	1.04[bc]	1.15[c]	± .07	P < .001
Leu	1.08[ab]	1.75[a]	1.80[b]	3.01[c]	1.40[ab]	1.72[b]	1.36[ab]	1.44[ab]	± .24	P < .001
Tyr	.80[a]	1.72[b]	2.43[c]	1.66[b]	1.94[bc]	2.16[c]	1.96[bc]	1.71[b]	± .16	P < .001
Phe	.52[a]	1.43[ab]	.98[c]	1.07[a]	1.40[bc]	1.62[d]	1.01[c]	.98[c]	± .07	P < .001
Lys	1.83[a]	7.81[b]	10.34[c]	1.41[a]	6.87[b]	6.94[b]	7.02[b]	7.36[b]	± .40	P < .001
His	.80[a]	1.11[bc]	1.23[bcd]	1.54[d]	1.36[cd]	1.09[bc]	7.02[ab]	1.18[bc]	± .28	P < .001
Arg	1.95[a]	1.97	2.23[a]	2.41[a]	4.23[b]	4.19	3.90	4.46	± .26	P < .001
TEAA[k]	14.70	23.81	30.92	20.87	26.60	27.82	24.12	27.58		
Non-essential amino acids										
Asp	.73	.55	.53	.56	.62	.59	.55	.65	± .06	N.S.
Ser	6.37[e]	4.23[bc]	3.64[b]	5.01[bcd]	5.15[cd]	2.78[a]	2.34[a]	2.78[a]	± .26	P < .001
Asn	.90[a]	.90[a]	1.21[ab]	1.03[ab]	1.30[b]	1.18[ab]	.92[a]	1.05[ab]	± .09	P < .05
Glu	5.54	5.98	5.88	6.21	5.94	5.94	5.49	5.99	± .50	N.S.
Pro	3.51[ab]	5.08[a]	4.41[c]	3.69[c]	2.75[b]	2.31[ab]	2.23[ab]	2.22[ab]	± .19	P < .001
Gly	3.61[c]	2.44[b]	1.48[a]	2.11[b]	2.37[b]	2.09[b]	1.99[b]	1.99[b]	± .14	P < .001
Ala	7.17[d]	6.20[c]	5.07[b]	5.36[bc]	5.21[b]	4.58[ab]	4.08[a]	4.59[ab]	± .27	P < .001
Cys	.45[c]	.43[c]	.32[ab]	.32[ab]	.40[bc]	.32[ab]	.32[ab]	.45[c]	± .03	P < .001
Orn	1.64[b]	1.14[a]	1.11[a]	1.36[ab]	1.31[a]	1.42[ab]	1.33[ab]	1.32[ab]	± .10	P < .01
TNEAA[l]	28.90	23.85	23.76	25.66	25.15	21.31	19.31	21.05		
TAA[m]	43.60	47.66	54.68	46.53	51.75	49.13	43.43	48.63		

[g]Values are the average for 10 rats and are expressed as mg/100 ml

[h]Treatments are listed in Table 4

[i]Values with similar superscripts, form a statistically homogenous grouping (P <.05)

[j]Determined by analysis of variance

[k]Total essential amino acids

[l]Total non-essential amino acids

[m]Total amino acids

the rats in this experiment was 24.55 mg/100 ml, whereas it was 13.82 mg/100 ml for the rats in experiment two. Likewise, the PAA levels for casein fed rats in this experiment were double those in experiment two. The higher PAA levels, in this experiment may be due to the shorter fasting period before blood was collected (8 hours vs. 16 hours in experiment two).

In comparison to the saline infused rats, the glucose infused rats had significantly ($P < .05$) lower plasma levels of all amino acids except alanine, cystine and ornithine (Table 16.) The effect of glucose on the PAA levels of each dietary group are shown in Appendix III, while the PAAI calculated from these values appear in Table 17. The PAAI procedure of determining the limiting amino acid (lowest PAAI) failed to identify the recognized limiting amino acids in the diets except for diet 2 where either leucine or valine was predicted as limiting. Furthermore, when the PAAI from rats fed the other diets were expressed as a percent of the PAAI for either whole egg- or casein-fed rats the recognized limiting amino acid was not predicted (Table 18). The recognized limiting amino acids are those determined by growth response of rats (Mitchell, 1959; McLaughlan et al., 1967 and Mitchell and Smutts, 1932 and Russel et al., 1946).

Results of these experiments indicate that neither the PAAI method nor the procedure using a ratio of PAAI (PAAI from rats fed test proteins divided by PAAI from rats fed high quality proteins) are useable for determination of the limiting amino acid of the diet.

Since the dietary limiting amino acids appeared to be reflected by a low level in the plasma, PAA concentrations were again used to calculate another index which might identify the limiting amino acid. The PAA concentrations of rats fed each test diet in experiment three

Table 16

Mean[a] Concentrations of Plasma Amino Acids According to Saline or Glucose Treatment and Overall PAAI, Experiment Three

Amino acid	Treatment: Saline	Treatment: Glucose	SEM	Significance of F value		PAAI[b]
Essential amino acids						
Thr	6.15	5.22	±.23	P <.01		85
Val	2.33	1.83	±.08	P <.001		79
Met	1.02	.69	±.04	P <.001		68
Ile	1.19	.91	±.04	P <.001		76
Leu	1.86	1.27	±.12	P <.001		68
Tyr	2.04	1.58	±.08	P <.001		77
Phe	1.06	.84	±.04	P <.001		79
Lys	6.72	5.77	±.20	P <.001		86
His	1.22	1.10	±.04	P <.05		90
Arg	3.48	2.86	±.13	P <.001		82
TEAA[c]	27.07	22.07			Ave EAAI	79
Non-essential amino acids						
Asp	.66	.53	±.03	P <.005		80
Ser	4.31	3.75	±.13	P <.005		87
Asn	1.21	.92	±.05	P <.001		76
Glu	6.24	5.50	±.25	P <.05		88
Pro	3.08	2.45	±.10	P <.001		80
Gly	2.38	2.14	±.07	P <.05		90
Ala	5.47	5.09	±.14	N.S.		93
Cys	.39	.40	±.02	N.S.		103
Orn	1.45	1.27	±.05	N.S.		88
TNEAA[d]	25.19	22.05			Ave NEAAI	87
TAA[e]	52.26	44.12			Ave TAAI	83

[a]Mean of 40 rats on 8 different diets and expressed as mg/100 ml

[b]PAAI are glucose-group values divided by saline-group values x 100

[c]Total essential amino acids

[d]Total non-essential amino acids

[e]Total amino acids

Table 17

PAAI for Dietary Treatment Groups, Experiment Three

Amino acid	Dietary treatment group							
	WE 1	WE + AA 2	Cas 3	CGM 4	Soy 5	Soy + AA 6	Soy + AA 7	Soy + AA 8
Thr	89[a]	91	72	103	81	63	97	97
Val	91	85[c]	71	76	85	65	87	83
Met	77	98	68	63	100	51[b]	68[b]	57[b]
Iso	77	84	66[b]	71	83	71	87	80
Leu	61[b]	55[b]	67	57	87	72	79	81
Tyr	72	105	81	61[b]	79[b]	70	77	78
Phe	64	82	83	72	89	65	88	94
Lys	62	109	78	98	94	78	83	83
His	79	101	81	101	88	86	86	90
Arg	84	103	85	80	84	82	79	73
Ave EAAI	76	91	75	78	87	70	83	82
Ave TAAI	87	90	81	82	83	76	89	85

[a] Index values = $\frac{\text{PAA concentration from glucose infused group}}{\text{PAA concentration from saline infused group}} \times 100$

[b] Lowest index indicates the predicted limiting amino acid

[c] The limiting amino acid as determined by rat growth response or in theoretical calculation is underlined (Rao et al., 1964)

Table 18

PAAI from Rats Fed Other Diets as a Percent of PAAI from Rats Fed Whole Egg or Casein Diets

	Expressed as a percent of whole egg PAAI						
	Dietary treatment[a]						
Amino acid	WE + AA 2	Cas 3	CGM 4	Soy 5	Soy + AA 6	Soy + AA 7	Soy + AA 8
Thr	102	81	116	91[b]	71	109	109
Val	93[c]	78[b]	84[b]	93	71	96	91
Met	127	88	92	130	66[b]	88[b]	74[b]
Iso	109	86	92	108	92	113	104
Leu	90[b]	110	93	143	118	130	133
Tyr	146	113	85	110	97	107	108
Phe	128	130	113	139	102	138	147
Lys	176	126	158	152	126	134	134
His	128	103	128	111	109	109	114
Arg	123	101	95	100	98	90	87

