METHIONINE, LYSINE, AND PHENYLALANINE INFUSION AND THE EFFECT ON PLASMA AMINO ACID CONCENTRATIONS AND MAMMARY UPTAKE

by

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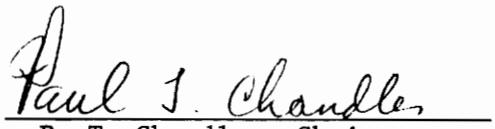

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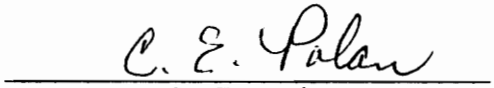

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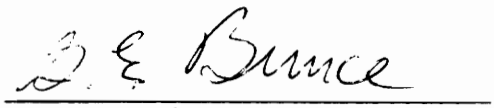

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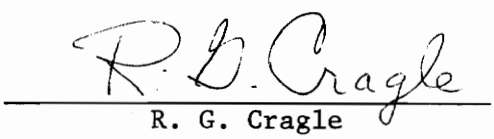

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INTRODUCTION

Protein and energy are two of the most important nutritional considerations necessary for attaining high productive responses in all mammalian systems. The high demand for protein and the economics of protein nutrition draw increased attention to this nutrient.

Initially dietary protein requirements were set above the determined values to compensate for varied responses of different dietary protein sources. It was necessary to accept some inefficiency in protein utilization to insure adequacy of amino acids. A more scientific approach in monogastric nutrition refined the protein requirement to the biological value of each protein source. Requirements were set for the potentially limiting amino acids and the diet was formulated to supply the required amount of each amino acid by balancing them with different protein sources or by individual amino acid supplementation.

Consideration of the amino acid requirement of ruminants is more complex. Microbial fermentation alters the dietary amino acid complement and the amounts presented for absorption.

The supply of fermentable carbohydrate, solubility of dietary protein, and time elapsed before passage from the rumen are just a few of the factors that can alter the amount of protein converted to microbial protein. As a result, only in recent years has the quality of protein been considered a factor in ruminant nutrition.

Recent comparisons of amino acid requirements based on plasma amino acids, growth rate, and nitrogen retention have indicated agreement for all three methods in poultry and swine. Some have concluded that

plasma amino acids could be utilized to evaluate the adequacy of dietary amino acids.

In ruminants the jugular and arterial amino acid concentrations have been variable and unreliable as an indicator of amino acid adequacy. However, arterial-venous differences enabled researchers to conclude that the free essential amino acids of the blood were incorporated directly into the major fractions of milk protein. Recent usage of amino acid uptake for evaluation of amino acid limitations seems promising.

After consideration of these points, a detailed study of plasma amino acids of lactating cows seemed desirable. Thus, the objectives of this study were: 1) to study the effect of the infusion of different essential amino acids on arterial and venous blood levels of amino acids; 2) to determine the effect of infusion on the amino acid uptake by the mammary gland; 3) to attempt to establish a possible order of limitation among the amino acids infused; and 4) to compare the above mentioned responses of cows at different productions as represented by early, mid, and late lactation.

REVIEW OF LITERATURE

Classical Theory of Amino Acid Usage

Researchers have known for some time that a specific protein contains amino acids in fixed proportions (21, 55). It has been established that a protein molecule is synthesized in a specific sequence according to the genetic code (30, 44). Thus, the amino acid present in the smallest amount in respect to the requirement of the synthesized protein limits the amount of protein synthesized and determines the efficiency of utilization of all other amino acids. Since storage of free amino acids is limited (55, 58), those amino acids supplied in excess of the complements required are catabolized and the nitrogen is excreted as a waste product (21).

Several amino acids can be synthesized in mammals from other amino acids in varying degrees while others must be supplied almost exclusively in the diet. Amino acids whose respective carbon skeletons must be supplied in the diet are considered essential or indispensable. Amino acids which can be synthesized in sufficient quantities are considered non-essential or dispensable (1, 55). If a diet devoid of one essential amino acid was fed and the deleted amino acid was supplied six h later, the response to the added amino acid was reduced in the rat (33, 55). In swine a longer time lapse was required for this to occur (55). However, the results emphasized that each animal has a requirement for complements of amino acids.

Determination of Amino Acid Requirements for Non-Ruminants

The digestive system of ruminants and non-ruminants have some

similarities. However, the most significant difference is that ruminants have a four compartmental stomach, allowing microbial fermentation of high fiber diets. This results in more variability during attempts to define the nutritional requirements of ruminants. Microbial fermentation distorts the relationship between the animal's requirements and the dietary components. However, after nutrients are presented to the post-ruminal intestinal tract, ruminants and non-ruminants have many similarities in physiological function (22, 72). Thus, it is reasonable to consider the responses of non-ruminants to protein and amino acid supplementation as a basis for understanding and predicting responses of ruminants.

Growth and Nitrogen Retention

Early determinations of protein and energy requirements relied heavily on rate of gain (1). Growth rate was plotted against varying dietary nutrient contents. The growth rate, as gain per unit increase in dietary supplementation, was generally high with initial increments but a diminishing response resulted as additional increments were added. High inputs sometimes reduced the response slightly from the maximum. Growth curves were later plotted on a log scale to emphasize the point at which the curve plateaued.

This approach assumed that the gain in weight was due primarily to protein storage. Body weight gain does not indicate how protein is being metabolized. Nitrogen retention was used to indicate storage of body protein and to give consideration to the excretion of nitrogen in the urine (55). Experiments, called titration studies, measured the responses to increasing dietary protein. Responses in weight gain and nitrogen retention were found to be very similar. The break point at

which maximal response was reached corresponded closely for weight gain and nitrogen retention (35).

Inconsistent results were noted with previously established protein requirements unless protein sources were balanced with each other. Attempts to refine the responses to protein supplementation focused on the amino acid components of protein. Almquist (1) obtained adequate growth and nitrogen retention responses with crystalline amino acids and concluded that either nitrogen retention or rate of growth could be applied to amino acid supplementation. By routinely supplying all other amino acids in excess and supplementing graded levels of the remaining amino acids in titration studies, Almquist and other nutritionists approximated the requirements for each amino acid by the point of maximal growth rate and nitrogen retention. Each amino acid requirement was further refined by repeating the previous titration studies and measuring the responses when all other amino acids were supplemented just above the approximated requirement. This refined the amino acid requirements of growing chicks. Eventually, reference diets of crystalline amino acids were established, which approximated the chick's requirements (79). The amino acid content of reference diets was modified as it became apparent the requirements differed slightly from previously established reference standards. Growth rate on the reference diets was comparable to adequately supplemented diets.

Plasma Amino Acids

Studies with crystalline amino acid diets soon led to a keener interest in the physiological utilization of protein. Amino acid absorption from the intestines, transport throughout the body, and incorporation

into tissue received considerable attention. A high degree of correlation was found between patterns of blood amino acids and dietary protein (1, 48, 54, 88).

a. Classical theory of plasma amino acid limitation

The responses of growth rate, nitrogen retention, and plasma amino acids had similarities and several workers concluded that plasma amino acids could be used to determine dietary amino acid requirements (48, 54, 57, 88).

The classical theories can be summarized (27, 54, 57, 79, 88) by the following points: 1) An amino acid supplied in insufficient quantities will remain relatively low irrespective of the deficiency. With increments of dietary supplementation, plasma concentrations will not rise appreciably until the requirement is met; 2) Dietary increments of the amino acid supplemented in excess of the requirement will result in a rapid, linear accumulation in the plasma; 3) When plasma amino acid is plotted against dietary amino acid, the point of accumulation coincides with the break in growth and nitrogen retention response curves. The point of inflection of the plasma concentration was interpreted to be the requirement for that amino acid; 4) Supplementation of a limiting amino acid results in more efficient utilization and a decline in plasma concentrations of other essential amino acids. The one showing the most rapid rate of decline would be a candidate for the next limiting amino acid.

As this theory implies, supplementation of a non-limiting amino acid would produce only its accumulation in plasma. Similarly, once the first limiting amino acid is supplied in excess, a second limiting amino

acid should follow the expected pattern of limitation. Elevated plasma concentrations imply that these amino acids exceed requirements.

b. Application of plasma amino acid theory

Researchers have observed that supplementation of the limiting amino acid initially results in a decline in plasma concentration of other essential amino acids. This decline continues until that amino acid is provided in excess. With higher supplementation essential amino acids increase resulting in an overall curvilinear response to amino acid supplementation (42, 57). Plasma concentrations reach their low near the point of inflection in the growth or nitrogen retention response curve for the supplemented amino acid (57). Simultaneously, excessive supplementation depressed growth rate. Total non-essential amino acids have been noted to follow a similar curvilinear response, although individually the responses varied. Some amino acids such as glycine appear to be totally unrelated to the supplementation of essential amino acids.

When deficiencies of amino acids were known to exist, feed intake was depressed and plasma concentration of the deficient amino acid was low. Force feeding the diet resulted in drastic reduction of that amino acid in plasma of chicks despite its already low level (58). Thus, plasma amino acid application cannot be isolated from appetite regulation and other related factors.

Although plasma amino acids followed certain patterns, precise determinations of amino acid requirements through plasma amino acid concentrations were difficult. The coefficient of variation was much

higher than that detected on growth and nitrogen retention studies, so detection of significance was elusive.

Attempts were made to determine amino acid sufficiency through reference comparisons. First attempts used a comparison of plasma amino acids on an experimental diet to that after a fasting period (48). The resulting changes in plasma amino acids were not always concise when results were compared to established requirements. However, studies of plasma amino acids indicated an extreme variation in the concentrations existed in a 24 h fasting period (27, 36, 45). The quantities of plasma amino acids present in relation to the requirement are distorted by fasting, feeding schedule prior to fasting (27, 36, 45), and diurnal variations (25). Lewis and Speer (45) indicated that postprandial amino acid concentrations were more responsive than fasting concentrations. Time of fasting could have a critical effect on interpretation of plasma amino acid results by this method. Animals were placed in a critical energy demand so plasma amino acids were a partial reflection of the stress of energy need and the metabolic changes that resulted.

The total amount of plasma amino acid does not appear to be entirely dependent on absorption of amino acids by the intestine or time of feeding. However, researchers have noted a gradual linear increase in plasma amino acid concentrations after periods of absorption (58). This increase parallels but is smaller than changes noted in the portal vein.

McLaughlan and Illman (54) graphically illustrated the relationship between plasma amino acid and dietary amino acid content. The graphic response was compared to proven dietary requirements. In some cases,

agreement between requirement and the break in the plasma amino acid response curve to dietary supplementation occurred. Under other conditions little resemblance in requirement occurred. This lack of agreement pointed out the problem that exists if plasma amino acids are used to establish dietary requirements (27).

Others used reference comparisons of the experimental diet and a protein-free diet (36). The results appear to be more compatible with those obtained by growth and nitrogen retention responses since changes in plasma amino acids are not as abrupt as those produced by fasting. However, both ratio methods predicted amino acid limitation with some degree of success.

Scott (79) refined this ratio technique further by comparing plasma amino acids of comparable groups of chicks fed a modified amino acid mixture (Diet B) and the reference standard (Diet A). He calculated the relative difference in amino acids by the following ratio:

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

Others used the reference diet as the denominator (23). The ratio indicated excesses and deficiencies from the reference standard diet as positive and negative ratios, respectively. The magnitude of the ratio should be an indication of the relative limitation or excess of dietary amino acids. All of the ratio methods assume that the base level used represents a normal, balanced, and optimum diet and plasma amino acid content.

The use of this ratio by Scott and coworkers enabled them to pinpoint first and second limiting amino acids on experimentally manipu-

lated diets. Further usage of this ratio with dietary protein sources gave conflicting results and drew attention to potential excesses that existed in the reference standard diet. The suggested modifications were verified in later feeding trials (79).

Responses to Amino Acid Supplementation in Ruminants

As eluded to earlier, ruminants differ from non-ruminants by preferential presentation of the dietary amino acid complement to rumen micro-organisms. Deamination of amino acids in the rumen to ammonia and subsequent synthesis of microbial protein alters net dietary amino acid balance. Because of rumen microbial distortion of amino acid patterns, early researchers saw no response in gain or milk production with protein sources of different quality and concluded that protein quality was of little importance for ruminants (55, 82). Virtanen (86) proved that amino acids could be synthesized in the rumen without an α-amino source of nitrogen, but milk production on these rations was limited to 4,217 kg milk per year.

Some studies of amino acid composition of diet, abomasal contents, and blood plasma revealed that diet composition was not closely related to the other two parameters (61, 64). The amino acid composition of abomasal contents and plasma was more closely related to microbial than dietary composition, although some dietary relationship still existed (15, 47, 58, 70, 82).

Point of Supplementation

Australian workers utilized abomasal infusion and chemical treatment

of proteins to bypass the rumen while studying the production responses to protein supplementation in sheep. Improved nitrogen retention, daily gain, and wool growth were associated with these treatments. Wool growth of mature sheep was increased by 123 to 181% with rumen bypass of casein (74). Abomasal infusions of cystine or methionine resulted in 35 to 130% more wool growth than controls (73).

Little and Mitchell (46) studied effects of oral and abomasal administration of soybean and zein proteins on wethers. Abomasal administration of zein significantly lowered protein digestibility while nitrogen retention was non-significantly lower. Nitrogen retention was significantly higher when soy protein was infused abomasally. When casein and gelatin were administered by the same methods, results indicated that protein digestibilities were equal, yet nitrogen retention was significantly higher for casein abomasally. Nitrogen retentions were negative for oral administration and positive for abomasal administration with both protein sources. Their results were related to solubilities of protein in the rumen (46) and reasonable agreement existed with digestibilities reported for rats when protein sources were administered abomasally. Australian workers had obtained similar digestibilities (51). Thus, some protein sources have a greater potential value if the rumen could be bypassed. This has resulted in the suggestion that the rumen may be a factor limiting productive response in ruminants (34, 72).

