# The Roles of Amino Acids in Milk Yield and Components

*Brian J. Bequette[1] and Katie Nelson*
*Department of Animal and Avian Sciences*
*University of Maryland, College Park 20742*

## Introduction

Unlike the situation in monogastric species (Fuller et al., 1989), it has proven more difficult to predict with accuracy the amino acid (AA) requirements of ruminants for growth and milk production. The current NRC publications for dairy and beef cattle recognise this, and do not explicitly allow for diets to be balanced for essential (EAA) or limiting AA contents, except to recommend a ratio of 3:1 lysine to methionine in metabolizable protein (NRC 2000, 2001). With greater pressures being placed on dairy and beef production to reduce nitrogen wastes and the dairy industries desire to manipulate milk components to meet market demands, the need is there to define not just the limiting AAs, but the general requirement for all AA for production. Current feeding schemes sub-divide EAA requirements into net requirements for maintenance functions, i.e., the first priority, and for growth, lactation and/or reproduction. Therefore, the metabolizable protein requirement reflects only the roles AA serve as precursors for synthesis of proteins, e.g. muscle, milk caseins, conceptus. Conceptually, this approach is satisfactory since a major component of the metabolizable protein prediction will largely be determined from the AA composition of endogenous protein losses, and that needed for net synthesis of muscle, milk and fetal tissue proteins. Where there is less of a conceptual basis and agreement are the conversion or "efficiency" factors used to partition AA in the metabolizable protein to the various fates. It could be argued, therefore, that defining the basis of the "inefficiency" portion of the conversion factors is a goal that holds much greater potential for improving production and predictions than determining the pattern of limiting AA.

To that end, there is limited, yet intriguing, information on what comprises the AA requirements for producing ruminants. In recent years, we have come to realize the significance of EAAs, not only in terms of their nutritional availability for anabolic use, but also the fact that they are involved in a number of metabolic pathways that serve important functions in protein and energy metabolism, gluconeogenesis, lipid-fatty acid metabolism, and in mammary synthesis of milk protein and lactose. Defining and quantification of these "other requirements" will, in future, become important considerations in next generation feeding schemes for dairy cattle (and other species) beyond current emphasis on identification of limiting AA.

## Nutritional and Metabolic Classification of Amino Acids

[1]Contact at: 4147 Animal Science Bldg., Department of Animal and Avian Sciences, University of Maryland, College Park; 301-405-8457-office; 301-314-9059-fax; bbequett@umd.edu
February 1-2, 2006 Florida Ruminant Nutrition Symposium, Best Western Gateway Grand, Gainesville FL

Before discussing physiological requirements for any AA, it is important to consider the classification of AAs in the context of the animal's physiology and general metabolism. There are 20 AAs commonly found in animal proteins, and all of these are incorporated as the L-isomer. Unfortunately, the terms EAA and indispensable, and likewise non-EAAl and dispensable, are used interchangeably. An important qualifier is that these categories were initially established for growth, in fact rapid growth. For producing animals, the dietary requirement for EAAs is dominated by the composition and amounts of the proteins accreted (e.g. skeletal proteins in growing animals) or secreted (e.g. milk proteins in lactating animals). An EAA was defined by William C. Rose as, '*One which cannot be synthesized by the animal organism out of materials ordinarily available to the cells at a speed commensurate with the demands for normal growth'* (Borman et al., 1946). As we will illustrate, the availability of intermediates of AA metabolism within tissues does have a bearing on the rate of synthesis of other AAs, glucose and milk component synthesis.