	Expressed as a percent of casein PAAI						
	Dietary treatment[a]						
Amino acid	WE 1	WE + AA 2	CGM 4	Soy 5	Soy + AA 6	Soy + AA 7	Soy + AA 8
Thr	124	126	143	113	88	135	135
Val	128	120	107	120	92	123	117
Met	113	144	93	147	75[b]	100	84[b]
Ile	117	127	108	126	108	132	121
Leu	91	82[b]	85	130	107	118	121
Tyr	89	130	75[b]	98[b]	86	95	96
Phe	77[b]	99	87	107	78	106	113
Lys	79	140	126	121	100	106	106
His	98	125	125	109	106	106	111
Arg	99	121	94	99	96	93[b]	86

[a]Dietary treatments corresponds to diets in Table 4

[b]Lowest index indicates the predicted limiting amino acid

[c]The limiting amino acid as determined by rat growth response or in theoretical calculation is underlined (Rao et al., 1964)

were expressed as a percent of "reference PAA concentrations". The "reference PAA concentrations" were obtained by averaging the PAA concentrations of rats fed whole egg, whole egg plus amino acid and casein since these diets supported excellent growth. The values expressed in this manner are referred to as "plasma amino acid reference indices". The reference PAA concentrations and PAA reference indices for experiment three are shown in Table 19. The lowest PAA reference index did correspond to the recognized limiting amino acid of diets 1, 2, 3, 4, 5, 6 and 7. The limiting amino acids were those determined in growth trials with rats, or by calculation (percent in diet divided by requirement). The reference index of diet 8 was lowest for leucine. The limiting amino acid in diet 8 has not been determined by growth trial but was calculated as being phenylalanine.

The reference PAA concentrations obtained in experiment three were used as a reference to calculate the reference indices for experiments 1 and 2 (Table 20). The expected limiting amino acid was predicted (lowest index) for 3 of the 4 diets of experiment two. Similarly when PAA reference indices were calculated from Bergen et al. (1968) data, the limiting amino acids were identified in 2 of 3 cases. While the limiting amino acid is not known for the sheep in experiment 1, the PAA reference indices predicted lysine. This result must be viewed with caution as the reference concentrations were obtained from rats, not sheep.

Experiment Four

Experiment four was designed to evaluate the use of PAA concentrations in determining the limiting amino acid in proteins reaching the

Table 19

Plasma Amino Acid Reference Indices[a] for Each Dietary Treatment Group, Experiment Three

Amino acid	concentration mg/100 ml	Dietary groups: WE 1	WE + AA 2	Cas 3	CGM 4	Soy 5	Soy + AA 6	Soy + AA 7	Soy + AA 8
Thr	4.70	87	138	146	141	135	115	88	117
Val	2.35	77	66	117	76	82	109	71	100
Met	.86	65	106	102	66	51	147	116	141
Ile	1.15	72	68	119	75	98	108	90	100
Leu	1.48	71	51	122	203	95	116	89	95
Tyr	1.80	50	96	135	92	108	120	109	95
Phe	.89	73	82	111	116	106	141	113	110
Lys	7.11	26	110	145	20	97	97	99	108
His	1.11	72	100	113	135	123	98	89	103
Arg	2.16	90	91	105	112	196	194	181	206

[a]Index values = $\frac{\text{PAA conc. for the saline infused rats fed test protein diets}}{\text{Reference PAA concentration}} \times 100$

[b]Reference concentration refers to the average PAA concentration of the saline treated rats on diets 1, 2 and 3

[c]Lowest index indicates the predicted limiting amino acid

[d]The limiting amino acid as determined by rat growth response or in theoretical calculation is underlined (Rao et al., 1964)

Table 20

Plasma Amino Acid Reference Indices[a] Determined for Other Experiments Using Reference PAA Concentration in Table 20

	Experiment two				Bergen et al. (1968)			Expt. One
Amino acid	Egg albumin	Casein	Soy	Zein	Protozoal	Bacterial	Casein	Saline
Thr	31	91	61	88	103	52	101[b]	40
Val	51	50	37	33	61	57	138	129
Met	24[b] [c]	17[b]	19[b]	37	73	48[b]	114	41
Ile	48	48	37	33	159	75	124	116
Leu	66	63	48	79	95	58	132	117
Tyr	25	33	25	28[b]	116	49	122	58
Phe	54	55	42	66	148	92	125	79
Lys	61	68	62	43	134	61	159	27[b]
His	36	48	54	79	40[b]	50	144	70
Arg	50	48	59	73	123	81	118	95

[a] $\text{Index value} = \frac{\text{PAA concentration from treatment listed}}{\text{Reference PAA concentration}} \times 100$

[b] Lowest index indicates the predicted limiting amino acid

[c] The limiting amino acid as determined by rat growth response or in theoretical calculation is underlined (Rao et al., 1964)

small intestine of sheep. Mean PAA concentrations of sheep are shown in Tables 21, 22, 23 and 24 according to duodenal infusion treatment. Tables 21 and 22 show the pre-glucose (T_0) and one hour post-glucose (T_1) PAA concentrations on day 4 of the protein infusions while Tables 23 and 24 show the T_0 and T_1 PAA concentrations from day 6 of the infusion, respectively. The T_0 PAA concentrations from days 4 and 6 of the infusion were averaged as were the T_1 PAA concentrations from days 4 and 6, these appear in Appendix IV.

PAAI calculated from the average PAA concentrations of days 4 and 6 are shown in Table 25. These PAAI failed to identify the expected limiting amino acids in the protein infusions except in the case of the soy plus methionine infusion (infusion 5). PAA reference indices were calculated for each of the infusion treatments by expressing T_0 PAA levels as a percent of reference PAA concentrations. Reference PAA concentrations were calculated by averaging the T_0 PAA concentrations from sheep being infused with whole egg and casein. Both the reference PAA concentrations and PAA reference indices are shown in Table 26. The lowest reference indices for the whole egg and casein infused sheep corresponded to threonine and histidine respectively. While neither of these amino acids are the limiting amino acids of those proteins, these results were not unexpected as both whole egg and casein are of high quality and both were used in calculating the PAA reference concentrations. The PAA reference index predicted methionine and lysine as the limiting amino acids in soy and corn gluten meal, respectively. These results agree with growth trial results indicating these as the limiting amino acids.

Calculation of theoretical limiting amino acids by dividing the amino acid composition of the protein by the amino acid requirements

Table 21

Mean PAA Concentration[g] for Sheep Duodenally Infused with Different Proteins, Day Four Pre-Treatment. Experiment Four

Amino acid	Infusion treatment[h] WE 1	Cas 2	Soy 3	CGM 4	Soy + Met 5	EA 6	WE 2X 7	WE 3X 8	SEM	Significance of F value
Essential amino acids										
Thr	.53	.45	.84	.89	.25	.03	.72	.69	±.10	N.S.
Val	2.21	2.33	2.82	2.28	2.17	.97	2.68	2.54	±.22	N.S.
Met	.36[ab i]	.29	.30[a]	.39[ab]	1.32[d]	.44[b]	.34[ab]	.55[c]	±.03	P < .005
Ile	1.09	.86	1.41	.62	1.13	.37	1.01	1.37	±.11	N.S.
Leu	1.65	1.55	1.98	2.63	1.33	.21	1.91	1.69	±.25	N.S.
Tyr	.93	.88	1.11	1.36	.89	.24	1.12	1.47	±.12	N.S.
Phe	1.16	.88	1.06	1.46	1.55	.67	1.02	1.19	±.08	N.S.
Lys	1.38	1.26	2.23	.57	1.75	.61	1.14	.81	±.18	N.S.
His	.87[c]	.57[ab]	.93[c]	1.15[d]	.45[a]	1.42[e]	.83[c]	.65[b]	±.04	P < .01
Arg	2.02	1.41	2.86	1.18	1.95	1.94	1.77	1.99	±.17	N.S.
TEAA[j]	12.20	10.48	15.54	12.53	12.79	6.90	12.54	12.95		
Non-essential amino acids										
Ser	.56	.53	1.00	.98	.41	.40	.70	.94	±.07	N.S.
Glu	2.03	1.68	1.99	1.33	1.79	1.75	1.54	1.65	±.13	N.S.
Gly	2.67[a]	3.72[abc]	5.89[de]	6.88[e]	3.39[ab]	3.19[ab]	4.46[b]	4.92[bcd]	±.38	P < .05
Ala	1.59	1.18	1.95	1.79	1.72	.70	1.51	1.48	±.27	N.S.
Cys	.67	.57	.32	.50	.49	.37	.58	.51	±.05	N.S.
TNEAA[k]	7.52	7.68	11.15	11.48	7.80	6.41	8.79	9.50		
TAA[l]	19.72	18.16	26.69	24.01	20.59	13.31	21.33	22.45		