Response to Amino Acids and Amino Acid Analogs

Nelson (61) thoroughly reviewed results from amino acid supplementation. The primary responses to dietary supplementation of unprotected amino acids have been with lysine to feedlot cattle and methionine

hydroxy analog (M-analog) to dairy cattle. Even with supplementation of lysine and M-analog, the results have varied and are not conclusive. Lysine is more resistant to microbial degradation and some is available for absorption in the intestine (61). M-analog is resistant to microbial degradation (4, 76) and can be converted to methionine by liver (4).

From Nelson's review (61) it appears 7 to 9 g/day of lysine may be beneficial to feedlot cattle. Most lysine responses were reported on urea rations, but responses were not sufficient to equal those of natural protein controls. Animals did not respond uniformly to lysine supplementation.

Similarly abomasal administration of certain amino acids to animals receiving some crude protein from urea produced responses above controls but did not exceed natural protein sources (61). This may reflect the smaller portion of α-amino nitrogen which reaches the abomasum with urea supplementation (16) and the adaptation period required for efficient urea utilization (15, 50). However, in limited trials at Iowa State, supplementation of M-analog produced a response in gain that exceeded natural protein controls in rapidly growing 182 kg heifers (61).

Milk production responses with M-analog were first reported for cows by Griel et al. (29). In trials designed to test effects of M-analog supplementation on prevention of ketosis, potentially ketotic cows were supplemented with 0, 40, and 80 g of M-analog/day. Supplementation with 40 g increased milk production, but at 80 g a breed interaction was noted. Two breeds increased production at 80 g, while the other two declined. Part of the response at 80 g supplementation was

attributed to feed intake.

Polan et al. (69) illustrated level of supplementation can alter responses observed. M-analog was supplemented at 0, .2, .4, and .8% of the concentrate resulting in daily intakes of approximately 0, 20, 40, and 80 g/day. M-analog consumed in excess of 45 g/day reduced milk production. Fat test increased over the entire range of supplementation. Quadratic regressions for milk and butterfat content were significant at $p < .01$ and .05, respectively. The highest supplementation depressed feed intake.

Bishop (6) conducted a field trial with 181 dairy cows at three locations. Cows were supplemented with 30 to 40 g/day of M-analog over the first three to four months of lactation. Responses to M-analog over controls were an additional 374 kg of milk, 21 kg of fat, and 463 kg of fat corrected milk for 2X, 305 day M.E. lactations. Responses were compared as deviations from the previous lactation. General responses were similar at all locations with only the magnitude changing. Further analysis revealed the response was greater for the high production group. Cows with 5,511 and 7,537 kg of milk produced 211 and 715 kg more fat corrected milk than controls.

In a similar field trial, Bishop and Murphy (7) obtained increments of 554, 22 and 554 kg of milk, fat, and fat corrected milk respectively. Records for the test year only revealed greater responses in mature than younger cows.

Researchers have documented an altered lipid metabolism (18, 37, 66, 69, 75). Patton et al. (67) observed an increase in protozoa concentrations in the rumen. However, some have reported decreased nitrogen

retention with M-analog supplementation (37). Others did not obtain a response to M-analog supplementation (61) or intravenous infusion of methionine (28). Bull and Vandersall (14) attributed M-analog response to effects of sulfur on microbial fermentation in the rumen. However, lack of response may be due in part to amounts infused and its effect on intake (69) and changes in limitation under differing dietary conditions. Munro (58) indicates that methionine affects adrenocortical function even at physiological ranges in plasma.

Several researchers have studied responses to abomasal administration of amino acids and methionine supplemented casein or dietary administration of protected casein in lactating dairy cattle. Some responses have resulted in increased milk production (12, 24, 84), increased protein production (11, 12, 24, 84), and increased protein content (11, 12, 24, 84). Broderick et al. (11) reported changes in percent milk protein was immediate. Halfpenney et al. (31) and Yousef et al. (87) reported protein content was increased by a higher plane of energy nutrition.

Yousef et al. (87) indicated that increased protein content of milk on high grain diets could not be explained through the uptake of α-amino nitrogen by the mammary gland. Vik Mo et al. (84) reported that the amino acid supply to the mammary gland was responsible for increased protein yields in his experiment and not energy.

Broderick et al. (12) fed graded amounts of formaldehyde treated casein to cows receiving a 10% crude protein basal ration. Milk production and protein content responses increased with initial increments and plateaued at 16% crude protein.

Broderick et al. (11) administered 800 g of methionine supplemented casein abomasally and obtained a 6.2% increase in milk protein content ($p < .10$) and a 11.6% increase in protein production. The infusion resulted in increased total essential amino acids, decreased total non-essential amino acids, and decreased grain intake.

Derrig et al. (24) infused sodium caseinate post-ruminally and significantly increased milk yield, milk nitrogen and nitrogen retained. Fecal and urinary nitrogen were significantly decreased. They obtained an increase in most essential and non-essential amino acids in plasma.

Several workers (11, 12, 19, 24, 85) studied plasma amino acid responses in lactating dairy cows and related it to amino acid uptake by the mammary gland. These responses will be further considered as related to productive function.

Plasma Amino Acid Responses of Ruminants

Nimrick et al. (62, 63) fed semipurified diets with urea as the sole source of nitrogen to abomasally cannulated wethers. In a series of experiments, they obtained responses in daily gain and efficiency of feed conversion to abomasal administration of methionine, lysine, and threonine in that order of limitation. No response to lysine was obtained until methionine was supplemented, or from threonine until methionine and lysine were supplemented. The peak nitrogen retention in each combination of experiments was near 3.3 g/day.

Abomasal administration of methionine alone resulted in drops in plasma lysine that were greater than other amino acids. Likewise supplementation with methionine and lysine resulted in a similar decline

in threonine, thus substantiating the theoretical response expected.

Schelling obtained responses to abomasal infusion of methionine and lysine separately (77). Supplementation with either resulted in precipitous declines in plasma concentration of the other. Apparently, both were nearly equally limiting and supplementation of either resulted in more efficient use of the other. Chalupa and Chandler (17) infused graded methionine abomasally. Their plasma methionine response curve followed the previously described response theory of limiting amino acid supplementation. The nitrogen retention response resembled the milk production response of Polan et al. (69) and was related to plasma amino acid response.

Methods which have been applied to non-ruminants have been attempted with ruminants. Ratio indexes have been utilized in several trials (71), but overall interpretations have been restricted by variations that occurred. Effects of diurnal variation could be a problem (81). This reflects many factors that affect plasma amino acids as reported by Harper (32). Derrig et al. (24) indicated that jugular amino acid concentrations were of little value when used alone.

For the lactating cow, amino acid uptake by the mammary gland has received renewed interest. This method looks promising because of its relationship to productive function and will be covered in the following section.

Relationship to Productive Function

Responses to supplementation cannot be isolated from productive function. Poultry responds to methionine, but flesh and feathers of birds contain higher levels of sulfur containing amino acids (2).

Likewise, response in wool growth shown by Australian workers (51, 73, 74) can be attributed to sulfur amino acid concentrations in wool. When g of each amino acid/100 g nitrogen is compared (2), muscle of beef cattle contains about 10% more lysine than milk. In growing lambs responses in daily gain to methionine and lysine may be a response influenced by both rates of gain and wool growth. Limitation can be expected to be determined by this need as compared to that supplied by microbial and feed bypass amino acids that are absorbed. Thus, plasma amino acids would be expected to be representative of changes in flux through this pool (32). The drain for protein synthesis should reflect proportions of amino acids present in the quantity of synthesized protein produced.

Several researchers have considered plasma amino acids in relation to amino acid content of milk (19, 24, 83, 85). Their calculations consider the drain across the mammary gland as reflected by arterial-venous differences. Derrig et al. (24) and Chandler and Polan (19) calculated the utilization efficiency of amino acid uptake when related to milk protein production and composition. Derrig et al. calculated amino acid uptake and percentage extraction by the mammary gland during rumen and abomasal infusion. To determine a relative limitation he calculated the ratio of uptake from plasma to output in milk. These ratios were used to determine the relative order of limitation with the smallest ratio reflecting the most limiting amino acid. Derrig et al. predicts that the potential order of limitation with his data is phenylalanine, methionine, lysine, threonine, leucine, isoleucine, and histidine.

Derrig et al.'s ratio is comparable to efficiency of utilization

calculated by Chandler and Polan. His calculation compares amino acid output in milk to uptake by the mammary gland, and expresses this as percent efficiency of amino acid incorporation into milk protein.

Broderick et al. (12) used differences in jugular and mammary vein amino acids and predicted that methionine, valine, and lysine were the order of limitation.

Previously, Chandler and Polan (18) calculated amino acid compositions reaching the abomasum. After comparing these calculations to amino acid content of milk they concluded methionine, valine, isoleucine, tryptophan, and lysine could be a potential order of limitation.

Amino acid oxidation is a direct result of productive function of an amino acid. Brookes et al. (13) conducted trials to study lysine oxidation over graded dietary lysine supplementation and indicated that oxidation remained at low levels until requirements are exceeded. However, limited numbers restricted conclusions drawn. This method assumes that amino acids supplied in excess of requirements are oxidized and could serve as guidelines for evaluating the total productive requirements of animals.

Site and Time of Sampling

Researchers have utilized different points of sampling in evaluating plasma amino acids. The majority have utilized the jugular vein, but results with dairy cattle have been inconclusive with jugular vein sampling (24). Early work in establishing source of amino acids for milk protein synthesis used arterial-venous differences across the mammary gland and blood flow rates to estimate amino acid uptake. This method enabled researchers to conclude that free amino acids in blood were incorporated directly into most fractions of milk protein in the mammary gland.

Studies relating to site and time of sampling have been reported (19, 68). Significantly higher plasma amino acid concentrations at jugular and tail sampling sites than at the subcutaneous abdominal mammary vein were observed. Tail samples were non-significantly higher than jugular samples, but this could be due in part to mixed arterial and venous sampling.

Sampling times included post milking, four h post milking and 10 h post milking. Large differences in amino acid uptake by the mammary gland occurred with time of sampling. This metabolite uptake appeared to be more dependent on time of feeding and absorption of nutrients and energy from the digestive tract. The metabolite uptake would appear to be especially dependent on production of VFA's in the rumen. Others who have measured metabolite uptake by the mammary gland have sampled between three to five h after feeding as well (19, 24, 85).

The site of sampling should give values that are representative of amounts presented to or absorbed by the mammary gland. The assumptions made when evaluating essential amino acid uptake are: 1) Complete amino acid requirement by the mammary gland is for milk protein; 2) Blood sampled is available only to the mammary gland; 3) Efficiency of utilization in the mammary gland is proportioned to limitations of that amino acid.

Some limitations must be considered as well. Mepham and Linzell (56) reported that the subcutaneous mammary vein can sometimes contain a variable amount of blood from the external pudic vein in goats. They clamped this vein prior to sampling (5, 56).

Aoki et al. (3) indicates that some error occurs in muscle of man due to consideration of only plasma instead of the entire blood pool. These previous two effects are probably minimal in the cow as compared to potential influence of metabolic and hormonal controls. Amino acids may be required in pathways other than production of milk protein, such as the function of methionine as a methyl donor in lipid metabolism.

Nutritional Status

a. Dietary effects

When ruminant rations are altered, allowances must be made for effects on the rumen microbial fermentation. Adaptation time is required for changes in microbial populations. This is particularly evident from reports of urea utilization (15, 50). Indications are that major changes may require as much as three weeks for complete adaptation. Changes in feed intake have resulted from such ration manipulations. It is reasonable to expect that lesser changes require a shorter adaptation period. Many have used a two wk pre-experimental adaptation period which should be more than adequate for minor changes. This should insure that any responses measured are not due to changes in microbial fermentation.

Time of feeding can affect quantities of VFA's and amino acids available for absorption at a specific time. Thus, time of feeding could result in higher or lower plasma amino acid concentrations at sampling time.

b. Physiological effects

Although there is no appreciable storage of free amino acids, the

adequacy of nutrition can affect amounts of amino acids stored as tissue protein (55, 58). Thus, the state of build-up or depletion of body protein could alter an animal's response to dietary supplementation. This is especially evident during early lactation when negative nitrogen balances have frequently been obtained.

Muscle protein is higher in concentrations of lysine and glycine than is milk (2). Some free amino acids may concentrate in certain tissues as well (58). It has been reported that when dietary lysine levels increase from 28% to 200% of the requirement, or approximately seven-fold, the plasma free lysine increased seven times while muscle-free lysine elevated 28 times (27, 58). The reversal of accumulation in tissues could be one reason for the confusing effect that is seen during fasting. Metabolic demand for energy is reflected in blood metabolites within a short period (58).

Although changes were not as abrupt in monogastrics on a protein-free diet (36), application to ruminants is questionable.

A change from high to low plane of nutrition results in metabolic adaptation. Initially, muscle protein is depleted until the animal reaches a "conservation" state. The animal's amino acid requirements during the adaptation period may be different than the requirements during the conservation state. This adaptation phenomena exists during adjustment to a higher or lower plane of nutrition.

Harper (32) has shown time is required for induction of liver enzymes. Feeding excessive amounts of casein resulted in initially high plasma threonine. However, sampling after three days indicated that plasma threonine had declined appreciably from the one to two day

high. Amino acid imbalance did not produce abrupt change.

This adaptation phenomena appears to vary considerably with parameters measured. Keith et al. (42) conducted an experiment with Lucas' design and found no carry-over effects when daily gain of growing swine was evaluated. Broderick et al. (11) noted that change in percent milk protein was abrupt.