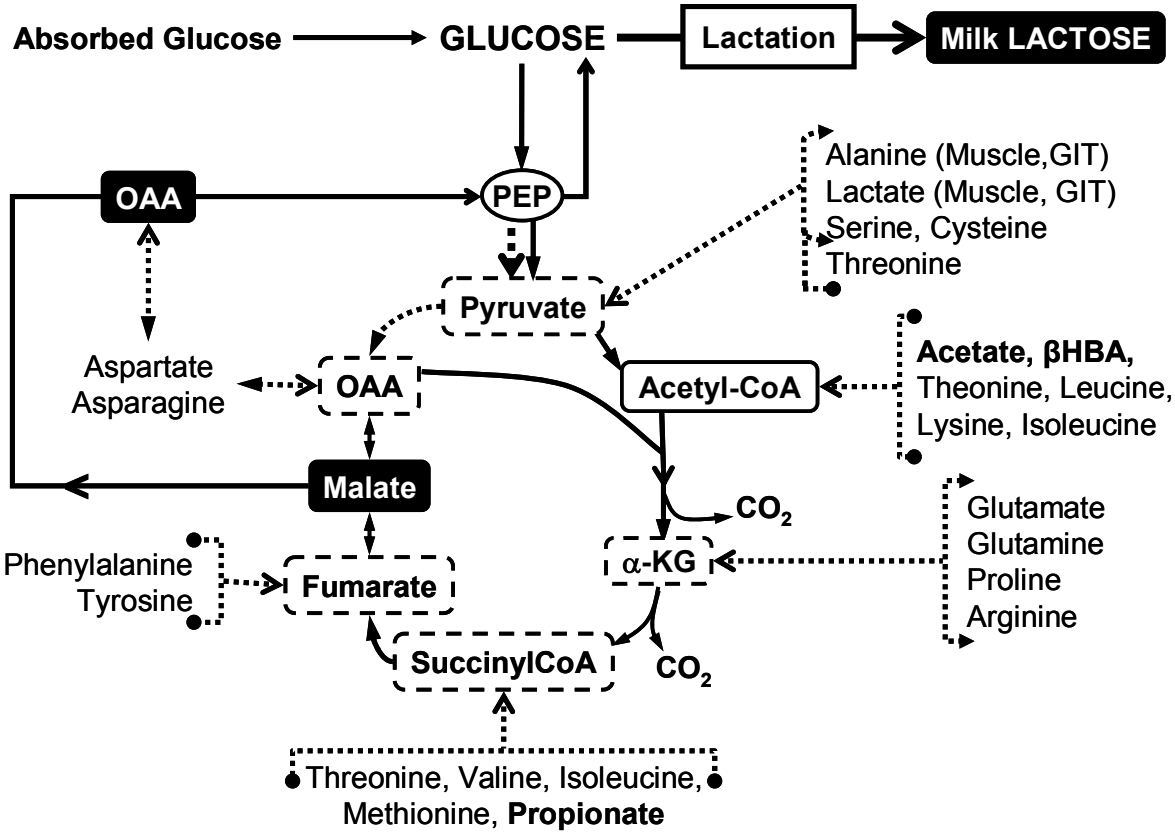


**Figure 1.** Primary pathways for synthesis and catabolism of amino acids, gluconeogenesis and their links to the Krebs cycle in ruminants. Dashed lines with arrows in both directions indicates points in the cycle where non-essential amino acids can be catabolized or synthesized from. OAA, oxaloacetate; PEP, phosphoenolpyruvate; α-KG, α-ketoglutarate; βHBA, β-hydroxybutyric acid.

Reeds (2000) pointed out that, from a metabolic standpoint, the only truly non-EAAs are glutamate and serine (**Fig. 1**). These can be synthesized from non-amino nitrogen (ammonium ions) and appropriate carbon skeletons derived from intermediates

of glycolysis (3-*phospho*glycerate) and the Krebs cycle (α-ketoglutarate). This should not be taken to infer that glutamate is not required in the diet. Indeed, diets devoid of glutamate have been shown to depress growth (Rose et al., 1948), suggesting that under normal growing conditions the materials normally available or the rate of glutamate synthesis may be limiting. All other non-EAA derive their amino group or carbon skeleton from other AAs. In this connection, glutamate and serine play a central role because they are the primary precursors for non-EAA synthesis. Nutritional essentiality has often been misinterpreted to mean that the animal is incapable of synthesizing it. Not true. In ruminants, essentiality relates to the supply of AAs leaving the rumen, of which microbial proteins supply the majority with variable contributions from undigested feed proteins. In the core essential group, the branched chain AAs, methionine and phenylalanine can be synthesized by animals from their corresponding keto-acids. However, the keto-acids can only be derived *de novo* from the original parent AA via transamination, and so there is in no new or net synthesis, unless the corresponding keto-acid is provided in the diet. Racemic mixtures of D and L-isomers of AAs can provide an effective source of supplemental AA to balance rations. The keto-acid produced following action of D-oxidase has no racemic center and so it can be reaminated to yield the L-isomer. Therefore, keto-acids can be supplemented in the diet to yield the corresponding L-isomer. The keto-acids of all AAs, except for lysine and threonine which are not transaminated, can be converted to the corresponding AA upon transamination.

Attempts to increase milk protein yield by increasing the supply of methionine by addition of rumen-protected methionine have given mixed results. One side effect of providing excess methionine is that it is one of the most toxic AA. Methionine can be synthesized via remethylation of homocysteine but this also does not represent new or net synthesis of methionine because the only source of homosysteine in the body from methionine catabolism. However, the synthetic hydroxyl-analogue of methionine, 4-thiomethyl-2-hydroxybutanoic acid (HMtBA), has been used effectively as a source of methionine in pig, poultry and dairy cow diets. The advantage with HMtBA is that is not toxic, as is free methionine, and the analogue does not appear to be removed by the gut and liver tissues to the same extent as methionine. Furthermore, there are no known mammalian transporters for HMtBA, so it readily diffuses into tissues where it can be converted to methionine. In sheep (Wester et al. 2000), the HMtBA is converted to keto-methionine and via transamination to L-methioine, with the greatest contributions to tissue methionine arising from the kidneys (22%), followed liver (14%) and the gastrointestinal tract (5-12%). The mammary gland is also a significant site of conversion of HMtBA to methionine with 20% of milk protein methionine derived from the supplemented HMtBA given to dairy cows (Lobley and Lapierre, 2001; Lapierre et al., 2002).