[g] Values are expressed as mg/100 ml
[h] Refers to the protein which was infused into the duodenum
[i] Values with similar superscript form a statistically homeogenous grouping (P < .05)
[j] Total essential amino acids
[k] Total non-essential amino acids
[l] Total amino acids

Table 22

Mean PAA Concentration[g] for Sheep Duodenally Infused with Different Proteins, Day Four Post Treatment. Experiment Four

Amino acid	Infusion treatment[h] WE 1	Cas 2	Soy 3	CGM 4	Soy + Met 5	EA 6	WE 2X 7	WE 3X 8	SEM	Significance of F value
Essential amino acids										
Thr	.40	.65	.67	.97	-.02	.03	.86	1.14	±.10	N.S.
Val	1.45	2.02	2.26	1.68	.89	1.70	1.87	2.20	±.21	N.S.
Met	.27	.45	.16	.31	.84	.54	.39	.64	±.09	N.S.
Ile	.41	.68	.79	.45	.27	1.31	.69	.56	±.08	N.S.
Leu	.76	1.02	1.26	1.84	.26	1.67	1.18	1.21	±.16	N.S.
Tyr	.74	1.18	.97	1.24	-.15	.28	1.16	1.86	±.13	N.S.
Phe	1.03	1.12	1.03	1.25	.46	.60	1.11	1.48	±.06	N.S.
Lys	1.16	1.96	2.02	.51	1.07	.75	1.13	1.17	±.24	N.S.
His	.34[ci]	.47[c]	.63[d]	.96[ab]	.27[a]	1.75	.68[bc]	.98[d]	±.13	N.S.
Arg	1.47[ci]	1.31[c]	2.00[d]	.93[ab]	.80[a]	2.08	1.27[bc]	1.82[d]	±.10	P < .05
TEAA[j]	8.03	10.68	11.79	10.14	4.69	10.71	10.34	13.06		
Non-essential amino acids										
Ser	.48	.81	.83	1.14	.18	.41	.71	1.12	±.14	N.S.
Glu	1.85	1.57	1.80	1.37	1.13	1.83	1.42	1.98	±.18	N.S.
Gly	3.42	4.11	4.66	8.00	1.72	3.14	4.25	7.41	±.72	N.S.
Ala	1.62	1.51	1.68	1.97	1.19	.62	1.90	2.47	±.19	N.S.
Cys	.31	.41	.42	.43	.50	.53	.33	.34	±.09	N.S.
TNEAA[k]	7.37	8.41	9.39	12.91	4.72	6.53	8.61	13.32		
TAA[l]	15.40	19.09	21.18	23.05	9.41	17.24	18.95	26.38		

[g]Values are expressed as mg/100 ml
[h]Refers to the protein which was infused into the duodenum
[i]Values with similar superscript form a statistically homeogeneous grouping (P < .05)
[j]Total essential amino acids
[k]Total non-essential amino acids
[l]Total amino acids

Table 23

Mean PAA Concentration[g] for Sheep Duodenally Infused with Different Proteins, Day Six Pre-Treatment. Experiment Four

Amino acid	Infusion treatment[h] WE 1	Cas 2	Soy 3	CGM 4	Soy + Met 5	EA 6	WE 2X 7	WE 3X 8	SEM	Significance of F value
					Essential amino acids					
Thr	.39[a i]	1.20[b]	.50[a]	1.35[b]	.36[a]	.24[a]	1.45[b]	1.32[b]	±.09	P < .05
Val	1.52[a]	3.10[b]	2.10[a]	2.13[a]	1.40[c]	1.32[b]	2.17[a]	2.52[b]	±.17	N.S.
Met	.27[a]	.40[b]	.21[a]	.26[a]	1.31[c]	.38[b]	.20[a]	.41[b]	±.03	P < .01
Ile	1.03[a]	1.39[f]	1.15[d]	.80[f]	.97[b]	.45[a]	.45[f]	.66[e]	±.09	N.S.
Leu	1.36[e]	2.45[f]	1.62[d]	2.55[f]	.84[b]	.51[a]	2.55[f]	2.12[e]	±.07	P < .005
Tyr	.75[a]	1.65[d]	.79[b]	1.71[e]	.63[cd]	.42[a]	1.75[d]	2.06[e]	±.15	N.S.
Phe	1.07[bc]	1.32[d]	.95[c]	1.61[e]	1.19[cd]	.48[ab]	1.00[b]	1.76[e]	±.05	P < .01
Lys	1.31[bc]	2.70[d]	1.78[c]	1.01[ab]	3.70[e]	1.10[ab]	.74[a]	1.10[ab]	±.13	P < .01
His	.99	.47	1.14	1.08	.86	1.12	1.02	.84	±.26	N.S.
Arg	1.80	1.69	1.98	1.49	1.49	1.76	.90	1.69	±.28	N.S.
TEAA[j]	10.49	16.37	12.22	12.91	12.75	7.78	12.23	14.48		
					Non-essential amino acids					
Ser	.76	1.09	.83	1.58	.38	.49	1.41	1.30	±.16	N.S.
Glu	1.96	2.53	1.49	2.00	1.78	2.16	1.60	2.31	±.15	N.S.
Gly	5.86	6.09	5.70	8.64	3.70	3.84	7.44	7.61	±.96	N.S.
Ala	1.73	2.32	1.49	1.40	1.86	.94	1.92	2.24	±.17	N.S.
Cys	.21	.55	.40	.51	.52	.52	.33	.51	±.09	N.S.
TNEAA[k]	10.52	12.58	9.91	14.13	8.24	7.95	12.70	14.02		
TAA[l]	21.01	28.95	22.13	27.04	20.99	15.73	24.93	28.50		

[g] Values are expressed as mg/100 ml
[h] Refers to the protein which was infused into the duodenum
[i] Values with similar superscript form a statistically homeogenous grouping (P < .05)
[j] Total essential amino acids
[k] Total non-essential amino acids
[l] Total amino acids

Table 24

Mean PAA Concentration[g] for Sheep Duodenally Infused with Different Proteins, Day Six Post-Treatment. Experiment Four

Amino acid	Infusion treatment[h] WE 1	Cas 2	Soy 3	CGM 4	Soy + Met 5	EA 6	WE 2X 7	WE 3X 8	SEM	Significance of F value
Essential amino acids										
Thr	.33	.80	.39	.99	.22	.50	.81	.61	±.08	N.S.
Val	.87	2.13$_{d}$	1.34$_{c}$	2.05$_{e}$	.65$_{f}$	3.15$_{g}$	1.15$_{a}$	1.06$_{b}$	±.17	N.S.
Met	.18^{ci}	.37^{d}	.21^{c}	.18^{c}	1.45^{f}	.68^{e}	.07^{a}	.14^{b}	±.04	P < .01
Ile	.55	.86	.78	.88	.45	1.01	.16	.35	±.07	N.S.
Leu	.64	1.28	1.10	1.10	.67	2.17	.35	.45	±.15	N.S.
Tyr	.49	1.58	.57	1.02	.75	.97	1.10	.88	±.18	N.S.
Phe	.62	1.09	.72	1.16	1.07	1.36	.89	.89	±.10	N.S.
Lys	1.32	3.14	1.51	1.90	1.56	.84	1.34	1.77	±.23	N.S.
His	1.02	.87	1.21	.97	.69	.66	1.38	.96	±.27	N.S.
Arg	.98	1.42	1.58	1.11	2.04	2.06	.45	.28	±.21	N.S.
TEAA[j]	7.00	13.54	9.41	11.36	9.55	13.40	7.70	7.39		
Non-essential amino acids										
Ser	.68	.69	.87	.98$_{d}$	.27$_{b}$	.80$_{c}$	.69$_{a}$	.54$_{a}$	±.13	N.S.
Glu	2.47^{d}	1.63^{bc}	2.05^{cd}	2.42^{d}	1.32^{b}	1.97^{c}	.18^{a}	.44^{a}	±.13	P < .05
Gly	4.49	4.81	4.96	7.17	2.75	4.71	5.50	4.17	±.45	N.S.
Ala	1.71	1.63	1.20	1.66	1.44	1.93	.76	.70	±.15	N.S.
Cys	.30	.46	.36	.46	.41	.51	.20	.18	±.05	N.S.
TNEAA[k]	9.35	9.22	9.44	12.69	6.19	9.92	7.33	6.03		
TAA[l]	16.35	22.76	18.85	24.05	15.74	23.32	15.03	13.42		