Considerations During Interpretation of Plasma Amino Acid Values

Plasma amino acid patterns have been highly variable. However, free amino acids represent only .5% of the total amino acid pool. Since the plasma pool represents only .2 to 6% of the free amino acid pool for individual amino acids, the overall effect cannot be minimized (58). When individual researchers compared plasma amino acid responses to nitrogen retention or daily gain, some have obtained the theoretical responses (17, 42, 57, 88). Featherson (27) noted that responses were not always in agreement. Dietary imbalances create excesses and deficiencies in plasma. McLaughlin and Campbell (53) state, "The true value of a protein supplement will depend not only on the limiting amino acid, but also on the excess of amino acids in the supplement ... and the makeup of deficiencies." When lysine greatly exceeds requirements, several amino acids tend to accumulate in plasma and there is a marked decline in arginine. Supplementation with arginine can overcome toxic effects of lysine (58, 88). Arginine will accumulate with a deficiency of lysine or valine (88). Yet a deficiency of lysine and arginine gives rise to a marked increase in plasma threonine (88). Interrelationships have been reported for valine, leucine, and isoleucine and supplementation of one affected the others. Many relationships have been shown

to exist, but understanding of the processes is limited. Certain amino acids are known to share common transport systems, and some of these interrelationships are involved.

Harper (32) reports supplementation of excessive amounts of protein resulted in high initial plasma levels. After two to three days the plasma concentration was considerably reduced. He indicates liver enzymes are induced by imbalance and alter plasma amino acid responses.

Concentrations of amino acids present in the plasma pool are influenced by several factors (32) as follows: 1) Changes in food intake (and protein level); 2) Changes in stomach emptying; 3) Changes in absorption in the intestine; 4) Changes in reabsorption from the kidney; 5) Changes in transport into and out of tissues; 6) Protein synthesis; and 7) Changes in amino acid degradation. Another factor that cannot be overlooked is tissue catabolism. The modifying effect of liver is an important part of both protein synthesis and amino acid degradation since the turnover rate of liver tissue is very high. Equally important in ruminants is the modifying effect of the rumen. However, effects of all these factors on plasma amino acids cannot be quantitatively predicted at this time.

Effects of metabolic controls of animals on plasma amino acids cannot be overlooked. Methionine and leucine have been shown to affect adrenocortical function at all levels of administration including physiological ranges of intake (58). Infusing amino acids can elicit secretion of insulin, glucagon, and growth hormone (58). Plasma amino acids are sensitive to a number of factors. These potential sources of variation must be considered during interpretation of results.

Dietary Requirements

Many nutrients are required for milk production. However, the primary ones that would influence the results of experiments relating to amino acid nutrition are energy, protein, and sulfur. For this reason only the effects of excesses, deficiencies, and responses to these three will be considered.

Experimental conditions for study of a nutrient such as protein require that all other nutrients be provided in reasonable excess. Otherwise, responses to protein or amino acid supplementation could be limited by restriction of another nutrient.

Energy

An energy deficiency could alter and reduce the response to amino acid supplementation. In response to energy demand, larger quantities of amino acids enter catabolic routes for oxidation (22). This could reduce responses to protein supplementation in two ways. First, supply and balance of amino acids available for protein synthesis is changed. Second, energy is required for optimum utilization of amino acids. Energy is involved in certain amino acid transport mechanisms (30, 58) and is required for activation of amino acids with transfer RNA during protein synthesis (44).

By supplying energy in reasonable excess, amino acids, especially the non-essentials, are spared through reduced amino acid oxidation. This allows the system to fully express its protein synthetic potential due to energy availability for required processes and complete amino acid supply. This should place greatest stress on the system and allow a response to the most limiting amino acid. The National Research Council

(NRC)(59) has established energy specifications of 1.4, 1.6, and 1.8 Mcal of Net Energy lactation per kg of ration dry matter for cows producing < 20, 20-30, and > 30 kg of milk/day, respectively. Chandler and Walker (20) used the correlation between Net Energy lactation and crude fiber content in feeds to establish crude fiber limits in formulating rations with adequate energy content. Calculations of crude fiber from their regression equation indicates that 28, 22, and 16% crude fiber would provide sufficient energy for the previously quoted production groups, respectively.

Protein

Deficiency of protein should be the ideal situation for obtaining a response to addition of a specific amino acid or combination of amino acids if the addition is the correct one. Some researchers use this method to reduce variation of responses and increase the amount of response to detect significance. However, this in itself may limit the maximum response to suboptimal amounts.

When protein is provided in excess, most amino acids are inefficiently utilized with the possible exception of the first limiting amino acid. A response at recommended protein levels would be a response to that amino acid or a more desirable balance of amino acids. NRC (59) recommends crude protein levels of 14, 15, and 16% for three production groups. They are appropriate to use when evaluating current protein recommendations, but could limit the response to amino acid supplementation. However, these crude protein percentages are comparable to amounts used by Broderick et al. (11) and Derrig et al. (24) in amino acid studies.

Monogastric nutritionists have been challenged to produce comparable growth rates with lower protein content in rations when it is supplemented with limiting amino acids. This promotes more efficient protein utilization and lowers true protein requirements. This may be possible in ruminant nutrition, but it is complexed with uncertainty of the complement of amino acids that reach the abomasum as well as our incomplete understanding of protein requirements. Jahn (41) evaluated response of body weight gain to ration fiber and protein content. He obtained a significant interaction between protein and fiber and concluded that protein requirements change with fiber content of the ration of growing Holstein calves. Because of extreme variations in fiber intakes, protein requirements are not totally defined for lactating cows.

Sulfur

Bull and Vandersall (14) suggested responses to M-analog supplementation may be due to sulfur provided by the synthetic compound, and its effect on rumen fermentation. If sulfur is deficient providing sulfur or sulfur containing amino acids could produce a response in one of two ways. First, there may be a unique bacterial requirement for sulfur which is different from that of the animal since bacteria synthesize most of their amino acids. This could explain in part the response to increased cellulose and crude fiber digestion that has been observed.

With sulfur deficiency, sulfur amino acids could cause a response, but similar responses could occur from an available inorganic sulfur source. This could be due to microbial reply to sulfur addition or to sparing methionine in certain pathways. However, when excess sulfur is provided, then responses would be due to the sulfur amino acid addition

itself rather than the source of sulfur. Although excess of sulfur could spare some methionine or cystine, the response to inorganic sulfur should be minimal.

Recently, sulfur requirements for dairy cattle have received closer scrutiny. Protein sources are naturally high in sulfur. Inclusion of urea in ruminant rations as a nitrogen source reduces the sulfur supplied in the ration.

Jacobson et al. (38, 39) studied sulfur supplementation at approximately .09 and .12% of ration dry matter. Supplementation with sulfur had little effect on negative sulfur balance. However, it did improve dry matter intake and persistency of milk production over a nine-week period, resulting in significantly higher milk production response by the ninth week. They concluded that dietary sulfur was inadequate at both levels.

Bouchard and Conrad compared sources and levels of sulfur (8, 9, 10). Their results with sulfur balance suggested that the ration should contain around .18 to .24% sulfur. One study compared responses to graded levels of sulfur supplementation from .06 to .42% sulfur when non-protein nitrogen furnished 60% of dietary nitrogen. The results indicated that sulfur balances between .15 and .26% sulfur were comparable, but excessive sulfur accumulation occurred above .30% sulfur. Another trial reduced feed intake at .30% sulfur. Reduced feed intake occurred with .18% sulfur from M-analog at 43 g/day suggesting the cause of response with this source may differ (8). Bouchard and Conrad indicated dry matter production in milk is related to sulfur intake, but this appeared

to be due to the effect of inadequate intake on the basal diet (.06% sulfur) as compared to the two treatments (.18 and .24% sulfur). Nitrogen retention data supported the response to adequate sulfur supplementation on one trial and suggested that deficiency of sulfur may limit microbial protein synthesis. The effectiveness of different sulfur sources appeared to vary on different trials.

Bull and Vandersall (14) compared amount of sulfur on in vitro cellulose digestion and observed optimum digestibility between .16 and .24% sulfur. Comparable cellulose digestion occurred at lower sulfur for calcium sulfate than for sodium sulfate. A separate trial indicated that rate of cellulose digestion was slower for M-analog than for sodium sulfate. Belasco (4) and Salsbury (76) reported that M-analog is available only to a limited degree in the rumen environment. Bull and Vandersall (14) suggested that solubility could be a factor for M-analog. At .32% sulfur, M-analog, dl-methionine, and sodium sulfate stimulated cellulose digestibility to the same degree. They suggested sulfur may be needed to "prime" the system, but that in an in vivo system sulfur is present at all stages of digestion. The required levels may not be as high. An in vivo trial indicated that responses to sulfur supplementation from M-analog was different than supplementation from dl-methionine or sodium sulfate.

NRC (59) recommends .2% dietary sulfur in the ration dry matter, but indicates that there is inadequate proof of sulfur requirement.

EXPERIMENTAL PROCEDURE

Cows

Three rates of production were studied by evaluating four cows per group at 30, 120, and 240 days of lactation. A total of 10 Holstein-Friesian cows were utilized with two cows appearing in both the 30 and 120 day studies. The 30 day group consisted of three cows in their second lactation and one in her third lactation, while the 120 day group consisted of three cows in their second lactation and one cow in her fourth lactation. The 240 day group consisted of a second lactation cow and three cows in their first lactation, but their age at calving for the current lactation only ranged from 29 to 35 mo.

Animals were of acceptable body size resulting in minimal differences within requirements for maintenance and growth. However, two animals in the 240 day group were not at comparable stages of gestation due to breeding problems. This was reflected by milk production and created some conflict in productive function within this group. Applicable information on experimental animals is shown in Table 1.

High production was a prime consideration during selection of the experimental animals for the 30 and 120 day groups so first lactation animals were excluded. The mean milk production in the pretreatment period was 37.0, 29.2, and 18.7 kg/day for the 30, 120, and 240 day groups, respectively. Individual cow production shown in Table 1 was reasonably uniform within groups with the exception of 240 day.

Cows were housed and milked in a stanchion barn for the entire experimental period.

Table 1. Description of Cows Used in Infusions

Production Group	Cow No.	Lactation No.	Age at Calving (Mo)	Pretreatment Milk Production (Kg/day)	Calving to Conception Interval (Mo)
30	662	3	49	37.0	-[1]
	663	2	36	38.6	-
	718	2	38	34.3	-
	731	2	35	38.2	-
		AVE.	39.5	37.0	
120	577	4	62	27.1	-
	620	2	43	27.3	-
	718	2	38	31.4	-
	731	2	35	31.1	-
		AVE.	44.5	29.2	
240	650	1	29	21.2	7
	655	1	31	17.7	2
	684	2	35	14.8	2.25
	696	1	35	21.1	4.5[2]
		AVE.	32.5	18.7	

[1]Not applicable.

[2]Subsequently aborted.

Rations

Rations were formulated to meet NRC recommendations (59) as shown in Table 2. Crude fiber was set at 15, 18, and 21% of dry matter for 30, 120, and 240 day groups to insure adequate energy for response to amino acid infusions. Corn silage served as the only source of roughage in the ration. A concentrate was formulated for each group to supply the remainder of the nutrients required. This pelleted concentrate was fed separately at silage:concentrate dry matter ratios shown in Table 2. Silage was offered ad libitum, allowing approximately two kg of weighback each day.

Phosphorus was in excess of the minimum requirements due to balance of calcium and phosphorus in the premix. Actual phosphorus content was .55, .48, and .40% of ration dry matter for 30, 120, and 240 day groups. However, calcium:phosphorus ratios were greater than 1:1 on all rations. NaCl was .58% of the total ration dry matter for the 30 day group.

The cows were placed on the appropriate rations two wk prior to beginning treatments and continued until completion of experimental periods.

Experimental Design

Experimental design was a 4 x 4 balanced Latin square with an extra period included for estimation of carry-over effects (49). The fourth treatment in the sequence was replicated in a fifth period. Thus, each treatment was preceded by all other treatments including itself. Blood sampling was scheduled prior to amino acid infusion so plasma amino acid concentrations and mammary gland uptake could be compared to

Table 2. Ration Specifications and Formulations

	30 Day	120 Day	240 Day
	(kg)		
Ration Specifications			
Dry Matter	100	100	100
Crude Protein	16	15	14
Crude Fiber	15	18	21
Sulfur	.2	.2	.2
Calcium, Min.	.6	.6	.6
Phosphorus, Min.	.4	.4	.4
Magnesium	.2	.2	.2
Salt, Trace Mineralized	.5	.5	.5
Concentrate[1], 1,000 kg			
Corn, Shelled	413.5	299.1	57.7
Soybean Oil Meal	394.8	499.6	717.9
Sodium Sulfate	4.2	5.7	8.8
Ground Limestone	6.1	11.3	22.2
Premix	179.5[2]	179.5	179.5
Magnesium Oxide	1.9	3.2	6.0
Trace Mineralized Salt	0.0[3]	1.6	8.0
Dry Matter Feeding Ratios			
Corn Silage, %	49.5[4]	62.5	75.5
Concentrate, %	50.5	37.5	24.5

[1]Formulation specifications result in the following nutrient content of concentrate for the 30, 120, and 240-day groups, respectively: crude protein, 25.8, 30.0, and 38.6%; and crude fiber, 4.2, 4.6, and 5.5%. Dry matter content of the concentrate was 90%, based on the tabled estimates used (60).

[2]179.5 kg of premix consisted of the following: 10 kg iodized salt, 20 kg dicalcium phosphate, 12.5 kg dried molasses, 2 kg trace minerals, 30 kg molasses, 25 kg distiller's dried grains, 57.5 kg wheat, 20 kg pellet binder, and 2.5 kg of vitamin A and D mix containing 6×10^6 and 8×10^6 I.U., respectively.

[3]In addition to the salt provided by the premix.

[4]Corn silage was sampled at ensiling. Rations were formulated with these analysis results on a dry matter basis: dry matter, 38.0%; crude protein, 6.0%; and crude fiber, 26.0%. Subsequent analysis at feeding indicated the following results: dry matter, 34.0%; crude protein, 6.1%; and crude fiber, 22.8%.

pretreatment values.

Jugular infusions of amino acids were prepared with glutamic acid as control (C), methionine (M), methionine + lysine (ML), or methionine + lysine + phenylalanine (MLP) in balance with glutamic acid. Glutamic acid equalized the weight of amino acid infused and made the infusions nearly isonitrogenous. The amount of amino acid infused was determined by milk production of the cow during the pretreatment period and milk protein composition values derived from Crampton and Harris (21), Metabolism handbook (2), and McKenzie (52). Methionine, lysine, and phenylalanine content used as the basis is shown in Table 3. Amounts of amino acid infused were calculated at 25% of expected secretion in milk. Infusion compositions per kg of milk produced are shown in Table 3.