## Challenges to Predicting Amino Acid Requirements

Milk protein yield increases in a curvilinear manner in response to post-ruminal supplementation with protein (Guinard and Rulquin, 1994). However, the extra milk protein yield comes at a cost in terms of overall efficiency of recovery of the additional protein. The poor efficiency of utilization of the additional absorbed AA may be a consequence of energy limitations at the upper levels of supplementation, sequential

removal of AA as they pass through consecutive tissue beds, changing priorities for partition between tissues, an imbalance in the pattern of supply of EAA (i.e. creating limiting AA) to productive tissues (i.e. mammary gland) or the biological (genetic) limits of production have been reached. On balance, when one compares the EAA profile of microbial protein or typical rumen by-pass protein sources (e.g. corn gluten, soybean and fish meals) to the profile required for tissue gain or milk protein secretion, the patterns are similar with the exceptions of leucine and histidine, and possibly also methionine (NRC 2000, 2001). In the study of Guinard and Rulquin (1994), casein was infused and so we would not expect that the profile of the absorbed EAA would be a limitation. However, a compilation of studies in dairy cows where AA (in the pattern of casein) was infused into either the duodenum, the blood supply prior to the liver or the peripheral blood, suggests that AA supply to the udder is probably modified as it passes through the body (Bequette et al., 2003). Thus, the responses in milk protein output were lowest, as was the recovery of the EAA, when AA was provided to the gut. Furthermore, in reality, the profile of AA delivered to the mammary gland for milk protein synthesis does not reflect that which leaves the rumen and which is available for absorption. The same is true for any source of protein delivered to the small intestines. Therefore, if our goal is to define exactly the pattern of AA required to maximize production and efficiency, we need to consider the potential transformations of the AA supply, beginning at the level of the small intestines, traversing through the gut tissues and liver (in series with the gut) and how these are divided between the mammary gland and other tissues. Lastly, we will need to consider mammary metabolism of AA, and the factors and mechanisms that regulate or limit transport and incorporation of AA into milk protein.

## Maintenance Metabolic Needs for Amino Acids

### *Gastrointestinal metabolism*

Nutritionally significant quantities of EAAs are removed by the gastrointestinal tract during absorption. In ruminant sheep, the net appearance in the portal vein of EAA represented only 55-77% of that disappearing from the small intestines (MacRae et al., 1997). Here, threonine removal by the intestines was the highest, and it is likely that most of the threonine removed was directed at mucin synthesis. In the latter study, net metabolism by the gastrointestinal tract was determined at two levels of intake, which probably altered gut mass and endogenous losses. What we would really like to know is whether AA metabolism by the gut tissues remains constant across levels of intestinal protein supplies or whether at higher protein flows there is an added cost of doing metabolic business. We examined these possibilities in growing sheep given four levels of casein infusion into the intestines (**Table 1**, El-Kadi et al., 2006). Casein infusion raised metabolizable protein supply from 60% to 110% of growth requirements. Net uptake measurements across the portal drained viscera (i.e. rumen, small intestines, cecum, large intestines) were made and compared against the rate of casein-AA infusion. There were two important observations. First, casein infusion increased the net absorption of most EAA, and except for the branched chain AA (leucine, valine, isoleucine), 100% of the additional casein-EAA was recovered in blood. The implication is that, above maintenance, EAA needs of the gut have been met but that there may be additional requirements by the gut tissues to metabolised the branched chain AA.

**Table 1**. Net absorption of amino acids (μmol/(kg BW • h) across the portal drained viscera of sheep infused with increments (0, 35, 70 and 105 g/d) of casein into the duodenum (El-Kadi et al., 2006)[1]

| | Casein infusion (g/d) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 35 | 70 | 105 | SEM | *P*-value |
| Essential AA | | | | | | |
| Valine | 32 | 39 | 58 | 69 | 9.7 | NS |
| Leucine | 46$^{c}$ | 60$^{bc}$ | 86$^{ab}$ | 95$^{a}$ | 7.8 | 0.0043 |
| Isoleucine | 27$^{c}$ | 35$^{bc}$ | 49$^{ab}$ | 56$^{a}$ | 4.4 | 0.0035 |
| Methionine | 11$^{c}$ | 15$^{bc}$ | 27$^{ab}$ | 30$^{a}$ | 3.1 | 0.0043 |
| Threonine | 25 | 32 | 43 | 56 | 7.5 | NS |
| Phenylalanine | 31$^{b}$ | 38$^{b}$ | 51$^{ab}$ | 62$^{a}$ | 5.3 | 0.0113 |
| Lysine | 41 | 52 | 72 | 85 | 9.7 | NS |
| Histidine | 14 | 14 | 19 | 29 | 3.5 | NS |
| Tryptophan | 7 | 1 | 9 | 11 | 4.2 | NS |
| Non-essential AA | | | | | | |
| Alanine | 61$^{c}$ | 80$^{bc}$ | 101$^{ab}$ | 119$^{a}$ | 7.8 | 0.0020 |
| Glycine | 39 | 56 | 47 | 75 | 9.7 | NS |
| Proline | 24$^{c}$ | 42$^{bc}$ | 77$^{ab}$ | 93$^{a}$ | 9.1 | 0.0010 |
| Serine | 61 | 60 | 87 | 81 | 22.9 | NS |
| Aspartate | -2$^{b}$ | 4$^{ab}$ | 9$^{ab}$ | 22$^{a}$ | 7.1 | 0.0380 |
| Glutamate | 6 | 21 | 34 | 23 | 12.5 | NS |
| Glutamine | -6 | 10 | 73 | 13 | 44.1 | NS |

[1] Values are least-square treatment means, $n$ = 14. Positive values denote net release (absorption into blood) and negative values denote net removal from blood.

[abc] Means with different superscripts within a row are significantly different from one another ($P < 0.05$). SEM, pooled standard error of the means; NS, Not significant ($P > 0.05$).

Second, although there was net absorption of some non-EAA, except for alanine, the gut tissues metabolise from 40 to nearly 100% of the intestinal supplies of other non-EAA, namely glutamate, glutamine and aspartate.