[g]Values are expressed as mg/100 ml
[h]Refers to the protein which was infused into the duodenum
[i]Values with similar superscript form a statistically homeogenous grouping (P < .05)
[j]Total essential amino acids
[k]Total non-essential amino acids
[l]Total amino acids

Table 25

Plasma Amino Acid Indices[a] for Experiment Four

Amino acid	Infusion[b] WE 1	Cas 2	Soy 3	CGM 4	Soy Met 5	EA 6	WE 2X 7	WE 3X 8
Essential amino acids								
Thr	80	88	79	87	32[c]	193	77	87
Val	62	76	73	85	43	211	62	64
Met	72	117	73[c]	76	87	149	85	81
Ile	45[c]	68	62	94	34	283	59	45
Leu	46	58[c]	66	57[c]	43	33	34[c]	43[c]
Tyr	74	109	81	73	39	191	78	77
Phe	74	101	87	79	56	169	99	80
Lys	92[d]	129	88	153	48	93[c]	132	153
His	73	129	88	87	73	95	111	129
Agr	103	88	74	76	83	112	64	71
TEAA[e]	71	91	76	81	56	164	73	74
Non-essential amino acids								
Ser	88	93	92	83	58	67	66	74
Glu	108	76	111	113	69	114	51	61
Gly	93	91	83	98	63	74	82	92
Ala	101	90	84	114	74	98	77	85
Cys	70	79	108	88	90	149	59	48
TNEAA[f]	96	87	89	100	68	87	74	82
TAA[g]	82	89	82	90	61	121	73	78

[a] Indices = $\frac{\text{Post-treatment (glucose) PAA concentration}}{\text{Pre-treatment (glucose) PAA concentration}} \times 100$

[b] Refers to protein infused

[c] Lowest index indicates the predicted limiting amino acid

[d] The limiting amino acid as determined by rat growth response or in theoretical calculation is underlined (Rao et al., 1964)

[e] Total essential amino acids

[f] Total non-essential amino acids

[g] Total amino acids

of swine (Table 27), were made for lack of a better comparison. This comparison can be criticized because the amino acids in a protein which are available for utilization are not always the same as the amino acid composition.

Threonine had the lowest PAA reference index in sheep infused with soy plus methionine. According to the calculation in Table 27 threonine was the second amino acid in soy. Thus, the PAA reference index prediction of threonine was correct as the first limiting amino acid, methionine, had been added to the diet in amounts so that the final methionine concentration in the diet was above the requirement. The theoretical limiting amino acid for egg albumin is either threonine or lysine. The PAA reference index was lowest for threonine. The other two infusions were both whole egg, but differed from the first whole egg infusion as the amount of protein infused was 2 and 3 fold greater. The lowest PAA reference index corresponded to lysine for both the whole egg 2X and 3X infusions. Theoretically, methionine is the limiting amino acid of egg. However, if cystine fulfills 40% of the methionine requirement (NRC Nutrient Requirement of Swine, 1968) then lysine should be the limiting amino acid in whole egg. Furthermore, lysine was shown to be the limiting amino acid in whole egg growth studies (Mitchell, 1959). Thus, these results indicate that the limiting amino acid of the protein reaching the small intestine can be determined by expressing PAA concentrations as a percent of reference PAA levels which are obtained after duodenally infusing a high quality protein.

To evaluate further the PAA reference indices, plasma amino acid concentrations of conventional ruminants in other studies (without duodenal re-entrant cannulas) were expressed as a percent of the

Table 26

PAA Referenced Indices[a] for Each Protein Infusion Treatment in Experiment Four

Amino acid	Av[b] (1 + 2)	Protein infusion: WE 1	Cas 2	Soy 3	CGM 4	Soy + Met 5	EA 6	WE 2X 7	WE 3X 8
Thr	.65	71[c]	128	103	174	48[c]	22[c]	168	155
Val	2.30	81	118	107	96	78	50	106	110
Met	.34	94[d]	103	76[c]	97	388	121	79	141
Ile	1.10	96	103	116	65	95	37	66	93
Leu	1.76	117	132	119	172	72	24	148	126
Tyr	1.06	79	120	90	145	72	31	136	167
Phe	1.11	101	99	91	139	123	52	91	133
Lys	1.67	81	119	120	47[c]	163	51	56[c]	57[c]
His	.73	127	71[c]	142	153	90	174	127	103
Arg	1.73	101	90	140	77	99	107	77	106

[a] Referenced Indices = $\frac{\text{PAA conc from animals on test protein}}{\text{PAA conc from animals on reference protein}}$

[b] Average PAA concentrations from whole egg and casein infused sheep, pre-treatment. Expressed as mg/100 ml

[c] Lowest index indicates predicted limiting amino acid

[d] The limiting amino acid as determined by rat growth response or in theoretical calculation is underlined (Rao et al., 1964)

Table 27

Theoretical Limiting Amino Acid of Proteins

Amino acid	Amino acid requirement[a] of swine % of diet	Protein			
		Soy	Casein	Whole egg	Egg albumin
Thr	.45	8.7[bd]	10.0[d]	10.4	9.3[c]
Val	.50	10.4	14.8	14.0	16.2
Met[e]	.50	2.2[c]	6.6[c]	8.0[c]	9.8
Ile	.50	11.6	13.2	12.8	13.6
Leu	.60	12.6	16.8	15.0	15.0
Phe[f]	.50	9.6	11.6	12.0	14.2
Lys	.70	9.4	11.7	9.1[d]	9.3[c]
His	.18	13.9	16.7	13.9	12.2
Arg	.20	35.0	21.5	29.5	30.0

[a]Taken from N.R.C. Nutrient Requirements of Swine 1968

[b]Determined as follows: $\frac{\text{Percent amino acid in protein}}{\text{Amino acid requirement}}$

[c]Indicates first limiting amino acid

[d]Indicates second limiting amino acid

[e]Does not account for cystine being able to fulfill 40% of the methionine requirement

[f]Does not account for tyrosine being able to fulfill 30% of the phenylalanine requirement

Table 28

PAA Reference Indices[a] of Conventional Sheep

Amino acid	Purser et al. (1966) A	B	C	D	B faun	B def	Oltjen et al. (1966) Soy	Urea	Ely et al. (1966) Zein
Thr	254	302	-	-	197	569	49	44	247
Val	59	76	134	126	113	279	75	59	68
Met	65	79	59[b]	44[b]	35[b]	88[b]	43	43	83
Ile	68	96	107	91	74	146	75	63	63[b]
Leu	75	76	66	60	72	139	51	41	127
Tyr	167	109	-	-	78	198	23	21	97
Phe	79	66[b]	72	64	56	111	36[b]	30[b]	75
Lys	35[b][c]	73	122	131	86	161	60	46	105
His	186	216	214	195	119	218	64	74	391
Arg	39	79	-	-	77	161	55	44	68

Amino acid	Potter et al. (1968) Expt one	Glu T_0	Glu 6 hr	Schelling et al. (1967) Purified	SBM
Thr	286	351	232	174	354
Val	131	96	70	44	185
Met	103	106	71	29[b]	62[b]
Ile	121	103	83	39	134
Leu	98	100	76	30	206
Tyr	98	116	97	61	278
Phe	63[b]	81[b]	63[b]	39	146
Lys	114	129	81	48	129
His	107	186	163	104	127
Arg	119	-	-	46	145

[a] Referenced index = $\frac{\text{PAA conc in other studies}}{\text{PAA conc from whole egg and casein infused sheep}}$

[b] Designates the limiting amino acid as determined by the proposed reference index method

[c] The theoretical limiting amino acid(s) are underlined. These estimates are based upon the amino acid composition of the diet and upon the amino acid composition of rumen bacteria and are thus somewhat arbitrary

reference PAA concentrations determined in this study. The PAA reference indices predicted from these studies showed phenylalanine (6 times), methionine (6 times), lysine (once) and isoleucine (once) as the amino acids limiting in the protein reaching the small intestine of the ruminant (Table 28). In the study by Ely et al. (1969) lysine was expected as the limiting amino acid, since a zein diet was fed and zein is deficient in lysine. In the study by Schelling et al. (1967) methionine was expected limiting as rumen bacterial protein and soy protein are methionine deficient. The reference index correctly predicted methionine in both cases. The limiting amino acids in the other studies may have been methionine or phenylalanine, as bacterial protein is deficient in methionine and phenylalanine. However, this does not preclude other amino acids from being limiting to the sheep.