NaCl required to balance osmolarity to 295 mOsm was calculated from estimates of amino acid contribution to osmolarity. Calculated amounts of amino acids and NaCl for daily infusion were brought into solution in distilled water and pH was adjusted to 7.2. Daily infusions were brought to four liter, filtered, and autoclaved.

Treatments were infused via jugular vein using a Buchler[1] peristaltic action metering pump at the projected rate of four liter per day. Each infusion period started at 5:00 P.M. and continued for four days. On two occasions when pump breakdowns occurred, resumption of treatments included one day of infusion of the prior treatment.

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[1]Model #2-6100, Buchler Instruments, Fort Lee, N. J.

Table 3. Amino Acid Content of Milk and Infusion Makeup

Amino Acid Content of Milk (g/kg)	
Methionine	.93 g
Lysine	2.55 g
Phenylalanine	1.65 g

Infusion Makeup (g/kg of milk)[1]

	Treatments			
	(C)	(M)	(ML)	(MLP)
Methionine	-[2]	.2325	.2325	.2325
Lysine[3]	-	-	.7969	.7969
Phenylalanine	-	-	-	.4125
Glutamic Acid[4]	1.2825	1.0500	.4125	-

[1]Levels of amino acids infused daily were 25% of the amount expected to be secreted in the milk and were infused in four liter. NaCl was added to achieve physiological osmolarity.

[2]This amino acid was not present in this treatment.

[3]Lysine HCl (80% lysine).

[4]Glutamic acid balanced the amino acid weight infused.

Blood Sampling

Blood samples were taken approximately 3.5 h post feeding and milking, when high demand was expected to be placed on the mammary gland for milk synthesis. Blood samples were taken twice in P.M. and twice in A.M. of the last four days in intervals between sampling of 12, 24, and 36 h. Blood samples were taken from the subcutaneous abdominal mammary vein and tail artery/vein[2] in that order. The subcutaneous abdominal mammary vein was always sampled first because it was assumed that the sample could be altered significantly as result of cow activity (5). The samples were collected in heparinized tubes and hematocrits determined. Blood was centrifuged immediately and plasma collected. Plasma proteins were precipitated by combining four volumes plasma with one volume 20% w/v sulfosalicylic acid, containing .8333 μM norleucine per ml. Samples were frozen at -10 C in sealed tubes. After several days, samples were thawed, centrifuged, filtered through glass wool, and composited for each treatment period. The composited filtrates were held at -10 C for amino acid analysis on a Technicon Model TSM Autoanalyzer. Peak areas were measured by the height-width at half height method. Amino acid concentrations were calculated using norleucine as an internal standard and comparing each analysis to the nearest standards in the analysis sequence.

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[2]This site was sampled between vertebrae of the tail. The artery and vein lie adjacent to each other. As a result, the sample was arterial blood, venous blood, or a combination of both.

Calculations

Amino acid uptake by the mammary gland was calculated by the following two equations:

1) Daily serum flow (liter/day) = (1 - hematocrit) x (702 x 623.6 x daily milk).

2) Uptake (g/day) = $\frac{\text{Serum flow x (A-V difference in mg/l)}}{\text{1000 mg/g}}$

Equation 1) is adapted from values reported by Kronfeld (43) for normal cows.

Part of the theory of amino acid limitation deals with the effects that supplementation of a limiting amino acid has on other amino acids. Thus, arterial amino acid concentrations, venous amino acid concentrations, and amino acid uptake by the mammary gland were calculated as percent ratio to pretreatment for each observation. Extraction and utilization of amino acids by the mammary gland were predicted. Calculations are as follows:

3) % ratio to pretreatment = $\frac{\text{Treatment observation}}{\text{Pretreatment observation}}$ x 100

4) % extraction = $\frac{\text{Arterial a.a.}^{3} - \text{venous a.a.}}{\text{Arterial a.a.}}$ x 100.

5) % utilization = $\frac{\text{Amino acid uptake/day}}{\text{A.A.M.}^{4} \text{ x milk production}}$ x 100.

Statistical Analysis

Results were analyzed as an extra period Latin square design (49). Analysis included arterial and venous plasma amino acid con-

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[3] Amino acid concentration.

[4] Amino acid content of milk - see Appendix I.

centrations and uptake as a percent to pretreatment period. Treatment means were analyzed with three orthogonal contrasts by procedures of Snedecor (80). The first contrast compared C treatment against all others (M, ML, and MLP). The second orthogonal contrast compared M treatment against addition of lysine and phenylalanine (ML and MLP). The third contrast compared addition of phenylalanine (MLP) to ML treatment.

RESULTS AND DISCUSSION

In this manuscript differences that are quoted as significant will be at probability $p < .05$. All non-significant differences are at $p > .05$.

Effect of Period, Cow, and the Residual Effect of Treatment

The Latin square partitions out variance due to period and cow allowing a more precise measurement of treatment response. In addition the Lucas design partitions out residual treatment carry-over effects (49). These three factors will be discussed briefly at this point relative to the experiment.

Significant cow differences (columns) were observed on feed intake at all lactational stages. This was expected since body weight and milk production have been shown to influence feed intake (20). Significant differences between cows for plasma amino acid concentrations and amino acid uptake by the mammary gland were numerable. Cow differences again were expected when these variables were considered in relation to feed intake, productive requirement, and resulting amino acid flux through the plasma pool (32). However, amino acid utilization by the mammary gland differed from other responses in that the cow factor was detected as significant in a limited number of parameters.

Period effects (rows) for all amino acids were found to be significant infrequently. However, in the 30 day lactational group significant period differences existed for feed intake. This was due in part to allowing ad libitum intake throughout periods.

Residual treatment carry-over effects existed for concentrate dry matter and crude protein intake in the 30 day group. Carry-over effects were significant for arterial amino acid concentrations in the 240 day group. This could be explained by excess dietary protein compared to nutritional requirements (59) which will be discussed in the section on direct effects of treatment. Our results support conclusions of Keith et al. (42) who reported that plasma amino acid carry-over effects were non-significant for growing swine.

The Lucas (49) procedure allowed computation of least square means independent of effects due to cows (columns), periods (rows), and residual treatment carry-over effects. Thus, least square means for direct effects of treatments are presented hereafter.

Direct Effects of Treatment

Feed Intake

Feed intake data is shown in Table 4. In the 30 day lactation group, cows on M infusion had significantly increased silage and decreased concentrate intakes. However, as a result of this shift concentrate dry matter intake was significantly higher on C treatment than for others.

Total dry matter intake was similar across the four treatments. Crude protein intake in this group was significantly higher for C treatment in comparison to the other three and reflected differences in concentrate dry matter intake.

In the 120 day group treatment differences in feed intake were non-significant. However, in the 240 day group there were significant

Table 4. Feed Intake (Kg/Day)

	Pre[2]	Treatments[1] C	M	ML	MLP	C.V.
30 Day						
Silage D.M.	8.62	8.50	9.24^{B}[3]	8.30^{b}	8.63^{b}	6.23
Conc. D.M.	10.46	10.62^{A}	9.95^{aB}	10.33^{ab}	10.38^{ab}	2.21
Total D.M.	19.08	19.12	19.19	18.63	19.01	2.91
Crude Protein	3.22	3.26^{A}	3.13^{a}	3.17^{a}	3.20^{a}	1.97
Crude Fiber	2.41	2.38	2.52	2.33	2.40	5.06
120 Day						
Silage D.M.	10.28	9.94	9.67	9.65	9.80	11.89
Conc. D.M.	7.51	7.48	7.51	7.57	7.71	2.04
Total D.M.	17.79	17.42	17.18	17.22	17.50	7.50
Crude Protein	2.88	2.85	2.84	2.86	2.91	3.98
Crude Fiber	2.69	2.61	2.55	2.55	2.59	10.51
240 Day						
Silage D.M.	12.85	10.70	11.34	10.56	11.13	4.28
Conc. D.M.	5.78	5.25	5.31^{B}	4.88^{bC}	5.29^{bc}	2.31
Total D.M.	18.63	15.95	16.64^{B}	15.44^{bC}	16.42^{bc}	3.24
Crude Protein	3.02	2.68	2.74^{B}	2.53^{bC}	2.72^{bc}	2.33
Crude Fiber	3.25	2.73	2.88	2.68	2.83	3.93

[1] See Table 3.

[2] Two week pretreatment period.

[3] Significance of orthogonal contrasts is denoted at $p < .05$ by upper and lower case letters as follows: Control (A) vs. all others (a); M (B) vs. ML and MLP (b); and ML (C) vs. MLP (c).

differences for concentrate dry matter, total dry matter, and crude protein intakes for M vs. ML and MLP and for ML vs. MLP. This is due primarily to lower concentrate intake on ML treatment.

The crude protein of dry matter was 16.8, 16.5, and 16.6% at 30, 120, and 240 days of lactation. Thus, crude protein intake was above formulated levels, especially for 120 and 240 day groups. Concentrates were formulated with 25.8, 30.0, and 38.6% crude protein to be fed at the dry matter ratios shown in Table 2. Regulation of concentrate:silage dry matter feeding was not adequate under ad libitum feeding conditions. Dry matter supplied by forage was 45.7, 56.4, and 67.8% for 30, 120, and 240 day groups. The crude protein in the concentrate resulted in over-feeding of protein (59). Crude protein intake in g/day was above requirements in 120 and 240 day groups, but it was within acceptable limits for the 30 day group.

Crude fiber intake was at 12.7, 14.9, and 17.3% of dry matter for the same three lactational groups. This was lower than planned for the ration due to changes in dry matter intake ratios of silage and concentrate and to the difference in crude fiber analysis of corn silage at ensiling vs. feeding (Table 2). Calculation of Net Energy lactation in ration dry matter with the crude fiber regression of Chandler and Walker (20) and Net Energy lactation requirement from NRC (59) specifications revealed that energy intakes were more than adequate. Energy intakes were 37.1, 32.6, and 29.7 Mcal compared to 36.8, 29.8, and 22.3 Mcal calculated requirement. The ratio of energy to protein was significant to prevent an energy limitation.

Milk Production and Infusion Rate

Milk production differences between treatments were non-significant in all groups (Table 5). Broderick et al. (11, 12) obtained an immediate response in milk protein content, but their milk production response reached its maximum around 16% crude protein. Thus, lack of a milk production response could be expected when protein intake is considered.

Differences between infusion rates were non-significant (Table 5). An infusion of 1.00 indicated that the entire four liters were infused and hence 25% of the amino acids in the pretreatment milk production (Table 3). Infusions were maintained just below 1.00 to maintain continuous infusion. Uniformity of infusion rate supports equal infusion across treatments.

Plasma Amino Acid Responses

Since different cows were utilized to form squares at three lactational periods, a combined analysis was not possible. Responses at different lactational stages should not be identical due to potential differences in productive function. Thus, plasma amino acid responses are presented for each lactation stage.

a. Early lactation (30 days)

Arterial plasma amino acids suggested a lowering trend on ML treatment (Table 6). Total essential amino acids, arginine, phenylalanine and tyrosine were significantly lower on ML than for MLP, while leucine and asparagine were significantly lower on ML and MLP than on M treatment. Methionine was significantly lower while alanine and serine were significantly higher on C than for M, ML, and MLP. Both methionine and phenylalanine increased in plasma as a result of their inclusion in

Table 5. Milk Production, Hematocrit Readings, Mammary Plasma Flow and Infusion Rate Means

	Pre[2]	Treatment[1] C	M	ML	MLP	C.V.
30 Day						
Milk (Kg/Day)	37.02	37.56	39.61	38.88	38.78	5.15
Hematocrit	31.15	29.44[A3]	31.23[a]	30.31[a]	30.64[a]	2.90
Plasma Flow[4] (1000 L/Day)	16.44	17.09	17.52	17.45	17.32	3.85
Infusion Rate[5]	-	1.00	.99	.99	.97	6.69
120 Day						
Milk (Kg/Day)	29.22	27.28	27.51	28.72	28.90	7.30
Hematocrit	32.46	33.20	33.07	32.98	33.09	3.67
Plasma Flow (1000 L/Day)	12.85	11.90	12.03	12.55	12.59	6.50
Infusion Rate	-	.99	.97	.98	.98	2.07
240 Day						
Milk (Kg/Day)	18.70	16.66	16.88	17.39	17.34	4.55
Hematocrit	32.42	31.34	31.52	31.07	30.86	3.76
Plasma Flow (1000 L/Day)	8.43	7.73	7.78	8.04	8.04	3.90
Infusion Rate	-	.94	.95	.95	.94	6.54

[1]See Table 3.

[2]See Footnote 2, Table 4.

[3]See Footnote 3, Table 4.

[4]Mammary plasma flow is tabled as 1000 L/day units. Calculation of liters/day was as follows:

$$\text{Plasma Flow} = \left(1-\frac{\text{Hematocrit}}{100}\right)(792 + 623.6 \times \text{pre daily milk production}).$$

[5]Fraction of the planned daily infusion (see Table 3).