The gastrointestinal tract (and liver) is a major site of AA catabolism and synthesis (Wu, 1998). In all species examined to date, almost 100% of dietary glutamate, glutamine and aspartate are removed by the gastrointestinal tract during absorption. Surprisingly, we found that glucose makes a minor (2 to 5%) contribution to direct (via acetyl-CoA) oxidative metabolism by the rumen and small intestines of sheep (Bequette et al., 2004). Particularly noteworthy were the results for the intestines, whose lumen is exposed to mostly AAs and very little volatile fatty acids. Based on studies with isolated rumen epithelial and small intestinal cells (Oba et al., 2004) both glutamate and glutamine were found to be oxidative fuels for the gut of ruminants. Net removal across the mesenteric-drained viscera (the blood draining the small intestines only) of sheep seems to indicate that the small intestine catabolizes substantial glutamine (Gate et al., 1999), and in dairy cows, net removal of aspartate and glutamate across the same bed is high (Berthiaume et al., 2001). Gate et al. (1999) found that of the blood glutamine removed by the gut of sheep, 25% was metabolized to ammonia, 6% was used for nucleic

acid (RNA, DNA) synthesis in regions of the gut where protein turnover was highest (i.e. small intestines), and most of the remainder was used for gut protein synthesis. The demand by the ruminant gut for glutamine and glutamate is high with 25% to 50% of the whole body synthesis of glutamine and glutamate partitioned to the gut (Heitmann and Bergman, 1981; Gate et al., 1999).

What is the significance of non-EAA catabolism by the gut? Assuming that 100% of the intestinal supplies of glutamine and glutamate, and 70% of aspartate, are metabolized by the gut even before they reach the blood circulation, the practical consequence is that these so called non-EAA must be entirely synthesized in the body. In growing and lactating animals, synthesis of aspartate, glutamine and glutamate would be considerable. There are three consequences of the need to synthesise non-EAA. First, their synthesis requires catabolism of other substrates that can provide at least a 3-carbon skeleton, the most likely candidates being propionate, glucose and EAA. Thus, the implications are that there is an additional, if not obligatory, requirement for EAA if not for the carbon skeleton, certainly to provide the amino group. Second, a further penalty may be imposed on milk protein synthesis since glutamine plus glutamate comprises 22% of milk protein AA residues. And third, not only does their synthesis demand carbon and nitrogen, but the energetic costs are also high. For example, four ATP are required per mole of glutamate synthesised. Reeds et al. (1998) estimated that synthesis of glutamate alone could account for 10% of maintenance energy expenditures, and double that value when both glutamate and glutamine require synthesis.

### *The liver and gluconeogenesis*

Enzymes for the catabolism and synthesis of AAs are present in every tissue, but their levels of expression and activities vary in some species to suite the metabolic needs or functions of the tissue. Catabolism involves deamination reactions with the resulting carbon skeleton reaminated to form non-EAAs or the carbon skeletons can be channelled into the Krebs cycle either for complete oxidation, for gluconeogenesis via malate, or for fatty acid synthesis in adipose and mammary tissues. The excess nitrogen (amino groups) is ultimately transaminated to form alanine, aspartate, glutamine or glutamate for their synthesis or for entry into the ornithine cycle for urea or arginine synthesis. Metabolism in ruminants is orchestrated to conserve glucose, and so it is no surprise that the contribution of AA carbon to gluconeogenesis has been estimated from net balance and radio-tracer studies across the liver to be 12 to 35% (Annison and Bryden, 1999). In early lactation when glucose demands for lactose synthesis are high, and where glucose-precursor (propionate) supply is low, the partition of AA carbon towards gluconeogenesis is probably vital. Apart from dietary glucose (starch) and its most important precursor in ruminants (e.g. propionate in ruminants), all new glucose carbon must derive from AAs. Glycerol would not be considered a net contributor to new glucose synthesis since glycerol originates from glucose prior to storage in adipose.

We have revisited the question of 'how much do AAs contribute to gluconeogenesis?' to begin to address the requirements for AA other than for net protein synthesis. Instead of employing net balance and radio-tracers, both of which can lead to errors, we used [U-$^{13}$C] glucose (non-radioactive, stable isotope). The advantage is that substrates bearing the complete carbon-13 skeleton of the original precursor or pathway can be distinguished by mass spectrometry from those bearing fewer $^{13}$C atoms that may result from recycling in connected pathways (see Bequette et al., 2006). It is important to recognise that for an AA to make a net contribution to liver gluconeogenesis, they must be catabolised for entry into the Krebs cycle at pyruvate, oxaloacetate, succinyl-CoA, or α-ketoglutarate. Thus, at the minimum, the AA must be 3-carbons in length. Entry via these routes contributes to malate, which after exchange from the mitochondria to the cytosol, is converted to phosphoenolpyruvate and thence to glucose. A critical control point in this process is the activity of phosphoenolpyruvate carboxykinase (PEPCK), which in the lactating cow is dramatically increased in lactation (Agca et al., 2002) and is also high in non-lactating ruminants (E. Connor, K. Nelson and B. Bequette, unpubl.). In this preliminary study in sheep (**Fig. 2**), we determined