Experiment Five

This experiment, using sheep, was designed to determine whether the amino acid levels in jugular vein blood differed from levels in blood from the carotid artery, both before and one hour after the intravenous infusion of either saline, glucose or acetate. The mean concentration of venous and arterial PAA are shown for each sample time (pre-treatment "T_0" and post-treatment "T_1") in Table 29. There were no differences between the venous and arterial PAA levels at either T_0 or T_1. At T_0 the total EAA concentrations were 9.80 and 9.99 mg/100 ml for venous and arterial blood respectively, whereas the T_1 concentrations were 7.81 and 8.05 mg/100ml for venous and arterial samples, respectively. The venous and arterial PAA levels paralleled each other as they decreased from T_0 to T_1.

The mean T_0 and T_1 PAA concentrations for each infusion treatment

Table 29

Mean Concentration[a] of Venous and Arterial Plasma Amino Acids Before and One Hour After Treatment, Experiment Five

Amino acid	Pre-treatment (T_0) Sample site[b] Venous	Arterial	SEM	Post-treatment (T_1) Sample site Venous	Arterial	SEM
			Essential amino acids			
Thr	1.08	1.03	±.18	.79	.83	±.18
Val	1.69	1.82	±.21	1.40	1.46	±.26
Met	.23	.24	±.03	.19	.20	±.02
Ile	.88	.92	±.15	.59	.65	±.17
Leu	1.50	1.56	±.25	1.01	1.15	±.30
Tyr	.81	.84	±.11	.68	.69	±.07
Phe	.65	.63	±.11	.52	.53	±.07
Lys	.81	.87	±.29	.69	.70	±.17
His	.63	.64	±.13	.66	.58	±.15
Arg	1.52	1.44	±.48	1.28	1.26	±.35
TEAA[c]	9.80	9.99		7.81	8.05	
			Non-essential amino acids			
Asp	.26	.24	±.07	.20	.20	±.03
Ser	.99	.97	±.25	.89	.89	±.24
Glu	3.13	3.19	±.91	2.81	2.80	±.83
Ala	3.03	2.77	±.25	3.06	2.84	±.59
Pro	1.00	1.00	±.12	.89	.92	±.19
Cys	.42	.52	±.17	.35	.42	±.12
TNEAA[d]	8.83	8.69		8.20	8.07	
TAA[e]	18.63	18.68		16.01	16.12	

[a]Values are expressed as mg/100 ml

[b]Sample site refers to either jugular vein or carotid artery

[c]Total essential amino acid

[d]Total non-essential amino acid

[e]Total amino acid

are shown in Table 30. The T_0 methionine and tyrosine concentrations in the acetate infusion group were significantly ($P < .05$) higher than the methionine and tyrosine concentrations in the saline and glucose infusion groups. The T_1 plasma tyrosine concentration in the acetate infused group was significantly ($P < .05$) higher than the concentration in the saline and glucose infused groups. The T_1 plasma methionine concentration after glucose infusion was significantly ($P < .05$) lower than the methionine levels in the saline and acetate infused sheep.

The effects of the infusion treatments upon the combined venous and arterial PAA levels are shown in Table 31; first by expressing the T_1 PAA as a percent of the T_0 concentrations (PAAI) and then as absolute PAA concentration differences (T_0-T_1). The average essential amino acid PAAI are 90, 80 and 73 for the saline, glucose and acetate infusion groups. Potter et al. (1968) infused both glucose and acetate into the carotid artery of sheep and found lower PAAI in the glucose infused sheep. The results from these two experiments do not agree; such differences may be due to the small numbers of sheep used in this experiment or to different infusion sites (venous in this experiment vs. arterial in the 1968 study). A notable difference between the glucose and acetate treatments is that the acetate infusion resulted in a sizable decrease in the plasma non-essential amino acids levels whereas no decrease was noted after glucose infusion. This suggests that there was little non-essential amino acid synthesis when acetate was infused.

Table 30

Mean Concentration[c] of Plasma Amino Acid by Treatment[d] Before and One Hour After Treatment, Experiment Five

Amino acid	Sampling time: Pre-treatment (T_0) Saline	Glucose	Acetate	SEM	Significance of F value	Post-treatment (T_1) Saline	Glucose	Acetate	SEM	Significance of F value
					Essential amino acids					
Thr	.87	1.02	1.26	±.12	N.S.	.83	.76	.84	±.13	N.S.
Val	1.58	1.76	1.91	±.15	N.S.	1.47	1.42	1.40	±.19	N.S.
Met	.21[ae]	.22[a]	.31[b]	±.02	P <.05	.20[a]	.17[b]	.21[a]	±.02	P <.005
Ile	.91	.91	.88	±.10	N.S.	.72	.61	.53	±.12	N.S.
Leu	1.53	1.66	1.40	±.17	N.S.	1.24	1.09	.91	±.21	N.S.
Tyr	.62[a]	.73[a]	1.14[b]	±.08	P <.001	.58[a]	.57[a]	.82[b]	±.05	P <.001
Phe	.60	.65	.67	±.08	N.S.	.58	.52	.47	±.05	N.S.
Lys	.71	.68	1.13	±.20	N.S.	.58	.57	.92	±.12	N.S.
His	.62	.65	.65	±.09	N.S.	.64	.65	.57	±.11	N.S.
Arg	1.36	1.06	2.02	±.34	N.S.	1.18	.98	1.65	±.25	N.S.
TEAA[f]	9.01	9.34	11.37			8.02	7.34	8.32		
					Non-essential amino acids					
Asp	.20	.23	.33	±.05	N.S.	.20	.18	.21	±.02	N.S.
Ser	.77	.89	1.28	±.18	N.S.	.91	.91	.86	±.17	N.S.
Glu	2.74	3.03	3.71	±.64	N.S.	2.86	2.76	2.86	±.59	N.S.
Pro	.85[a]	.82[a]	1.33[b]	±.08	P <.001	.87	.77	1.08	±.13	N.S.
Ala	2.35[a]	2.50[a]	3.85[b]	±.18	P <.001	2.97	2.92	2.96	±.41	N.S.
Cys	.44	.35	.62	±.12	N.S.	.44	.42	.31	±.08	N.S.
TNEAA[g]	7.35	7.82	11.12			8.25	7.96	8.28		
TAA	16.36	17.16	22.49			16.27	15.30	16.60		

[c]Expressed as mg/100 ml

[d]Treatment refers to the infusion of either saline, glucose or acetate.

[e]Like letters indicate a stastically homogenous grouping, Duncan's multiple range (P <.05).

[f]Total essential amino acids

[g]Total non-essential amino acids

[h]Total amino acids

Table 31

Mean Plasma Amino Acid Indices[a] and Pre-Treatment to Post-Treatment Change in Concentration by Treatment, Experiment Five

Amino acid	Treatment			(T_0-T_1) Differences[ba]				
	Saline	Glucose	Acetate	Saline	Glucose	Acetate	SEM	Significance of F value
				Essential amino acids				
Thr	95	75	67	.04	.26	.42	±.13	P <.001
Val	93	81	73	.11	.34	.51	±.17	P <.05
Met	95	77	68	.01	.05	.10	±.03	P <.01
Ile	79	67	60	.19	.30	.35	±.08	P <.05
Leu	81	66	65	.29	.57	.49	±.16	P <.001
Tyr	94	78	72	.04	.16	.32	±.08	P <.01
Phe	97	80	70	.02	.13	.20	±.07	P <.005
Lys	82	84	81	.13	.11	.21	±.18	N.S.
His	103	100	88	-.02	.00	.08	±.15	N.S.
Arg	87	92	82	.18	.08	.37	±.27	N.S.
Ave EAAI[c]	90	80	73					
				Non-essential amino acids				
Asp	100	78	64	.00	.05	.12	±.04	P <.05
Ser	118	102	67	-.14	.02	.42	±.18	P <.05
Glu	104	91	77	-.12	.27	.85	±.35	P <.05
Pro	102	94	81	-.02	.05	.25	±.12	N.S.
Ala	126	117	77	-.62	-.42	.89	±.47	P <.05
Cys	100	120	50	.00	-.07	.31	±.17	N.S.
Ave NEAAI[d]	108	100	69					