Table 6. 30 Day Arterial Plasma Amino Acids[1] (Mg/liter)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
TAA[4]	294.36	293.70	298.61	273.46	294.57	5.37
TEAA	127.98	131.82	139.59	120.71[C][5]	139.27[C]	6.92
TNEA	166.38	161.88	159.02	152.76	155.30	4.94
EAA						
Arg	11.40	12.12	13.53	10.16[C]	13.77[C]	17.29
His	8.92	9.63	9.24	8.06	9.23	9.55
Ile	29.66	30.31	32.99	27.25	30.86	11.36
Leu	19.23	20.93	22.07[B]	18.90[b]	19.97[b]	5.79
Lys	10.10	10.53	10.81	11.68	13.45	14.92
Met	3.76	3.16[A]	4.03[a]	3.93[a]	4.08[a]	9.40
Phe	6.61	6.92	7.02	6.24[C]	8.88[C]	12.86
Thr	12.43	9.38	10.43	8.62	10.60	14.14
Val	25.86	28.84	29.46	25.86	28.43	7.87
NEA						
Ala	19.86	22.01[A]	18.42[a]	18.71[a]	17.09[a]	10.14
Asn	8.53	7.74	7.89[B]	7.22[b]	7.66[b]	3.73
Asp	1.66	1.69	1.71	1.57	1.69	13.45
Cit	13.64	14.11	14.43	13.04	13.78	6.89
CyS	4.78	4.34	4.90	4.62	4.90	10.23
Glu	7.45	8.86	8.66	7.98	7.47	10.44
Gln	30.49	27.46	24.75	26.47	25.44	9.11
Gly	30.57	25.01	25.45	26.07	27.94	13.17
Orn	6.32	7.25	6.79	6.39	7.09	11.71
Pro	10.55	11.31	11.13	9.91	9.40	14.54
Ser	11.58	10.74[A]	9.28[a]	9.10[a]	9.65[a]	6.43
Tau	1.24	1.38	1.79	1.76	2.10	26.65
Tyr	8.44	8.18	7.49	6.62[C]	8.78[C]	8.56

[1]Sampled at the tail arterial and/or venous site.

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]See Footnote 3, Table 4.

the infusion mixture. Although there was a trend for lysine to increase with infusion, the highest value, observed for the MLP treatment, was non-significant. All amino acids, with exception of methionine, that were significantly altered were lower for ML treatment.

Most of the significance with venous plasma amino acids was due to higher concentrations on controls (Table 7). Total amino acids, non-essential amino acids, histidine, leucine, alanine, asparagine, glutamine, ornithine, serine, and tyrosine were significantly higher on C. Lysine and phenylalanine were significantly increased with their inclusion in the infusion mixture. Tyrosine responded significantly with phenylalanine as well. However, venous methionine concentration did not change with its infusion.

The analysis of ratios to pretreatment added little to the interpretation of arterial and venous amino acid concentrations for any of the lactation groups. Thus, none of these ratios to pretreatment are presented. A more meaningful interpretation can be obtained by simultaneous evaluation of arterial and venous concentrations as related to production which will be described later.

b. Mid lactation (120 days)

Arterial amino acid concentrations for this group indicated only limited numbers of significant parameters among treatments (Table 8). The three treatment amino acids infused increased significantly with infusion. Lysine was significantly higher for M, ML, and MLP than with C due primarily to the increased plasma lysine for ML and MLP. Proline, the only other amino acid with significance, was lower on C than for M, ML, and MLP.

Table 7. 30 Day Venous Plasma Amino Acids[1] (Mg/liter)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
TAA[4]	221.26	233.14[A5]	213.40[a]	189.34[a]	210.68[a]	9.69
TEAA	81.57	96.67	88.23	72.05	87.41	13.65
TNEA	139.69	136.46[A]	125.17[a]	117.29[a]	123.28[a]	8.36
EAA						
Arg	6.03	6.46	5.77	4.96	6.44	19.71
His	7.78	9.15[A]	6.64[a]	6.14[a]	6.71[a]	13.35
Ile	18.53	22.90	23.24	16.86	20.47	20.59
Leu	10.96	14.96[A]	13.46[a]	10.01[a]	11.75[a]	18.47
Lys	3.67	4.93	3.31[B]	4.17[b]	5.11[b]	14.84
Met	1.37	1.47	1.93	1.50	1.75	32.20
Phe	3.58	5.02	3.60	3.03[C]	5.59[c]	25.02
Thr	8.89	6.94	6.56	5.19	7.16	24.29
Val	20.75	24.85	23.71	20.18	22.43	12.50
NEA						
Ala	17.62	22.26[A]	17.76[a]	15.42[a]	14.90[a]	20.23
Asn	6.04	6.35[A]	5.15[a]	3.87[a]	5.24[a]	18.77
Asp	1.23	1.16	1.10	1.52	1.07	42.20
Cit	13.26	13.27	13.43	12.58	13.06	12.08
CyS	4.24	4.14	4.73	4.19	4.66	8.59
Glu	3.22	3.36	2.69	3.07	2.70	17.31
Gln	20.85	21.58[A]	16.86[a]	18.46[a]	17.11[a]	11.05
Gly	30.30	25.18	24.42	25.23	27.04	10.63
Orn	4.30	4.47	4.25	3.92	4.32	14.60
Pro	9.29	10.14[A]	8.49[a]	7.57[a]	8.22[a]	11.04
Ser	9.61	9.28[A]	6.36[a]	6.78[a]	7.20[a]	11.22
Tau	1.15	1.37	1.66	1.75	1.56	25.74
Tyr	4.77	6.05[A]	3.70[a]	2.34[aC]	5.11[ac]	32.34

[1]Sampled at the subcutaneous abdominal mammary vein.
[2]See Table 3.
[3]See Footnote 2, Table 4.
[4]See Appendix I.
[5]See Footnote 3, Table 4.

Table 8. 120 Day Arterial Plasma Amino Acids[1] (Mg/liter)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
TAA[4]	313.07	305.89	305.84	310.98	317.91	7.58
TEAA	138.06	138.45	138.29	143.78	146.57	10.30
TNEA	175.01	167.44	167.54	167.20	171.34	6.34
EAA						
Arg	14.84	13.75	14.47	16.37	17.24	13.45
His	9.49	9.95	11.01	9.63	9.97	10.70
Ile	31.97	32.20	29.79	31.60	31.55	13.83
Leu	20.43	21.35	20.11	20.91	20.31	9.83
Lys	11.92	11.48^{A}[5]	12.77^{aB}	14.96^{ab}	16.21^{ab}	11.78
Met	3.19	2.87^{A}	3.89^{a}	4.22^{a}	4.10^{a}	16.39
Phe	7.32	7.13	7.22	6.51^{C}	7.97^{C}	7.71
Thr	10.84	9.92	10.04	10.22	10.62	13.64
Val	28.07	29.81	29.00	29.37	28.61	10.42
NEA						
Ala	18.76	17.87	18.68	18.16	17.28	11.79
Asn	8.33	7.71	7.75	7.80	8.05	12.88
Asp	1.88	1.62	1.67	1.64	1.63	12.72
Cit	18.43	18.59	18.11	18.98	19.00	12.72
CyS	4.82	4.75	5.31	5.18	5.37	9.46
Glu	7.32	8.16	8.39	7.51	7.56	11.61
Gln	32.68	32.50	33.17	33.50	33.21	13.93
Gly	22.44	21.43	19.62	20.52	21.24	10.29
Orn	7.40	7.97	8.54	8.81	9.25	13.25
Pro	10.59	9.86^{A}	10.50^{a}	10.91^{a}	10.73^{a}	3.12
Ser	9.52	8.83	8.71	8.35	8.29	3.80
Tau	1.45	1.61	1.68	1.72	2.04	14.30
Tyr	9.12	7.95	7.85	7.52	8.35	19.25

[1]See Footnote 1, Table 6.

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]See Footnote 3, Table 4.

When arterial plasma amino acid ratio to pretreatment was calculated, serine was significantly lower on M, ML, and MLP than for C and on ML and MLP than for M. This was due primarily to the inclusion of lysine in infusions.

In venous blood at 120 days, methionine and lysine increased significantly with their infusion (Table 9). Phenylalanine addition did not affect plasma phenylalanine or tyrosine. Cystine was significantly increased with infusion of methionine. Aspartic acid and glycine were significantly lower on M than ML and MLP. Taurine increased with infusion of lysine. With exception of serine, ratios to pretreatment did not appear to add to interpretation on either arterial or venous blood.

c. Late Lactation (240 Days)

Most significances in arterial plasma were due to lowered levels on ML treatment (Table 10). Total amino acids, essential amino acids, non-essential amino acids, histidine, isoleucine, leucine, valine, asparagine, glycine, ornithine, and serine were lower on ML than MLP. Lysine, methionine, and threonine were higher on MLP with the same contrast. Leucine and serine were significantly lower on M, ML, and MLP due primarily to the low arterial concentration on ML treatment. Taurine was significantly lower on M, ML, and MLP as well. Lysine and phenylalanine were significantly increased in plasma with infusion of lysine (ML and MLP vs. M).

Analysis of arterial ratios to pretreatment agreed with interpretations on actual concentrations. Isoleucine was significantly lower

Table 9. 120 Day Venous Plasma Amino Acids[1] (Mg/liter)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
TAA[4]	236.19	248.69	217.99	234.73	236.39	10.84
TEAA	91.26	95.95	85.56	94.57	94.54	15.78
TNEA	144.93	152.74	132.42	140.16	141.85	10.03
EAA						
Arg	8.09	7.76	7.70	10.67	11.70	30.48
His	7.75	8.25	8.72	7.98	8.07	16.90
Ile	21.77	22.93	18.84	21.02	20.49	17.06
Leu	13.09	14.07	11.16	12.12	11.33	19.84
Lys	4.80	5.29	5.44[B][5]	7.01[b]	7.41[b]	18.30
Met	.93	.94[A]	1.46[a]	2.05[a]	1.67[a]	29.49
Phe	4.07	4.59	4.35	3.80	4.41	17.16
Thr	7.88	7.39	5.73	6.88	6.86	21.57
Val	22.86	24.73	22.16	23.03	22.61	12.76
NEA						
Ala	16.81	16.63	17.08	16.82	16.02	18.65
Asn	6.47	5.89	5.29	5.89	5.71	16.11
Asp	1.15	1.20	1.02[B]	1.28[b]	1.27[b]	13.31
Cit	17.39	18.58	17.11	18.78	18.06	17.67
CyS	4.55	4.54[A]	4.97[a]	5.23[a]	4.95[a]	4.02
Glu	2.99	3.19	3.34	3.80	3.31	11.93
Gln	27.30	35.69	20.34	24.29	26.56	52.13
Gly	20.92	20.65	17.95[B]	20.13[b]	20.96[b]	4.48
Orn	4.09	5.24	4.44	5.95	6.02	25.67
Pro	8.49	8.77	8.90	9.42	9.31	15.89
Ser	6.79	7.38	6.45	6.33	5.86	17.74
Tau	1.24	1.51	1.52[B]	1.92[b]	1.83[b]	13.04
Tyr	5.04	5.35	4.08	3.66	4.69	34.01

[1]See Footnote 1, Table 7.

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]See Footnote 3, Table 4.

Table 10. 240 Day Arterial Plasma Amino Acids[1] (Mg/liter)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
TAA[4]	306.07	281.25	265.87	246.78^{C}[5]	291.97^{C}	4.42
TEAA	141.60	130.86	119.14	110.76^{C}	141.18^{C}	6.17
TNEA	164.48	150.39	146.73	136.02^{C}	150.79^{C}	5.98
EAA						
Arg	14.25	13.90	12.21	12.58	14.81	12.20
His	9.80	8.56	7.67	7.10^{C}	9.30^{C}	5.57
Ile	32.38	31.07	27.36	24.92^{C}	31.85^{C}	8.76
Leu	20.22	18.99^{A}	17.12^{a}	15.54^{aC}	18.81^{ac}	7.80
Lys	12.77	11.08	10.77^{B}	11.38^{bC}	14.54^{bc}	12.08
Met	3.74	3.24	3.47	3.01^{C}	4.07^{C}	13.78
Phe	6.63	6.85	6.46^{B}	7.21^{b}	7.63^{b}	5.47
Thr	11.67	9.43	8.71	7.34^{C}	10.55^{C}	10.19
Val	30.12	27.72	25.36	21.68^{C}	29.61^{C}	7.03
NEA						
Ala	19.57	17.75	17.94	16.77	17.47	5.84
Asn	8.08	7.46	6.50	5.56^{C}	7.50^{C}	17.66
Asp	1.81	1.39	1.51	1.30	1.53	19.43
Cit	13.13	12.76	10.63	12.13	12.45	11.41
CyS	4.75	4.83	4.98	4.67	5.07	9.65
Glu	10.11	9.06	10.48	9.68	9.82	10.60
Gln	31.47	28.08	30.29	26.37	28.34	13.68
Gly	19.44	16.36	15.53	14.20^{C}	17.05^{C}	6.58
Orn	8.02	7.13	7.30	6.57^{C}	8.60^{C}	13.91
Pro	10.08	9.21	9.45	7.76	9.51	13.97
Ser	9.14	8.49^{A}	7.55^{a}	6.53^{aC}	8.54^{ac}	9.04
Tau	2.72	1.68^{A}	1.39^{a}	1.35^{a}	1.35^{a}	16.84
Tyr	9.23	8.28	7.95	7.24	8.79	12.95

[1]See Footnote 1, Table 6.

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]See Footnote 3, Table 4.

on M, ML, and MLP than C while lysine was only significant in the comparison of ML vs. MLP. Tyrosine was significant when analyzed as a ratio to pretreatment in that a lowered concentration on ML was observed as compared to MLP. Both analysis of the arterial plasma amino acid concentrations and ratios to pretreatment suggested that arterial plasma amino acid concentrations decreased on the ML treatment at 240 days. Concentrations were also lower on the M treatment although this comparison was non-significant. The venous plasma amino acid concentration paralleled the arterial pattern (Table 11). Total amino acids, essential amino acids, arginine, isoleucine, leucine, threonine, valine, and glycine were significantly lower on ML than MLP. Aspartic acid was significantly lower on M than ML and MLP while for glutamine the opposite pattern was significant. Glycine was significantly lower on all treatments compared to C due to reduced concentrations on ML and MLP.

Analysis of venous ratios at 240 days of lactation showed significantly lower leucine and valine ratios on M, ML, and MLP than C. This was due to low ratios on M and ML. The ratios added little to the interpretation.

Amino Acid Utilization by the Mammary Gland

The high producing dairy cow is unique when compared to other ruminants. A large proportion of nutrient intake is directed toward milk production. Venous blood can be easily sampled via the subcutaneous abdominal mammary vein, and amino acid extraction is sizeable (56). Thus, uptake of amino acids by the mammary gland was of prime interest in this study.