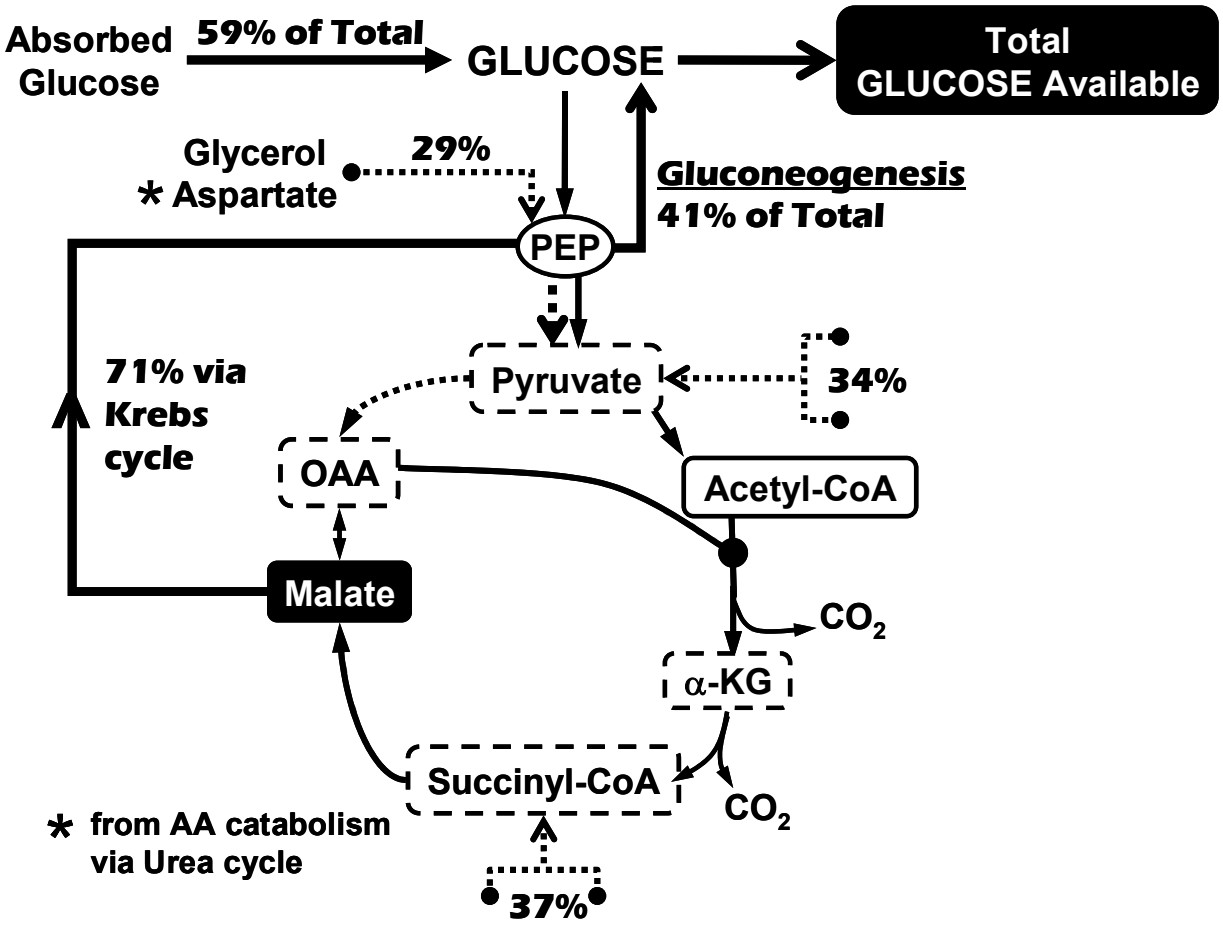


**Figure 2.** Contributions of substrates to liver gluconeogenesis in sheep fed to 1.1 times maintenance energy intake. Refer to Fig. 2 for substrate inputs to Krebs cycle. Total glucose available is the sum of absorbed glucose plus 'new' gluconeogenesis (excludes glucose carbon recycling which makes no net contribution).

that propionate contributed a maximum of 37% to gluconeogenesis via succinyl-CoA and that AA catabolism via pyruvate and oxaloacetate contributed a minimum of 34%. As the direct contributor to oxaloxacetate is aspartate, and recalling that aspartate must be synthesised nearly completely by the animal from EAA, the real impact is on EAA supply.

## The Mammary Gland and Milk Component Synthesis

### *A challenge to the limiting amino acid concept?*

It is not clear whether the relationship between AA supply and protein synthesis is simply a substrate effect or a reflection of regulatory events. Although acyl-tRNA are normally saturated in other tissues at prevailing intracellular AA concentrations (Shenoy and Rogers, 1978), the same does not appear to be the case for the udder (Elska et al., 1971). If the tRNA-acylating enzymes are not saturated with AA under normal conditions, then provision of additional AA should result in an increase in acylated-tRNA concentrations and more efficient rates of mRNA translation. Given that all 20 AA are required to synthesize milk protein, limitations may occur simultaneously for any of a number of amino acylated-tRNA, e.g. when an AA is at less than saturating concentrations the ribosome may 'hesitate' slightly at each codon that is specific for that AA. Consequently, the relative deficiency and the number of moles of that particular AA required to synthesize a mole of milk protein would determine the substrate response when the deficiency is alleviated. The implications are that multiple AA may be rate-limiting for milk protein synthesis at any one time. This is at odds with the traditional definition and use of the term 'nutritionally limiting AA' where only one AA can be limiting. However, a multiple limiting AA concept is consistent with the observations of Clark et al. (1978), wherein responses to three different AA were observed under identical culture conditions. If milk protein synthesis is sensitive to multiple AA at the same time, then changes in arterial concentrations of each AA could be very important when predictions of milk protein production are attempted.