[a] Indices = $\frac{T_1 \text{ plasma amino acid concentration}}{T_0 \text{ plasma amino acid concentration}} \times 100$

[b] Differences = T_0 PAA concentration-T_1 PAA concentration

[c] Average essential amino acid indices

[d] Average non-essential amino acid indices

GENERAL DISCUSSION

Protein synthesis is influenced by both amino acid and energy supplies to the cell. The quantity and quality (distribution) of amino acids supplied is a major factor determining rate of protein synthesis (Munro, 1968a). While withdrawal of protein from the diet of an animal decreased the stability of microsomal protein synthesizing machinery in the cell (Munro 1968b, 1969), increases in the liver amino acid levels increased polysome formation (Munro 1968a). The rate of sulfur amino acid incorporation into protein was directly correlated to RNA concentrations in liver, kidney and muscle (Allison et al., 1963). Energy, (ATP) is another main factor affecting protein synthesis (Munro et al., 1962). Protein synthesis appeared to account for decreases in plasma essential amino acid levels when glucose (substrate for ATP) was given to semi-fasted animals. The pattern of decrease in the concentration of the plasma EAA (decrease in each essential amino acid) resulting from glucose ingestion or infusion was correlated to the EAA composition of striated muscle (Munro and Thompson 1953 and Potter et al., 1968).

Blood amino acid levels are a reflection of the larger tissue free amino acid pool (Rogers and Harper, 1968). Since the concentrations of plasma and tissue free amino acids are influenced by digestibility, quantity and quality of the dietary protein, quantity and type of energy substrates in the diet and rate of cell protein synthesis, then limiting amino acid determinations based upon plasma amino acid concentrations will be influenced by these factors.

McLaughlan and Morrison (1968) emphasized the importance of reference PAA patterns when using PAA concentrations to determine the limiting amino acid. The methods of McLaughlan (1964) and Longenecker and Hause (1959) used fasting PAA levels as a reference, while Hill and Olsen (1963) used the PAA levels after a non-protein diet was fed. Reference PAA concentration patterns were used as a reference point to compare subsequent changes in PAA concentrations which occurred when test proteins were fed. Potter et al. (1968) used 24 hour "fasting" plasma amino acid concentrations as a reference point in studies with sheep. This 24 hour post-feeding sample was expected to represent the time at which energy supply dictated neither an anabolic or catabolic state and a time when protein supply was neither minimum or maximum in comparison to other post-feeding times.

The limiting amino acid determination method (Plasma Amino Acid Indices) reported by Potter et al. (1968) was based upon the following theoretical assumptions; that glucose infusion would provide more ATP, the presence of more ATP would stimulate protein synthesis and protein synthesis would withdraw free amino acids from the plasma and tissue pools as they were needed. Since the limiting amino acid is the amino acid in shortest supply relative to the metabolic need, then expressing the post-glucose PAA levels as a percent of the pre-glucose PAA levels (PAAI) will predict the limiting amino acid (lowest PAAI). The results of experiments 2 and 3 with rats and experiment 4 with duodenally cannulated sheep showed that the PAAI method does not predict the dietary limiting amino acid as was previously established in growth trials. Therefore, it is unlikely that the PAAI method (Potter et al., 1968) can identify the limiting amino acid in the protein reaching the duodenum of sheep.

The PAAI method assumed that glucose would stimulate protein synthesis and that the amino acid requirement for protein synthesis was equivalent to the total amino acid requirements of the animal. The results of these experiments suggest that the PAAI method does not accurately predict the limiting amino acid even though glucose induced protein synthesis. Thus it appears that the PAAI procedure will not identify the limiting amino acid because amino acid requirements for growth and maintenance differ. An example of an amino acid whose maintenance requirement differs from the requirement for protein synthesis is methionine. Methionine (S-adenosylmethionine) serves as a methyl donor in the synthesis of phosphatidyl choline from phosphatidyl ethanolanine. Thus the methionine requirement will differ in respect to the rate of lipogenesis in the liver (synthesis of phosphatidyl choline). Furthermore the methionine requirement is somewhat undefined as cystine can fulfill part of the methionine requirement. Likewise, tyrosine can replace part of the phenylalanine requirement. Thus, the PAAI procedure may have identified the limiting amino acid for protein synthesis, or at least the limiting amino acid for protein synthesis in a specific organ (Munro 1969) and may explain why the PAAI procedure did not identify the amino acid which was limiting in growth trials.

Since PAAI for leucine, isoleucine and phenylalanine in experiments 1, 2 and 3, were unduely low, the PAAI from rats fed poor quality diets were expressed as a percent of PAAI from rats fed high quality proteins. This procedure was expected to remove the "bias" effect of glucose on specific amino acids and allow determination of the limiting amino acids. This procedure failed to identify the limiting amino acid when PAAI from rats fed different proteins were compared to reference PAAI from rats fed either whole egg or casein.

Several research groups (Sauberlich and Salmon 1955, Grey et al., 1960, Hill and Olsen, 1963 and McLaughlan and Morrison, 1968) reported low plasma levels of the limiting amino acid. Results from experiments 2 and 3 are in agreement with these reports, as the plasma level of the limiting amino acid was lower than levels of that amino acid in rats fed other proteins. In experiment 3, the plasma levels of lysine and methionine were correlated with the dietary levels of these amino acids (these were the limiting amino acids when these proteins were fed to rats). This suggested that the plasma level of the limiting amino acid is dependent upon the dietary level. Zimmerman and Scott (1965) reported low plasma levels of the limiting amino acid when the dietary level of that amino acid is kept below the requirement.

In theory, an animal fed a high quality protein would not have a low plasma level of any one amino acid (either all low or all high), while a low quality protein would cause a low plasma level of the limiting amino acid. Consequently an animal should exhibit an "optimal" PAA concentration pattern when fed a high quality protein. The limiting amino acid of the dietary protein should be the essential amino acid with the lowest percentage value when the PAA levels of animals fed low quality proteins (less than "optimal" quality) are expressed as a percent of PAA concentrations from rats fed high quality proteins. These percent values were referred to as "PAA reference indices" and the PAA concentrations from animals fed the high quality protein diets as "reference PAA concentrations." When PAA reference indices were calculated for the 8 protein diets in experiment 3, using reference PAA concentrations from rats fed whole egg, whole egg plus amino acids and casein, the lowest PAA reference indices corresponded to the predicted amino acids shown to be limiting rat growth (Rao et al., 1964) in 7 of these diets.

To apply the reference indices method (determination of the limiting amino acid) to sheep, reference PAA concentrations had to be determined. Reference PAA concentrations were obtained from sheep duodenally infused with whole egg and casein. These proteins may not give the "optimal" reference concentrations but were used because they are high quality proteins. Crystalline amino acid infusions composed so that every amino acid is equally limiting should give the "optimal" reference PAA concentrations, but the composition of such a mixture for sheep is unknown. Reference indices calculated by expressing PAA concentration patterns from sheep infused with other protein as a percent of the whole egg and casein reference PAA concentration identified the known limiting amino acids in 6 of the 8 protein treatments.

The reference PAA concentrations, as determined in sheep infused with whole egg and casein, were compared with PAA concentrations of conventional sheep. Results showed methionine, phenylalanine, lysine and leucine as the limiting amino acids in the different studies (Table 28). A hypothetical limiting acid was proposed for the animals in each of these studies by taking into account the dietary protein source and the amino acid composition of rumen microbial protein. The amino acid composition of the dietary protein was considered because some of the dietary protein will escape rumen degradation and reach the duodenum (Smith 1969). The theoretical limiting amino acid of rumen bacteria was determined by dividing the amino acid composition of rumen bacteria (Purser and Buechler, 1966) by the essential amino acid requirements of swine (NRC Nutrient Requirement of Swine 1968). This calculation showed methionine and phenylalanine as the first and second limiting amino acid of rumen microorganisms.

The diets fed by Purser et al., (1966) contained corn, corn cobs and alfalfa meal. Diet A was high in corn, thus lysine which is limiting in corn may have been the limiting amino acid in the protein passing to the duodenum. In contrast diets B, C and D contained more alfalfa meal and less corn and corn cobs, thus very little dietary protein would have passed undegraded to the abomasum. Hence, the limiting amino acid(s) of bacterial protein were thus expected as limiting. Reference indices obtained using Purser's data with the reference PAA concentration from this study showed lysine, phenylalanine, methionine and methionine as the limiting amino acids on diets A, B, C and D respectively. Reference indices for faunated and defaunated sheep, fed diet B, showed methionine as the limiting amino acid in both faunated and defaunated sheep.