Table 11. 240 Day Venous Plasma Amino Acids[1] (Mg/liter)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
TAA[4]	234.82	214.24	204.24	185.67[C][5]	214.70[c]	5.72
TEAA	94.51	87.91	79.82	71.38[C]	93.88[c]	9.95
TNEA	140.30	126.33	124.42	114.30	120.82	5.65
EAA						
Arg	9.16	8.03	6.94	6.29[C]	9.33[c]	17.77
His	8.39	7.30	6.36	5.49	7.00	16.93
Ile	21.10	21.38	18.60	17.08[C]	22.25[c]	12.09
Leu	11.41	11.44	9.95	8.65[C]	11.10[c]	11.61
Lys	6.00	4.98	5.08	5.35	7.22	28.09
Met	1.32	.98	1.70	1.18	1.66	41.57
Phe	4.14	4.49	4.11	4.26	4.70	12.56
Thr	8.87	6.37	6.22	4.91[C]	6.80[c]	11.10
Val	24.10	22.92	20.84	18.17[C]	23.81[c]	7.61
NEA						
Ala	19.08	18.65	17.87	17.24	16.05	9.35
Asn	5.64	5.12	4.91	4.48	5.05	10.98
Asp	1.28	1.16	.91[B]	1.16[b]	1.17[b]	14.55
Cit	12.37	11.96	11.35	11.07	11.30	9.80
CyS	4.35	4.57	4.89	4.36	4.63	11.70
Glu	4.49	4.07	4.01	4.14	4.58	13.39
Gln	25.50	20.49	25.16[B]	19.41[b]	20.68[b]	11.34
Gly	19.26	16.56[A]	15.00[a]	14.12[aC]	16.25[ac]	4.82
Orn	5.50	4.79	5.13	5.19	5.94	16.42
Pro	8.48	8.84	8.00	6.85	7.24	16.85
Ser	8.21	7.98[A]	6.40[a]	6.45[a]	6.59[a]	7.29
Tau	1.67	1.66	1.79	1.81	1.60	35.53
Tyr	5.76	5.31	4.75	4.21	5.46	20.88

[1]See Footnote 1, Table 7.

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]See Footnote 3, Table 4.

a. Quantitative uptake by the mammary gland

Amino acid uptake by the mammary gland required an estimate of blood flow. This was calculated from data reported by Kronfeld (43) as previously described (Table 2). Estimates of plasma flow among treatments were non-significant, although hematocrit readings were significantly lower on C than for other treatments (Table 5).

In the early lactation group the primary response in amino acid uptake occurred with inclusion of methionine in the infusion (Table 12). Total amino acids, essential amino acids, non-essential amino acids, histidine, lysine, valine, asparagine, and serine uptakes were significantly higher with M, ML, and MLP than C. Isoleucine, leucine, phenylalanine, threonine, glutamine, and tyrosine uptakes were lower on C than M, ML, and MLP. Arginine uptake was significantly lower on ML than MLP, while glutamic acid was significantly higher on M than ML and MLP. Significance occurred even though the coefficient of variation increased considerably over that of arterial and venous concentrations. Uptake ratios to pretreatment (Table 13) also supported the above conclusion but indicated that uptake was lower on control than pretreatment period. Although this could be due to glutamic acid infused, glutamic acid in the infusion was reduced by only 20% with addition of methionine. Disruption of the cow's routine due to infusion and frequent attention could have caused altered responses during infusion periods. However, since crude protein intake was lower on M treatment than on C, this response becomes even more significant.

Only a limited number of amino acid uptakes were significant in the 120 day lactation group (Table 14). Total amino acids were

Table 12. Amino Acid Uptake by the Mammary Gland - 30 Day[1] (G/Day)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
30 Day						
TAA[4]	1190	1029^{A}[5]	1494^{a}	1470^{a}	1456^{a}	17.15
TEAA	757	595^{A}	905^{a}	852^{a}	900^{a}	18.79
TNEA	433	434^{A}	589^{a}	618^{a}	556^{a}	18.10
EEA						
Arg	87.71	96.42	134.67	90.95^{C}	126.33^{c}	17.72
His	18.19	7.47^{A}	45.04^{a}	33.64^{a}	43.98^{a}	29.23
Ile	181.37	125.73^{A}	173.42^{a}	182.15^{a}	180.44^{a}	24.66
Leu	135.13	101.23^{A}	152.74^{a}	155.75^{a}	142.72^{a}	23.12
Lys	105.02	95.02^{A}	130.94^{a}	131.65^{a}	144.20^{a}	22.06
Met	38.91	28.71	36.60	42.29	40.65	32.00
Phe	49.73	32.08^{A}	60.30^{a}	56.20^{a}	57.21^{a}	27.25
Thr	57.63	41.22^{A}	68.61^{a}	59.91^{a}	59.89^{a}	14.51
Val	83.32	67.43^{A}	102.39^{a}	99.47^{a}	104.37^{a}	19.22
NEA						
Asn	40.57	23.22^{A}	48.48^{a}	57.68^{a}	42.23^{a}	31.85
Gln	155.95	99.89^{A}	137.11^{a}	139.04^{a}	144.08^{a}	16.11
Glu	69.80	93.92	105.55^{B}	85.94^{b}	82.56^{b}	16.85
Ser	31.91	24.67^{A}	51.85^{a}	40.51^{a}	42.57^{a}	28.24
Tyr	60.00	35.82^{A}	66.55^{a}	74.54^{a}	64.17^{a}	27.24

[1]Amino acid uptake was calculated as follows:

$$\text{uptake (g/day)} = \frac{(\text{Arterial conc.} - \text{venous conc. in mg}) \times \text{plasma flow}^{6}}{1000 \text{ mg/g}}$$

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]See Footnote 3, Table 4.

[6]For plasma flow, see Footnote 4, Table 5.

Table 13. Ratio of Amino Acid Uptake to the Pretreatment Period - 30 Day[1]

	Treatments[2]				
	C	M	ML	MLP	C.V.
30 Day					
TAA[3]	95.1^{A}[4]	130.5^{a}	131.1^{a}	128.4^{a}	13.98
TEAA	80.3^{A}	120.8^{a}	114.4^{a}	119.7^{a}	17.28
TNEA	150.9^{A}	177.5^{a}	194.5^{a}	170.9^{a}	12.45
EAA					
Ile	74.6^{A}	97.6^{a}	104.6^{a}	105.8^{a}	20.04
Leu	77.5^{A}	112.3^{a}	116.5^{a}	106.8^{a}	20.55
Lys	90.3^{A}	128.2^{a}	126.1^{a}	137.9^{a}	21.49
Met	82.4	99.6	116.3	106.8	28.81
Phe	65.9^{A}	122.3^{a}	116.1^{a}	116.0^{a}	26.60
Thr	78.0^{A}	122.7^{a}	110.4^{a}	112.2^{a}	14.04
Val	83.6^{A}	125.0^{a}	123.9^{a}	131.0^{a}	18.38
NEA					
Asn	63.9^{A}	130.8^{a}	184.3^{a}	117.4^{a}	48.98
Gln	65.7^{A}	96.8^{a}	103.5^{a}	103.6^{a}	18.15
Glu	141.0	153.4^{B}	129.1^{b}	124.2^{b}	14.15
Ser	119.8^{A}	184.2^{a}	167.1^{a}	178.0^{a}	18.22
Tyr	62.7^{A}	111.8^{a}	127.7^{a}	107.8^{a}	24.65

[1]Ratio of amino acid uptake = $\frac{\text{uptake of observation}}{\text{uptake of pretreatment}} \times 100$
See Footnote 1, Table 12 for uptake calculation.

[2]See Table 3.

[3]See Appendix I.

[4]See Footnote 3, Table 4.

Table 14. Amino Acid Uptake by the Mammary Gland - 120 & 240 Day[1] (G/Day)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
120 Day						
TAA[4]	978	678[A5]	1068[a]	926[a]	1018[a]	19.64
TEAA	597	499	637	601	651	23.60
TNEA	382	180[A]	431[a]	325[a]	367[a]	23.25
EAA						
Ile	129.52	108.18	131.59	128.61	138.02	23.95
Leu	93.67	84.37[A]	107.11[a]	107.45[a]	112.03[a]	17.39
Lys	91.10	72.70	88.26	97.09	110.12	22.51
Met	28.99	23.19	29.54	26.91	30.63	23.70
Phe	41.60	30.68[A]	34.53[a]	33.57[aC]	44.74[ac]	14.22
240 Day						
TEAA	390	329	306	326	381	22.74
TNEA	201	190	176	176	238	32.16
EAA						
Lys	56.00	46.68	44.25	48.36	59.03	20.90
Met	20.70	17.73	13.84	15.15	19.62	19.89
Phe	21.07	18.51	18.77	23.87	23.33	26.33
Thr	23.56	22.61	19.10	20.75	30.82	29.20
Val	48.56	36.60	34.89	29.49[C]	46.18[C]	22.56
NEA						
Asp	4.40	1.56	4.54[B]	1.25[b]	2.83[b]	66.75
Ser	7.08	3.33	8.29	1.44[C]	15.26[C]	101.93

[1]See Footnote 1, Table 12.
[2]See Table 3.
[3]See Footnote 2, Table 4.
[4]See Appendix I.
[5]See Footnote 3, Table 4.

significantly lower on C than M, ML, and MLP. The essential and non-essential amino acids followed this trend as well. Leucine was significantly higher on M, ML, and MLP than on C. Phenylalanine was significantly lower on C than M, ML, and MLP, but this was largely due to the significant increase of MLP over ML with infusion. The pattern observed is similar to the uptake pattern at 30 days. Some of the comparable ratios are shown in Table 15.

At the 240 day lactational stage, amino acid uptake was reduced somewhat compared to pretreatment by the C, M, and ML treatments (Tables 14 and 15). This may be due to the decline in milk production with advancing stages of lactation. The pretreatment period comparison is the least comparable in this production group. Amino acid uptake tended to increase with the MLP treatment (Table 14). However, this uptake trend was significant only for valine and serine due to a higher variability. Aspartic acid uptake was significantly higher on M than for ML and MLP. Methionine and threonine uptake were significantly higher on MLP than for ML when the 240 day uptake ratios were analyzed. However, the serine uptake lost its significance when expressed as a ratio.

Increased variability at 240 days of lactation is probably due to protein intake in relation to productive requirements. A sampling of amino acid uptake ratios are presented (Tables 13 and 15) primarily to emphasize the relative response that occurred. They were calculated as a percent of each observation to the pretreatment period and then analyzed by the Lucas procedure.

Table 15. Ratio of Amino Acid Uptake to Pretreatment Period - 120 & 240 Day[1]

	Treatments[2]				
	C	M	ML	MLP	C.V.
120 Day					
TAA[3]	71.0^{A}[4]	109.9^{a}	94.9^{a}	106.4^{a}	19.71
TEAA	85.2	105.7	101.2	111.0	24.52
TNEA	51.2^{A}	117.0^{a}	85.6^{a}	99.8^{a}	21.42
EAA					
Leu	90.6	113.0	114.6	120.3	18.08
Lys	81.0	94.6	107.7	122.6	23.46
Met	80.9	99.3	93.1	106.2	24.66
Phe	72.9^{A}	82.6^{a}	80.9^{aC}	106.8^{ac}	13.95
Thr	78.8	146.2	108.6	131.7	43.32
NEA					
Tyr	58.9^{A}	86.7^{a}	91.7^{a}	88.4^{a}	27.92
240 Day					
TAA	87.9	82.6	82.5	104.5	22.86
EAA					
Lys	89.2	85.4	90.8	112.6	20.52
Met	86.0	69.6	73.3^{C}	95.7^{c}	15.49
Phe	88.8	88.2	118.8	115.3	27.57
Thr	111.9	94.8	86.5^{C}	140.8^{c}	28.49
Val	84.4	80.8	63.2^{C}	107.5^{c}	25.99
NEA					
Asp	55.3	131.5^{B}	28.7^{b}	82.4^{b}	73.88
Glu	87.8	116.0	98.0	91.5	18.34

[1]See Footnote 1, Table 13.

[2]See Table 3.

[3]See Appendix I.

[4]See Footnote 3, Table 4.

b. Extraction by the mammary gland

The extraction by the mammary gland was calculated by the method of Derrig et al. (24) as previously described. Extraction percentages and the calculation formula are shown in Table 16. The values are comparable to results reported (24). Again the response occurred primarily with addition of methionine in the infusion at 30 days. Percent extraction of arginine, histidine, threonine, total amino acids, and non-essential amino acids were significantly increased by M, ML, and MLP over C. Leucine, lysine, phenylalanine, valine, and essential amino acids had significant treatment differences due to lower extraction on C than M, ML, and MLP. Arginine extraction was greater on M than ML and MLP while phenylalanine extraction decreased with its infusion in the 30 day group. Although the non-essential amino acids are not presented, asparagine, glutamine, serine, and tyrosine were increased significantly by M, ML, and MLP as well. Tyrosine extraction decreased significantly with the infusion of phenylalanine as well. Extraction of methionine did not decrease with its infusion, while lysine extraction decreased non-significantly with lysine infusion.

Only a limited number of amino acid extractions were significant at 120 days (Table 17). Presence of methionine in the infusion mixture was the primary factor causing significance. Leucine extraction was significantly increased with M, ML, and MLP over C. Total amino acids and non-essential amino acid extraction was significantly lower on C than M, ML, and MLP. These results are related to higher plasma amino acid concentrations and lowered uptake noted previously on control treatment.