### *What other roles do amino acids serve in the mammary gland?*

It is apparent from net uptake measurements across the mammary gland that not all the supply of EAA is used for milk protein synthesis. On the other hand, many non-EAA are not extracted in adequate amounts and so there must be additional synthesis to support milk protein synthesis. For example, net removals by the cow, ewe and goat mammary gland of lysine, leucine, threonine, valine, isoleucine and arginine are always far in excess of milk protein synthesis requirements, whereas the removals of glutamate, glutamine, serine and proline (and often alanine) are considerably less (by 40-100%) than requirements (see Bequette et al., 1998).The question is, 'Do EAA contribute to the synthesis of non-EAA in the mammary gland and is this process regulated?' Considering that the pathways of EAA catabolism intersect with non-EAA synthesis via the Krebs cycle (see **Fig. 1**) the likelihood is that the 'excess' carbon and nitrogen derived from EAA (including arginine) catabolism contributes to the synthesis of these deficient non-EAA.

Lysine is an anomaly. Although it is often first- or second-limiting on most corn-based dairy rations, at the same time it is almost always taken up in excess by the udder. Like leucine, almost all the excess lysine taken up is oxidized (Mabjeesh et al., 2000), and furthermore, levels of oxidation are higher in late than in early lactation. Does oxidation of lysine serve a role? In short, it must. One wouldn't expect that such an important substrate is oxidized for no reason! Along with glutamine and arginine, lysine is a major nitrogen carrier in the body. Complete catabolism of lysine by the dairy cow mammary gland yields two nitrogens which are transferred to glutamic acid (H. Lapierre, unpubl.). On the other hand, lysine carbon skeleton is catablised via acetyl-CoA, and so it is not a source of non-EAA carbon.

Along with lysine, methionine is often considered one of the limiting AA of corn-based rations, particularly when heated soybeans make up most of the protein source. Methionine is involved in multiple pathways leading to synthesis of phospholipids, carnitine, creatine and polyamines. At the same time, methionine provides methyl groups for transmethylation reactions involved in regulation of DNA activity and oncogene status, and it provides sulfur for cysteine synthesis. In goats, 28% of whole body methionine contributes to the synthesis of choline, an important component of cell membranes, while 10% of methionine is irreversibly lost through oxidation (Emmanuel and Kelly, 1984). One consequence of this catabolism is the synthesis of cysteine. In the goat udder, 10% of methionine-sulphur contributes to the synthesis of cysteine (Lee et al., 1996), which is also not removed in adequate amounts.

Arginine is extracted in the greatest quantities relative to milk protein outputs (150 to 200% in excess). Arginine has other metabolic functions in addition to being a precursor for protein synthesis. Recently, its role as a precursor of nitric oxide has received attention because of the potential role of nitric oxide in regulating mammary tissue nutrient perfusion (Lacasse et al., 1996). In rat mammary tissue, arginase, which hydrolyses arginine to form ornithine and urea, increases three-fold in activity in lactation (Jenkinson and Grigor, 1996). The activity of this pathway may be important for the synthesis of proline. In the sheep and goat udder, citrulline, arginine, and ornithine contribute ~20% to casein-proline synthesis (Verbeke et al., 1968). This pathway may serve to provide an alternative and perhaps critical supply of proline, an AA that is also not extracted in adequate quantities. The synthesis of proline from arginine may be inherently limited, however. In bovine mammary tissue, the key enzyme in this pathway, ornithine-$\delta$-transferase, has a high $K_m$ (8.4 mM), which would require high intracellular concentrations of ornithine to maintain maximal rates of conversion through this pathway (Basch et al., 1995). This could explain observations that abomasal infusion of proline (Bruckental et al., 1991) increases milk protein production. The study involving proline should perhaps be treated with caution as it involved only two cows and since its publication this result has not been followed up.

For the branched chain AAs, their catabolic pathways in the udder are the same, or very similar, to pathways that occur in other tissues. Leucine, valine, and isoleucine are catabolized by mammary cells to yield amino-groups, $CO_2$ and organic acids (keto- and iso-acids, propionate, acetate, and citrate), and thus potential carbon skeletons and amino-groups for alanine, glutamate and glutamine synthesis. Substantial transamination of the branched-chain AA occurs in the mammary gland with reamination representing

20% to 50% of leucine flux (Bequette et al., 2002). The second rate-limiting step is decarboxylation of the respective keto-acid, catalyzed by the branched-chain keto-acid dehydrogenase. This dehydrogenase is shared by all branched-chain AA and methionine. Regulation of branched-chain keto-acid dehydrogenase is dependent upon phosphorylation status. When insulin levels or tissue sensitivity are high or when branched-chain AA concentrations are low, the enzyme is inactive (phosphorylated) and catabolism is inhibited. This regulatory system appears to operate in the mammary gland. In lactating goats, insulin infusion depressed mammary leucine oxidation and transamination (Bequette et al., 2002).