Oltjen and Putman (1966), fed soy and urea diets to steers. Methionine might be the limiting amino acid in both of these diets as methionine limits rat growth in soy-fed rats and methionine is the limiting amino acid (theoretical calculation) of bacterial protein. Reference indices showed phenylalanine as the first limiting amino acid for both diets. This difference may be due to the fact that plasma amino acid concentrations from steers were compared to reference PAA concentrations from sheep. Furthermore, sheep are expected to have a higher methionine requirement than steers as wool contains fairly large quantities of sulfur amino acids. References indices from a similar study with sheep (Schelling et al., 1967) shows methionine as limiting when soy and urea diets were fed.

Ely et al. (1969) fed sheep a zein protein diet. Since zein is devoid of lysine, lysine was expected as the limiting amino acid. Reference indices using data from these sheep did not show lysine as limiting.

When the reference plasma amino acid concentration pattern was compared with data from experiment one and data from Potter et al. (1968), phenylalanine was the limiting amino acid. The reference indices calculated from Potter et al. (1968) agrees with reference indices recalculated from diet B, Purser et al. (1966), as both indicated phenylalanine as limiting (both diets were identical).

The amino acids identified as limiting by the PAA reference index did in most instances agree with the estimates of theoretical limiting amino acids. Although unequivocal proof as to the accuracy of the PAA reference index procedure has not yet been attained, the reference index method appears to be able to identify the limiting amino acid in proteins infused into the duodenum of sheep. Proof as to the accuracy of this method will require further investigation in both rats and sheep.

The final study of these experiments suggested that blood collected from either the jugular vein or carotid artery would be of equal value for use in determining plasma amino acid concentrations.

CONCLUSIONS

1. Glucose infusion into the jugular vein of sheep or into the stomach of rats resulted in a decrease in the plasma amino acid concentrations.
2. The plasma essential amino acid decrease pattern (decrease in each EAA), which occurred after the infusion of glucose in sheep, was significantly ($P < .05$) correlated ($r = .80$) to the amino acid composition of striated muscle of lambs.
3. The plasma amino acid index method described by Potter *et al.* (1968) did not identify the dietary limiting amino acid in rats.
4. In rats, the limiting amino acid of dietary proteins was reflected by low levels of that amino acid in the plasma.
5. Supplementation of the limiting amino acid into the diets of rats at levels which exceed the animals requirement for that amino acid resulted in increases in the plasma level of that amino acid.
6. The limiting amino acids of some dietary proteins can be determined by comparing the plasma amino acid levels of rats fed those proteins with the plasma amino acid levels of rats fed a high quality protein diet (whole egg and casein).
7. Sheep with duodenal re-entrant cannulas can be maintained, for a period of at least 6 weeks when a protein-mineral-vitamin infusion replaced the normal abomasal ingesta flowing into the duodenum.
8. The limiting amino acids of proteins can be determined by comparing the plasma amino acid concentrations of sheep duodenally infused with these proteins to plasma amino acid concentrations (reference

CONCLUSIONS (cont.)

PAA concentration) of sheep infused with high quality proteins (whole egg and casein).

9. The limiting amino acid of conventional ruminants may be determined by expressing their plasma amino acid concentrations as a percent of plasma amino acid concentrations (reference PAA concentration) from sheep duodenally infused with high quality proteins (whole egg and casein).

10. The plasma amino acid concentrations of blood taken from the jugular vein did not differ from those in blood from the carotid artery.

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APPENDIX I

SAMPLE CALCULATION

Appendix I Table 1

Sample Calculation

Experiment One: Effect of Glucose on PAA Isoleucine One Hour After Glucose Infusion

Restricted

	d.f.	Mean Square	F	Approximate Level of Significance
Treatment	2	0.5491	18.723	$P < 0.0005$
Regression about Mean	19	0.0589	0.625	$P < 0.837$
Error	16	0.0943		
Total	35			

Unrestricted

	d.f.	Mean Square	F	Approximate Level of Significance
Regression about Mean	21	0.1056	3.60	$P < 0.009$
Error	14	0.0293		
Total	35			

$$\text{Standard Error} = \sqrt{\frac{0.0293}{2}} = \pm .12$$

Duncan's New Multiple Range Test

Critical Value ($P < .05$)		.35	.37
		P = 2	P = 3
Ranked Means:	1.16	0.30	
	0.86	0.12	0.42*
	0.74		

APPENDIX II

PLASMA AMINO ACID CONCENTRATIONS FOR SALINE AND GLUCOSE TREATED RATS, EXPERIMENT TWO

Table 1: Egg Albumin and Casein Diets
Table 2: Soy and Zein Diets

Appendix II Table 1

Mean[a] PAA Concentration of Saline and Glucose Treated Rats for Each Dietary Treatment, Experiment Two

Amino acid	Dietary treatments: Egg albumin, Saline	Egg albumin, Glucose	Casein, Saline	Casein, Glucose
	Essential amino acids			
Thr	1.36 ±.16[b]	1.38 ±.14	4.34 ±.36	4.21 ±.39
Val	1.32 ±.05	1.12 ±.10	1.30 ±.04	1.05 ±.10
Met	.20 ±.04	.23 ±.03	.23 ±.02	.16 ±.03
Ile	.64 ±.04	.46 ±.04	.62 ±.02	.48 ±.05
Leu	1.11 ±.05	.84 ±.11	1.11 ±.03	.76 ±.10
Tyr	.49 ±.02	.42 ±.03	.55 ±.02	.58 ±.05
Phe	.54 ±.03	.43 ±.03	.55 ±.02	.42 ±.04
Lys	4.45 ±.30	4.26 ±.50	4.58 ±.30	5.11 ±.47
His	.39 ±.03	.41 ±.03	.51 ±.05	.54 ±.05
Arg	1.03 ±.08	1.13 ±.07	1.03 ±.10	1.02 ±.07
TEAA[c]	11.53	10.67	14.84	14.33
	Non-essential amino acids			
Asp	.90 ±.08	.86 ±.04	.78 ±.13	.63 ±.08
Ser	3.42 ±.26	2.97 ±.08	3.95 ±.17	3.53 ±.25
Glu	3.23 ±.19	3.09 ±.29	3.17 ±.21	2.40 ±.13
Pro	1.45 ±.08	1.57 ±.09	1.66 ±.10	1.57 ±.17
Gly	4.68 ±.43	3.38 ±.14	3.35 ±.16	3.07 ±.20
Ala	3.49 ±.15	4.86 ±.37	3.24 ±.18	4.21 ±.12
Cys	.33 ±.04	.40 ±.05	.38 ±.02	.40 ±.03
Orn	.45 ±.03	.51 ±.03	.44 ±.01	.50 ±.04
TNEAA[d]	17.95	17.64	16.97	16.29
TAA	29.48	28.31	31.81	30.62

[a]Mean of 6 rats expressed as mg/100 ml

[b]Standard error

[c]Total essential amino acids

[d]Total non-essential amino acids

[e]Total amino acids

Appendix II Table 2

Mean[a] PAA Concentration of Saline and Glucose Treated Rats for each Dietary Treatment. Experiment Two

Amino acid	Dietary treatments: Soy, Saline	Soy, Glucose	Zein, Saline	Zein, Glucose
	Essential amino acids			
Thr	2.89 ±.22[b]	2.83 ±.35	4.52 ±.31	3.77 ±.28
Val	.88 ±.05	.87 ±.12	.80 ±.09	.74 ±.07
Met	.15 ±.01	.10 ±.02	.32 ±.05	.34 ±.03
Ile	.43 ±.02	.42 ±.07	.40 ±.05	.35 ±.04
Leu	.75 ±.04	.66 ±.11	1.19 ±.19	.92 ±.13
Tyr	.43 ±.03	.47 ±.07	.55 ±.10	.48 ±.06
Phe	.38 ±.02	.36 ±.05	.65 ±.08	.53 ±.06
Lys	3.97 ±.89	5.05 ±.87	3.29 ±.36	3.27 ±.68
His	.54 ±.06	.66 ±.10	1.09 ±.20	1.01 ±.17
Arg	1.12 ±.09	1.45 ±.14	1.60 ±.12	1.65 ±.25
TEAA[c]	11.36	12.92	14.41	13.06
	Non-essential amino acids			
Asp	.78 ±.05	.64 ±.07	.80 ±.04	.67 ±.13
Ser	4.27 ±.30	3.78 ±.31	6.57 ±.40	5.38 ±.29
Glu	2.56 ±.11	2.12 ±.09	3.00 ±.18	3.13 ±.35
Pro	1.41 ±.08	1.32 ±.07	2.13 ±.28	1.69 ±.11
Gly	3.32 ±.18	2.95 ±.25	4.34 ±.27	3.33 ±.41
Ala	2.55 ±.14	3.68 ±.36	3.49 ±.43	3.95 ±.38
Cys	.26 ±.03	.31 ±.03	.24 ±.03	.41 ±.04
Orn	.43 ±.03	.52 ±.07	.85 ±.12	.78 ±.10
TNEAA[d]	15.58	15.31	21.42	19.34
TAA[e]	26.94	28.23	35.83	32.40