Table 16. Essential Amino Acid Extraction by the Mammary Gland[1] - Early Lactation (30 Days)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
Arg[4]	48.0 (3)[5]	46.9^{A}[6] (3)	58.9^{aB} (2)	48.5^{ab} (4)	53.9^{ab} (3)	9.04
His	11.6 (9)	4.5^{A} (9)	28.2^{a} (8)	24.9^{a} (8)	28.4^{a} (8)	32.51
Ile	37.3 (6)	25.4 (7)	30.0 (7)	39.3 (7)	34.7 (6)	24.30
Leu	43.2 (5)	30.5^{A} (4)	39.3^{a} (5)	47.6^{a} (5)	42.3^{a} (4)	18.50
Lys	63.8 (1)	53.8^{A} (1)	70.3^{a} (1)	63.9^{a} (1)	63.0^{a} (1)	8.86
Met	62.8 (2)	53.0 (2)	53.3 (3)	61.5 (2)	57.5 (2)	26.37
Phe	45.6 (4)	28.5^{A} (5)	48.8^{a} (4)	51.5^{aC} (3)	37.1^{ac} (5)	21.80
Thr	28.1 (7)	26.8^{A} (6)	37.1^{a} (6)	40.5^{a} (6)	34.2^{a} (7)	20.89
Val	19.8 (8)	14.0^{A} (8)	19.5^{a} (9)	22.1^{a} (9)	21.8^{a} (9)	21.09
TAA	24.5	21.1^{A}	28.7^{a}	30.9^{a}	28.8^{a}	15.92
TEAA	36.2	27.4^{A}	37.0^{a}	40.5^{a}	38.0^{a}	16.35
TNEA	15.4	16.1^{A}	21.5^{a}	23.4^{a}	20.7^{a}	18.39

[1]Calculated as follows: % Extraction = $\frac{\text{Arterial - venous conc. in plasma}}{\text{Arterial conc. in plasma}} \times 100$

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]Number in parenthesis indicates a potentially limiting sequence of amino acids.

[6]See Footnote 3, Table 4.

Table 17. Essential Amino Acid Extraction by the Mammary Gland[1] - Mid Lactation (120 Days)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
Arg[4]	45.5 (3)[5]	42.9 (3)	46.8 (3)	34.9 (5)	33.9 (7)	43.57
His	18.0 (9)	16.6 (9)	20.4 (9)	16.0 (9)	18.8 (9)	51.03
Ile	31.7 (6)	28.6[A][6] (6)	36.2 (7)	32.8 (6)	35.9 (5)	16.61
Leu	35.8 (5)	34.4[A][6] (5)	44.1[a] (4)	41.8[a] (4)	46.4[a] (3)	16.40
Lys	59.8 (2)	54.5 (2)	57.2 (2)	52.9 (1)	54.5 (2)	13.70
Met	71.0 (1)	68.3 (1)	61.8 (1)	51.9 (2)	59.1 (1)	17.49
Phe	44.6 (4)	36.3 (4)	40.8 (6)	42.1 (3)	44.4 (4)	15.82
Thr	27.3 (7)	26.3 (7)	43.0 (5)	32.6 (7)	35.5 (6)	31.43
Val	18.8 (8)	17.5 (8)	23.2 (8)	21.5 (8)	21.1 (8)	31.41
TAA	24.5	18.9[A]	28.5[a]	24.2[a]	25.5[a]	18.43
TEAA	33.8	30.9	37.8	33.8	36.0	19.89
TNEA	17.3	8.5[A]	20.9[a]	16.0[a]	16.8[a]	32.43

[1]See Footnote 1, Table 16.

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]See Footnote 5, Table 16.

[6]See Footnote 3, Table 4.

At 240 days of lactation no apparent pattern or significance existed (Table 18). Even infused amino acids did not suggest any type of response.

c. Efficiency of amino acid utilization

This ratio of amino acid uptake to output by the mammary gland was calculated by the method of Mepham and Linzell (56) and Derrig et al. (24)(Table 19) and our results are comparable to those of Derrig et al. As ratios increase above 100, efficiency of amino acid utilization decreases. Ratios below 100 indicate that more of the amino acid is secreted in milk than is extracted from blood by the mammary gland.

At 30 days of lactation the ratio increased significantly with M, ML, and MLP over C for histidine, leucine, phenylalanine, threonine, and valine (Table 19). Other amino acids followed a similar trend but differences were non-significant. Non-essential amino acids are not presented, although tyrosine and serine were significantly higher on M, ML, and MLP. Glutamic acid decreased significantly from approximately 35% for ML and MLP to 30% for M and reflected the lowered quantity of glutamic acid infused. Many amino acid efficiency ratios on C infusion were lower than 100 and indicated a deficiency of amino acid uptake relative to output by the mammary gland.

At 120 days of lactation the overall trend followed the 30 day pattern (Table 20). Leucine and phenylalanine were significantly increased in M, ML, and MLP compared to C. The phenylalanine difference was due to a significant increase of MLP over ML. Tyrosine (not presented) was increased by M, ML, and MLP over C, while glutamic acid followed the

Table 18. Essential Amino Acid Extraction by the Mammary Gland[1] - Late Lactation (240 Days)

	Pre[3]	Treatments[2]				C.V.
		C	M	ML	MLP	
Arg[4]	35.5 (5)[5]	42.2 (3)	43.6 (3)	49.2 (3)	37.1 (5)	25.58
His	14.2 (9)	14.8 (9)	17.2 (9)	22.0 (8)	25.2 (8)	78.26
Ile	34.5 (6)	31.4 (7)	32.8 (6)	31.8 (6)	30.3 (7)	14.86
Leu	43.2 (3)	40.0 (4)	42.3 (4)	44.6 (4)	41.3 (4)	11.73
Lys	52.1 (2)	55.6 (2)	53.1 (1)	55.1 (2)	51.7 (2)	15.53
Met	64.6 (1)	69.8 (1)	51.1 (2)	62.2 (1)	59.8 (1)	18.49
Phe	37.3 (4)	34.5 (5)	36.8 (5)	40.8 (5)	38.4 (3)	20.44
Thr	24.4 (7)	32.9 (6)	29.0 (7)	31.6 (7)	35.4 (6)	21.96
Val	19.5 (8)	17.4 (8)	18.3 (8)	16.1 (9)	19.7 (9)	23.78
TAA	23.2	24.0	23.1	24.6	26.5	15.65
TEAA	32.9	33.0	33.2	35.4	33.7	15.12
TNEA	14.8	16.1	15.1	15.7	19.8	22.32

[1]See Footnote 1, Table 16.
[2]See Table 3.
[3]See Footnote 2, Table 4.
[4]See Appendix I.
[5]See Footnote 5, Table 16.

Table 19. Essential Amino Acid Utilization by the Mammary Gland[1] - Early Lactation (30 Days)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
Arg[4]	209.9 (8)[5]	226.2[6] (9)	301.4 (9)	205.1 (8)	287.6 (9)	21.20
His	60.8 (1)	24.6[A] (1)	133.8[a] (7)	100.0[a] (2)	130.6[a] (6)	28.83
Ile	249.4 (9)	171.2 (8)	217.9 (8)	236.0 (9)	235.0 (8)	22.86
Leu	116.7 (6)	86.6[A] (5)	121.0[a] (5)	126.9[a] (5)	116.6[a] (3)	20.79
Lys	111.6 (5)	99.7 (7)	129.7 (6)	131.7 (6)	145.4 (7)	23.03
Met	130.4 (7)	94.5 (6)	114.2 (2)	133.9 (7)	127.8 (5)	32.67
Phe	85.4 (2)	55.3[A] (2)	95.9[a] (1)	91.6[a] (1)	93.5[a] (1)	26.22
Thr	105.6 (4)	75.0[A] (3)	115.1[a] (3)	103.8[a] (3)	103.6[a] (2)	14.49
Val	104.7 (3)	84.0[A] (4)	117.0[a] (4)	117.5[a] (4)	123.5[a] (4)	19.70

[1]Calculated as follows: $\text{Ratio} = \frac{\text{Uptake from plasma}}{\text{Output in milk}} \times 100$

See Footnote 1, Table 12 for amino acid uptake; see Appendix I for amino acid concentrations in milk.

[2]See Table 3.

[3]See Footnote 2, Table 4.

[4]See Appendix I.

[5]See Footnote 5, Table 16.

[6]See Footnote 3, Table 4.

Table 20. Essential Amino Acid Utilization by the Mammary Gland[1] - Mid Lactation (120 Days)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
Arg[4]	260.6 (9)[5]	229.5 (9)	259.7 (9)	218.2 (8)	212.3 (8)	47.62
His	88.8 (2)	86.6 (3)	116.7 (2)	83.4 (2)	96.4 (1)	38.45
Ile	226.4 (8)	204.9 (8)	242.0 (8)	232.7 (9)	244.0 (9)	20.06
Leu	102.5 (4)	101.2[A][6] (4)	124.2[a] (3)	121.7[a] (5)	124.6[a] (5)	13.02
Lys	122.2 (7)	105.7 (7)	125.4 (4)	135.1 (7)	150.0 (7)	19.69
Met	122.0 (6)	103.7 (6)	130.6 (7)	116.4 (4)	130.0 (6)	21.63
Phe	90.7 (3)	70.5[A] (1)	79.8[a] (1)	75.1[aC] (1)	98.6[ac] (2)	11.83
Thr	87.6 (1)	74.2 (2)	127.0 (5)	98.1 (3)	110.6 (3)	36.54
Val	105.5 (5)	102.0 (5)	137.4 (6)	127.0 (6)	120.7 (4)	35.33

[1]See Footnote 1, Table 19.
[2]See Table 3.
[3]See Footnote 2, Table 4.
[4]See Appendix I.
[5]See Footnote 5, Table 16.
[6]See Footnote 3, Table 4.

decrease noted at 30 days from M with ML and MLP. At 240 days methionine, threonine, and valine ratios were higher on MLP than ML (Table 21).

Glutamic acid present in C appeared to have a negative effect on utilization of amino acids for milk production at 30 and 120 days. The calculated efficiency ratios result primarily from uptake differences (Tables 12 and 14). This effect cannot be isolated from overall effect of infusion, quantity of α-amino nitrogen present, and potential disruption of cows through frequent monitoring and changing of infusion bottles. As a result methionine substitution for glutamic acid increased the ratio of amino acid uptake to output by the mammary gland in these two groups. The increase in the phenylalanine ratio with its infusion at 120 days was the only other change that existed with treatment amino acids.

Interpretation

Mixed responses noted by different criteria confirms that interpretation of plasma amino acid responses are difficult. Interpretation hinges on causes of lowered amino acid uptake on C treatment. Nimrick et al. (63) infused glutamic acid abomasally in graded amounts. Their results indicated that nitrogen retention began to plateau around .6% glutamic acid equivalent in the diet which was their largest intake. This peak intake was .378 g of glutamic acid/kg of metabolic body weight ($B.W.^{.75}$). For a 600 kg cow the glutamic acid equivalent would be 45.8 g per day.

Intestinal tissue and liver would alter the amino acid balance that reaches the plasma pool and hence tissue with abomasal infusion. Glutamic

Table 21. Essential Amino Acid Utilization by the Mammary Gland[1] - Late Lactation (240 Days)

	Pre[3]	Treatments[2] C	M	ML	MLP	C.V.
Arg[4]	202.0 (8)[5]	239.9 (9)	213.9 (9)	255.8 (9)	223.7 (8)	35.45
His	73.9 (2)	66.5 (1)	70.1 (2)	87.9 (4)	125.2 (5)	85.86
Ile	257.6 (9)	224.6 (8)	203.1 (8)	184.1 (8)	225.0 (9)	12.94
Leu	126.6 (6)	110.4 (6)	105.1 (7)	101.7 (5)	113.5 (2)	15.29
Lys	119.8 (4)	109.5 (5)	102.2 (6)	109.1 (7)	132.5 (6)	18.59
Met	134.2 (7)	128.2 (7)	100.6 (5)	104.8[C6] (6)	137.5[C] (7)	14.60
Phe	71.3 (1)	69.6 (2)	69.2 (1)	86.5 (3)	86.6 (1)	21.64
Thr	85.6 (3)	94.2 (3)	77.3 (3)	77.2[C] (2)	117.5[C] (3)	22.93
Val	125.5 (5)	101.4 (4)	95.6 (4)	76.0[C] (1)	122.9[C] (4)	22.21

[1]See Footnote 1, Table 19.
[2]See Table 3.
[3]See Footnote 2, Table 4.
[4]See Appendix I.
[5]See Footnote 5, Table 16.
[6]See Footnote 3, Table 4.

and aspartic acids are not recovered among products of absorption (26). After transamination the carbon skeleton was metabolized as well. This indicates that glutamic acid can be readily metabolized by individual cells.

Liver has been demonstrated to modify amino acid supply from the gut as observed in the plasma pool (25). Thus, the effect of glutamic acid in C treatment is uncertain. Although glutamic acid was infused in large quantities (49.5 g/day cow maximum) at 30 and 120 days, glutamic acid enters the metabolic pathway more readily than most amino acids (26). Quantities fed by other researchers suggest that there should be no problems with the amount of glutamic acid infused (65, 78). However, the number of significant parameters would be reduced at 30 and 120 days if amino acid uptake and extraction responses were due to glutamic acid.

Nimrick et al. (62, 63) fed purified diets with urea as the sole source of nitrogen. Part of their reported increased nitrogen retention response could be due to provision of non-specific α-amino nitrogen. Thus, glutamic acid may have very little effect, and lowered amino acid uptake may be due to physical disturbances previously mentioned. No effect from glutamic acid was noted at 240 days although protein was overfed. In this group glutamic acid would be a natural end product of transamination reactions, while productive requirements are smaller than at 30 and 120 days.

Limitation of Infused Amino Acids

Supplementation of the limiting amino acid results in more efficient utilization of other essential amino acids (63). When a combination of amino acids are supplemented, inclusion of the most limiting amino acid

should produce a similar response. Amino acid imbalances affect amino acid utilization as well (32, 33, 58). In turn a productive response could be expected to follow a change in amino acid balance through supplementation of any amino acid. Supplementation of non-limiting amino acids results in substantial plasma increases that are proportional to excesses (12, 24). Supplementation of limiting amino acids should result in only minimal increases in plasma (24). Nimrick's results (62, 63) indicated that an amino acid should produce a productive response only if potentially limiting.