***Essential amino acids: The carbon currency in the mammary gland?***

To date, there is limited evidence that EAA catabolism by the udder serves to provide carbon and nitrogen for non-EAA synthesis. In our view, in addition to the excess uptake of EAA and the deficiency in uptake of some non-EAA, there are two additional observations that seem to support a role for EAA other than as milk protein precursors. First, studies in dairy cows (Bickerstaffe et al., 1974) and in lactating women (Sunehag et al., 2003) indicate that only ~50-70% of lactose (glucose + galactose) derives from plasma glucose. In humans, the remainder of glucose and galactose is derived from *de novo* mammary hexoneogenesis, in part from glycerol and from AA that provide 3 to 5-carbon skeletons (Sunehag et al., 2003). As indicated above, the non-EAA are in deficit and unlikely to be net carbon skeleton donators. Thus, glycerol and EAA are prime candidates as precursors for lactose synthesis, which requires involvement of multiple regulated enzyme steps to coordinate their metabolism for synthesis of lactose. Here, the controlling steps would involve the cytosolic enzymes NAD-dependent malate dehydrogenase and PEPCK.

Second, we (E. Connor, K. Nelson and B.J. Bequette, unpubl.) have recently determined that both the cytosolic and mitochondrial isoforms of PEPCK are expressed in the bovine mammary gland, just they are in dairy cow liver (Agca et al., 2002). PEPCK-cytosolic promotes channeling of propionate and AA through the Krebs cycle towards gluconeogenesis or glycerolneogenesis, whereas PEPCK-mitochondrial promotes “sequestration” of AA carbon skeletons within the mitochondrion (i.e. the Krebs cycle) for non-EAA synthesis or for complete oxidation. In the dairy cow liver (Agca et al., 2002), expression of both PEPCK isoforms increase at initiation of lactation, but it is the PEPCK-cytosolic isoform that is elevated the most. PEPCK-cytosolic is considered to be a key pace-setting enzyme, serving a critical role in maintaining high rates of gluconeogeneis to support high rates of glucose supply for mammary lactose synthesis. By contrast, in the cow mammary gland we observed that the PEPCK-mitochondrial gene expression was 30-fold greater than PEPCK-cytosolic expression. With PEPCK-mitochondrial >> PEPCK-cytosolic, carbon skeletons from catabolism of EAAs would be channeled towards either oxidation (energy) or used for synthesis of deficient non-EAAs.

To determine the sources of carbon skeletons for non-EAA synthesis and whether glucose supplies a portion or all of the galactose for lactose synthesis, we (Bequette et al., 2005) used bovine mammary explants incubated with increasing concentrations of [U-$^{13}C_6$] glucose (0.67 to 27.7 m*M*). First, we found that glucose made