[a]Mean of 6 rats expressed as mg/100 ml

[b]Standard error

[c]Total essential amino acids

[d]Total non-essential amino acids

[e]Total amino acids

APPENDIX III

MEAN CONCENTRATIONS OF ESSENTIAL AMINO ACIDS FOR SALINE AND GLUCOSE TREATED RATS, EXPERIMENT THREE

Appendix III Table 1

Mean[a] Concentration of Plasma Essential Amino Acids for Saline Treated Rats by Dietary Treatment, Experiment Three

Amino acid	Dietary treatment group[b] WE 1	WE + AA 2	Cas 3	CGM 4	Soy 5	Soy + AA 6	Soy + AA 7	Soy + AA 8
	Essential amino acids							
Thr	4.32	6.86	8.24	6.88	7.04	6.64	4.20	5.60
Val	2.16	1.68	3.21	2.02	2.08	3.10	1.79	2.56
Met	.63	.92	1.04	.70	.44	1.66	1.19	1.55
Iso	.94	.85	1.65	1.00	1.24	1.44	1.11	1.27
Leu	1.30	.98	2.15	3.85	1.47	2.00	1.52	1.55
Tyr	1.03	1.68	2.69	2.07	2.17	2.55	2.22	1.93
Phe	.79	.80	1.08	1.20	.99	1.53	1.08	1.01
Lys	2.17	7.58	11.60	1.43	7.10	7.92	7.70	8.42
His	.85	1.10	1.39	1.49	1.45	1.18	1.07	1.20
Arg	2.12	1.95	2.40	2.68	4.59	4.61	4.36	5.18
TEAA[c]	16.31	24.40	35.45	23.32	28.57	32.63	26.24	30.27
TAA[d]	45.26	49.54	61.17	44.82	58.08	56.04	45.90	52.58

Mean[a] Concentration of Plasma Essential Amino Acids for Glucose Treated Rats by Dietary Treatment, Experiment Three

Amino acid	Dietary treatment group[b] 1	2	3	4	5	6	7	8
	Essential amino acids							
Thr	3.82	6.26	5.95	7.09	5.70	4.16	4.06	5.40
Val	1.96	1.44	2.27	1.54	1.76	2.01	1.56	2.11
Met	.49	.90	.70	.44	.44	.85	.80	.89
Iso	.72	.71	1.09	.71	1.03	1.03	.96	1.02
Leu	.79	.53	1.45	2.18	1.29	1.44	1.20	1.25
Tyr	.74	1.76	2.17	1.26	1.72	1.77	1.71	1.50
Phe	.51	.66	.90	.87	.88	1.00	.95	.95
Lys	1.35	8.24	9.07	1.40	6.64	6.07	6.39	6.95
His	.67	1.11	1.12	1.50	1.27	1.01	.92	1.08
Arg	1.79	2.00	2.05	2.14	3.87	3.77	3.44	3.80
TEAA[c]	12.84	23.59	26.77	19.13	24.60	23.11	21.99	24.95
TAA[d]	39.51	44.49	49.24	36.57	48.44	42.70	40.94	44.64

[a]Mean of 5 rats expressed as mg/100 ml

[b]Refers to diets in Table 4

[c]Total essential amino acids

[d]Total amino acids

APPENDIX IV

AVERAGE OF DAY FOUR AND DAY SIX PLASMA AMINO ACID CONCENTRATIONS IN SHEEP DUODENALLY INFUSED WITH PROTEINS

Appendix IV Table 1

Plasma Amino Acid Concentrations[a] of Sheep Duodenally Infused with Different Proteins, Average of Days Four and Six, Pre-Treatment Experiment Four

	Infusion treatment[b]							
Amino acid	WE 1	Cas 2	Soy 3	CGM 4	Soy + Met 5	EA 6	WE 2X 7	WE 3X 8
				Essential amino acids				
Thr	.46	.83	.67	1.13	.31	.14	1.09	1.01
Val	1.87	2.72	2.46	2.21	1.79	1.15	2.43	2.53
Met	.32	.35	.26	.33	1.32	.41	.27	.48
Ile	1.06	1.13	1.28	.71	1.05	.41	.73	1.02
Leu	1.51	2.00	1.80	2.59	1.09	.36	2.23	1.91
Tyr	.84	1.27	.95	1.54	.76	.33	1.44	1.77
Phe	1.12	1.10	1.01	1.54	1.37	.58	1.01	1.48
Lys	1.35	1.98	2.01	.79	2.73	.86	.94	.96
His	.93	.52	1.04	1.12	.66	1.27	.93	.75
Arg	1.19	1.55	2.42	1.34	1.72	1.85	1.34	1.84
TEAA[c]	10.65	13.45	13.90	13.30	12.80	7.36	12.41	13.75
				Non-essential amino acids				
Ser	.66	.81	.92	1.28	.40	.91	1.06	1.12
Glu	2.00	2.11	1.74	1.67	1.79	1.67	1.57	1.98
Gly	4.27	4.91	5.80	7.76	3.55	5.32	5.95	6.27
Ala	1.66	1.75	1.72	1.60	1.79	1.31	1.72	1.86
Cys	.44	.56	.36	.51	.51	.35	.46	.54
TNEAA[d]	9.03	10.14	10.54	12.82	8.04	9.47	10.76	11.77
TAA[e]	19.68	23.59	24.44	26.12	20.84	16.83	23.17	25.52
No. of[f] Animals	5	4	7	4	1	3	1	1

[a]Values are expressed as mg/100 ml

[b]Refers to different protein infusions

[c]Total essential amino acids

[d]Total non-essential amino acids

[e]Total amino acids

[f]Number of animals per infusion

Appendix IV Table 2

Plasma Amino Acid Concentrations[a] of Sheep Duodenally Infused with Different Proteins, Average of Days Four and Six, Post-Treatment Experiment Four

	Infusion treatment[b]							
Amino acid	WE 1	Cas 2	Soy 3	CGM 4	Soy + Met 5	EA 6	WE 2X 7	WE 3X 8
	Essential amino acids							
Thr	.37	.73	.53	.98	.10	.27	.84	.88
Val	1.16	2.08	1.80	1.87	.77	2.43	1.51	1.63
Met	.23	.41	.19	.25	1.15	.61	.23	.39
Ile	.48	.77	.79	.67	.36	1.16	.43	.46
Leu	.70	1.15	1.18	1.47	.47	1.92	.77	.83
Tyr	.62	1.38	.77	1.13	.30	.63	1.13	1.37
Phe	.83	1.11	.88	1.21	.77	.98	1.00	1.19
Lys	1.24	2.55	1.77	1.21	1.32	.80	1.24	1.47
His	.68	.67	.92	.97	.48	1.21	1.03	.97
Arg	1.23	1.37	1.79	1.02	1.42	2.07	.86	1.05
TEAA[c]	7.54	12.22	10.62	10.78	7.14	12.08	9.04	10.24
	Non-essential amino acids							
Ser	.58	.75	.85	1.06	.23	.61	.70	.83
Glu	2.16	1.60	1.93	1.90	1.23	1.90	.80	1.21
Gly	3.96	4.46	4.81	7.59	2.24	3.93	4.88	5.79
Ala	1.67	1.57	1.44	1.82	1.32	1.28	1.33	1.59
Cys	.31	.44	.39	.45	.46	.52	.27	.26
TNEAA[d]	8.68	8.82	9.42	12.82	5.48	8.24	7.98	9.68
TAA[e]	16.22	21.04	20.04	23.60	12.62	20.32	17.02	19.92

[a]Values are expressed as mg/100 ml

[b]Refers to different protein infusions

[c]Total essential amino acids

[d]Total non-essential amino acids

[e]Total amino acids