Using these criteria an attempt was first made to establish an order of limitation among essential amino acids infused. Responses considered were arterial and venous plasma amino acid concentrations of methionine, lysine, and phenylalanine, amino acid uptake by the mammary gland, and effect on plasma concentrations of unrelated essential and non-essential amino acids. Consideration of the effect of infusion on remaining essential and non-essential amino acids requires that predominant effects of infusion amino acids be deducted. Thus, methionine, lysine, and phenylalanine were subtracted from essential amino acids and cystine, glutamic acid, and tyrosine were deducted from non-essential amino acids. Interpretations are based partially on these modified totals. This limitation ranking is by judgment alone.

a. Early lactation (30 days)

Lysine is the only amino acid which does not result in a significant increase in arterial blood with infusion. However, a substantial lysine increase and a significant tyrosine increase resulted with inclusion of phenylalanine.

In venous blood significant increases occurred in lysine and phenylalanine with their infusion. Tyrosine reflection with phenylalanine was again significant. Methionine followed no apparent pattern.

Amino acid uptake significantly increased for lysine and phenylalanine with infusion of methionine. Methionine increased non-significantly in uptake over C with its infusion and again with lysine infusion. The modified essential and non-essential amino acid patterns suggested lysine results in the lowest plasma amino acid concentrations followed by methionine or phenylalanine.

After weighing all of these factors the limitation would be lysine or methionine. Plasma amino acid concentrations pointed to lysine, while uptake calculations suggested methionine.

b. Mid lactation (120 days)

The considerations required are stated for the previous group. Responses of all parameters except modified totals were discussed previously. Little effect was noted in arterial blood as measured by modified total essential and non-essential amino acids at 120 days. Methionine substantially reduced concentrations in venous blood, while lysine and phenylalanine resulted in similar but higher concentrations. Amino acid uptake was 460, 804, 677, and 728 g/day on C, M, ML, and MLP. Overall consideration of limitation in this group presented many conflicts. If any of the three amino acids infused were limiting at 120 days, it would be methionine.

c. Late lactation (240 days)

The modified arterial amino acid totals suggested a lowering effect with M, followed by a larger drop on ML, and an increase with

MLP. The venous amino acid pattern was similar. Amino acid uptake was constant for essentials on C, M, and ML and increased on MLP. The non-essential pattern differed only in an uptake decline that was present from C to M.

The arterial and venous plasma amino acid patterns were not very definitive, but uptake suggests that phenylalanine was limiting. However, most plasma amino acid concentration increases were noted on MLP. Thus, results at 240 days are inconclusive.

Derived Orders of Amino Acid Limitation

Experiments with dairy cattle have compared uptake of amino acids as related to production of milk (19, 24, 56, 83, 85) and amounts present in arterial blood (19, 24, 56).

Several assumptions are basic to both methods: 1) Arterial and venous plasma pools were representative of amino acid supply that was available and not utilized by the mammary gland. Sampling time was selected for an expected high response from the available energy supply. 2) No preferential transport of amino acids occurred. 3) Alteration of amino acids within the gland was minimal (little degradation or inter-conversion). 4) Measurements are related to requirement.

The above assumptions are common to both methods. However, each method has an additional assumption that makes it unique. Extraction assumes that removal from the blood is proportional to the mammary cell's total requirements for milk production. Ratio of amino acid uptake to output in milk assumes that the mammary cell's amino acid requirement is a primary function of milk protein production and is proportional to output in milk.

a. Extraction by the mammary gland

To obtain the potential order of limitation, amino acids were ranked from highest to lowest percent (Tables 16, 17, and 18). Relative order was similar in all groups. Little change was noted with infusion, so overall limitation was obtained by summing limitation orders over pretreatment and treatments. Thus, the following orders were established for each lactational stage:

30 days - lysine, methionine, arginine, phenylalanine, leucine, threonine, isoleucine, histidine, and valine.

120 days - methionine, lysine, arginine or phenylalanine or leucine, isoleucine, threonine, valine, and histidine.

240 days - methionine, lysine, arginine or leucine, phenylalanine, isoleucine, threonine, valine, and histidine.

Similarity of this order to conclusions drawn from the plasma amino acid effects was noted. Further comparisons with other researchers will be held until after the following discussion.

b. Utilization for milk production

Ratio of amino acid uptake to output by the mammary gland relates plasma amino acid usage to milk production (Table 19). Limitation order was determined within treatment by ranking amino acids from the smallest to the largest ratio (Tables 19, 20, and 21). Again little change in overall order of limitation was noted with infusion treatments. Thus, overall order was determined across all treatments and follows:

30 days - phenylalanine, threonine, histidine, valine, leucine, methionine, lysine, isoleucine, and arginine.

120 days - phenylalanine, histidine, threonine, leucine, valine, methionine, lysine, isoleucine, and arginine.

240 days - phenylalanine, histidine or threonine, valine, leucine, lysine, methionine, isoleucine, and arginine.

Order of limitation between lactational groups was similar within method of ranking, but a discrepancy existed between the two methods. This apparently represents an invalidity within the assumptions. However, confidence limits of selection order are wide.

c. Relationship of limitation order to observations of others

Chandler and Polan (48) calculated minimum essential amino acid transfer efficiencies of the mammary gland as related to milk protein. They reported that the five most critical amino acids were methionine, lysine, phenylalanine, tyrosine, and threonine. Methionine was always the most critical amino acid while lysine increased in importance at high milk production. This calculation is comparable to efficiency of utilization ratios.

Derrig et al. (24) calculated order of limitation from both potential milk production and ratio of amino acid uptake to output by the mammary gland. Both expressed the same relationship and suggested order of limitation on abomasal infusion was phenylalanine, histidine, methionine, threonine, lysine, leucine, isoleucine, valine, and arginine. Order from two rumen infusion periods was phenylalanine, methionine, lysine, threonine, leucine, isoleucine, histidine, valine, and arginine. Our ratio is an equivalent calculation but order of methionine and lysine changed considerably.

After ranking Derrig et al.'s data (24) by extraction, potential limitation order with rumen infusion was lysine, methionine, leucine, arginine or phenylalanine, isoleucine, threonine or valine, and histidine. Potential order on abomasal infusion of sodium caseinate was methionine, lysine, arginine, phenylalanine, isoleucine, leucine, threonine, valine, and histidine. This order was quite similar to ours. However, results of both suggest that arginine, histidine, and phenylalanine order changes considerably with the two methods. Less discrepancy existed for differences in limitation of methionine and lysine for Derrig et al. (24) than with our results. This suggests that in our case extraction more nearly corresponds to the basic assumptions.

SUMMARY AND CONCLUSIONS

Cows were utilized at three stages of lactation to study responses to jugular infusion of methionine, lysine, and phenylalanine. Four cows were assigned to 4 x 4 Latin squares with an extra period for estimation of carryover effects at 30, 120, and 240 days of lactation. Cows in 30 and 120 day groups were in their second, third, or fourth lactation. The 240 day group contained first and second lactation cows that calved at 29 to 35 mo of age. Average milk production was 37.0, 29.2, and 18.7 kg/day in the pretreatment period at 30, 120, and 240 days of lactation.

Rations were formulated to contain 15, 18, and 21% crude fiber and 16, 15, and 14% crude protein for 30, 120, and 240 day groups with corn silage serving as the forage. Calcium, phosphorus, and sulfur were supplied at .6, .4, and .2% of ration dry matter. Cows were placed on experimental rations two wk prior to the experimental period.

Jugular infusions were methionine (M), methionine + lysine (ML), and methionine + lysine + phenylalanine (MLP) in balance with glutamic acid which served as control (C). Amino acid weights infused were calculated at 25% of amino acid content in pretreatment milk. Osmolarity was balanced with NaCl.

Blood samples were collected in heparinized tubes from subcutaneous abdominal mammary vein and tail artery/vein in P.M. and A.M. of days 3 and 4 of each infusion period and daily alternating between A.M. and P.M. the last four days of pretreatment period. Hematocrit readings were taken, and plasma was deproteinized using four volumes of plasma to

one volume of 20% sulfosalicylic acid containing norleucine as internal standard. Amino acid filtrates were subsequently prepared and analyzed on a Technicon TSM Autoanalyzer.

Results were analyzed with the Lucas procedure by arterial and venous sites, amino acid uptake and extraction by the mammary gland, and efficiency of amino acid utilization for milk production. These results were also compared as a ratio to pretreatment period. Means were tested for significance by orthogonal contrasts which tested each amino acid addition. Crude protein and crude fiber contents were 16.8, 16.5, and 16.6% and 12.7, 14.9, and 17.3% of ration dry matter for 30, 120, and 240 day groups. Protein intake could be a factor limiting our responses to infusion.

Differences in milk production were non-significant, although a slight trend did exist for an increase with methionine infusion. Hematocrit results suggest that these determinations may not be necessary in experimental designs that remove cow effects. Carry-over effects were nonexistent with exception of 240 day arterial plasma. This appeared to be due to protein intake relative to requirements.

Plasma amino acid responses were examined for patterns reported in monogastric animals and dairy cattle. At 30 days arterial and venous plasma amino acid concentrations were lowest on ML. However, amino acid uptake increased with M. Amino acid extraction followed the uptake trend, while amino acid uptake to output ratio increased with M and suggested lowered efficiency due to response on C.

Little significance was noted at 120 days although amino acid uptake followed the trend noted at 30 days. At 240 days, arterial and

venous plasma amino acid concentrations were lowest with ML while the trend in amino acid uptake was to increase with MLP. However, efficiency as noted from an increased mammary amino acid uptake to output ratio decreased with MLP.

Potential orders of amino acid limitation were considered for extraction and ratio of uptake:output by the mammary gland. Order was similar among lactation groups, but differed between methods of evaluation. Overall order of limitation by the amino acid extraction method was methionine, lysine, arginine, leucine, phenylalanine, isoleucine, threonine, valine, and histidine. However, histidine was extremely variable. Order of limitation by amino acid uptake to output ratio was phenylalanine, threonine or histidine, valine, leucine, methionine, lysine, isoleucine, and arginine. This change in order by the two methods could be due to limited understanding of the relationship of plasma amino acids to productive requirement in the lactating dairy cow and the width of confidence intervals of such rankings.

Conclusions with plasma amino acid are as follows:

1) Of amino acids infused either lysine or methionine could be considered the most limiting from plasma concentrations and amino acid uptake at 30 days.
2) If any of these three amino acids were limiting at 120 days, it would be methionine. However, few significant parameters were observed.
3) Results at 240 days are inconclusive.
4) Considerable disagreement exists between methods of ranking amino acids in potential orders of limitation. Further study of

the relationship between these methods and physiological function is required.

5) Since there was no substantial change in order of limitation as a result of infusion, either protein intakes were above requirements or amino acids not infused are candidates for limitation.

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Appendix I. Abbreviations of Tabled Amino Acids and the Amino Acid Content of Milk

Amino Acid	Abbreviation	Milk Amino Acid
		(g/kg)
Total Amino Acids	TAA*[1]	-[2]
Total Essential Amino Acids	TEAA*	-
Total Non-Essential Amino Acids	TNEA*	-
Essential Amino Acids	EAA*	-
Arginine	Arg	1.138
Histidine	His	.860
Isoleucine	Ile	1.978
Leucine	Leu	3.144
Lysine	Lys	2.560
Methionine	Met	.813
Phenylalanine	Phe	1.572
Threonine	Thr	1.484
Valine	Val	2.170
Non-Essential Amino Acids	NEA*	-
Alanine	Ala	1.104
Asparagine	Asn	-
Aspartic Acid	Asp	2.449
Citrulline	Cit	-
Cystine	CyS	.272
Glutamic Acid	Glu	7.290
Glutamine	Gln	-
Glycine	Gly	.641
Ornithine	Orn	-
Proline	Pro	3.384
Serine	Ser	1.852
Taurine	Tau*	-
Tyrosine	Tyr	1.641

[1] Amino acids are abbreviated by standard nomenclature as shown in Metabolism (2) with the exception of those indicated by an asterisk.

[2] The amino acid content of milk is tabled only where an efficiency of utilization was calculated. Values reported here are derived from Metabolism (2), and are the mean of estimates utilized by Jacobson (40) and Chandler (19).

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METHIONINE, LYSINE, AND PHENYLALANINE INFUSION AND THE EFFECT ON PLASMA AMINO ACID CONCENTRATIONS AND MAMMARY UPTAKE

by

Alfred W. Norman

(ABSTRACT)

Ten cows were used in 4 x 4 Latin squares with an extra period for estimation of carry-over effects to study plasma amino acid responses to jugular infusion of amino acids at early, mid, and late lactation. Methionine (M), methionine + lysine (ML), and methionine + lysine + phenylalanine (MLP) in a balance with glutamic acid (C) were infused at 25% of the amino acid content of pretreatment milk via the jugular vein. Rations were formulated at 16, 15, and 14% crude protein and 15, 18, and 21% crude fiber for 30, 120, and 240 day lactational groups.

Carry-over effects were present only in arterial blood at 240 days. Differences in milk production were not significant, although production increased on M, ML, and MLP at 30 days.

Plasma amino acid responses of nonruminants were utilized to aid in evaluation. Arterial and venous plasma amino acid concentrations were lowest on ML at 30 days, while amino acid uptake was significantly increased by M. At 120 days plasma responses were inconclusive and non-significant. The amino acid uptake trend followed that observed at 30 days. At 240 days plasma amino acid concentrations were lowest with ML, while uptake was increased on MLP.

Essential amino acids were ranked in potential orders of limitation by amino acid extraction and utilization for milk protein by the mammary gland. Order of limitation differed between calculation methods but was similar for all three stages of lactation. Orders of limitation were as follows:

Amino acid extraction - methionine, lysine, arginine, leucine, phenylalanine, isoleucine, threonine, valine, and histidine.

Amino acid utilization - phenylalanine, threonine or histidine, valine, leucine, methionine, lysine, isoleucine, and arginine.

Since there was no substantial change in order of limitation with infusion, either protein intakes were above requirements or amino acids not infused are candidates for limitation. However, among amino acids infused, the parameters observed suggested lysine or methionine at 30 days, methionine at 120 days, and no apparent choice at 240 days.