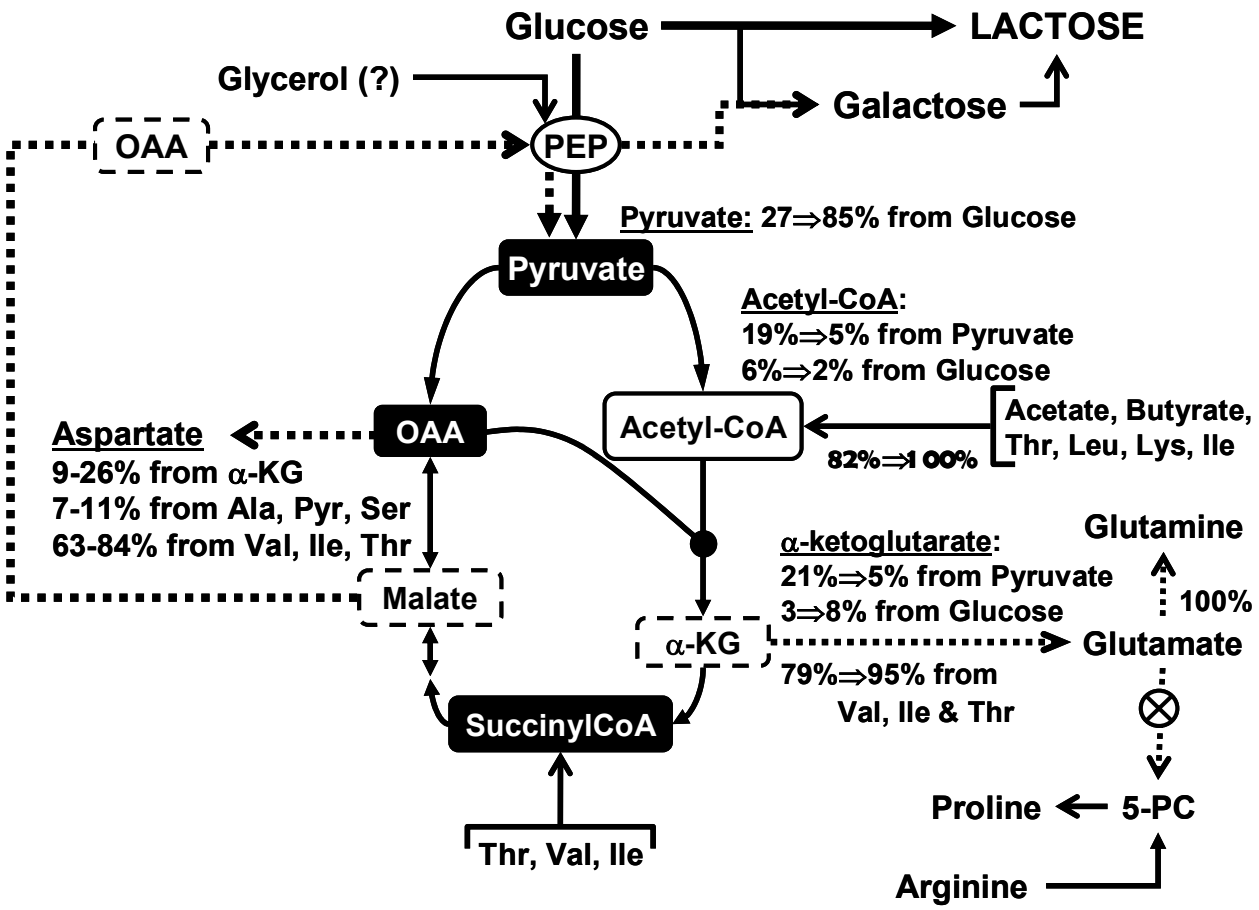


**Figure 3.** Substrate utilization for synthesis of non-essential amino acids and lactose, and Krebs's Cycle metabolism by Bovine mammary gland explants (Bequette et al., 2005).

only a minor (3-9%) contribution to the synthesis of aspartate, glutamate and glutamine and that proline could be derived exclusively from arginine catabolism, but not from glutamate (**Fig. 3**). Thus, we found that 79 to 95% of glutamate and glutamine, and 63 to 84% of aspartate, synthesis was derived from catabolism of threonine, valine and isoleucine. All are AA extracted in excess.

Second, we observed that within the physiological range of plasma glucose (1.11 to 5.55 m*M*), most (46 to 86%) of the galactose in lactose was synthesized *de novo* from non-glucose carbon sources, namely EAA and glycerol. Based on the amount and position of carbon-13 label in galactose, we estimated that 10 to 42% of galactose derived from glycerol with 4 to 12% arising from EAA catabolism. Overall, the results of this investigation provided compelling evidence of the role of EAAs in the synthesis of substantial amounts of non-EAAs and to some extent contributions of carbon skeletons to lactose synthesis. Furthermore, the results provided support for the presence of PEPCK activity in the cytosolic and mitochondrial compartments.

## Some Parting Thoughts

In this brief account of AA metabolism in growing and lactating ruminants, we did not attempt to cover all the roles and routes of AA metabolism. For example, the regulatory roles that the branched chain AA serve in protein synthesis and that which methionine appears to serve in fatty acid metabolism via transmethylation reactions and carnitine synthesis. What we hope to have made clear is that part and parcel of determining the AA requirements of growing and lactating ruminants is to realize their intimate connection to many central pathways of metabolism, namely the Krebs cycle. From the Krebs cycle evolves the pathways for glucose, lactose, fatty acid, glycerol and non-EAA synthesis. The synthesis of these latter must, of course, be balanced to ensure optimal function of the cell, organ and animal. More importantly, these processes must be balanced against the requirement to generate energy by the Krebs cycle. In this overall process, AAs appear to serve a vital role, one that must be appreciated when tallying the AA requirement balance sheet.

## References


Agca, C., R.B. Greenfield, J.R. Hartwell and S.S. Donkin. 2002. Cloning and characterization of bovine cytosolic and mitochondrial PEPCK during transition to lactation. Physiol. Genomics 11: 53.

Basch, J.J., E.D. Wickham, H.M. Farrell, Jr. and J.E. Keys. 1995. Ornithine-$\delta$-aminotransferase in lactating bovine mammary glands. J. Dairy Sci. 78: 825.

Bequette, B.J., F.R.C. Backwell and L.A. Crompton. 1998. Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. J. Dairy Sci. 81: 2540.

Bequette, B.J., C.E. Kyle, L.A. Crompton, A.G. Calder and M.D. Hanigan. 2002. Protein metabolism in lactating goats subjected to the insulin clamp. J. Dairy Sci. 85: 1546.

Bequette, B.J., H. Lapierre and M.D. Hanigan. 2003. Amino acid uptake by the mammary gland of lactating ruminants. In *Amino Acids in Animal Nutrition* [J.P.F. D'Mello, ed.] Wallingford: CABI Publishing, pp. 347-365.

Bequette, B.J., M. Oba, S.L. Owens and R.L. Baldwin, VI. 2004. Assessment of Krebs cycle metabolism by sheep rumen and intestinal cells employing [U-$^{13}$C] glucose and mass isotopomer analysis. FASEB J. 18: A493.

Bequette, B.J., S.L. Owens, S.W. El-Kadi, N.E. Sunny and A. Shamay. 2005. Use of $^{13}$C-mass isotope distribution analysis to define precursors for lactose and amino acid synthesis by bovine mammary explants. J. Dairy Sci. 88(Suppl. 1):289.

Bequette, B.J., N.E. Sunny, S.W. El-Kadi and S.L. Owens. 2006. Application of stable isotopes and mass isotopomer distribution analysis to the study of intermediary metabolism of nutrients. J. Anim. Sci. 84: (Suppl. 1, on-line)

Berthiaume, R., P. Dubreuil, M. Stevenson, B.W. McBride and H. Lapierre. 2001. Intestinal disappearance and mesenteric and portal appearance of amino acids in dairy cows fed ruminally protected methionine. J. Dairy Sci. 84: 194.

Bickerstaffe, R., E.F. Annison and J.L. Linzell. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci. (Camb.) 82: 71.

Borman, A., T.R. Wood, H.C. Black, E.G. Anderson, M.J. Oesterling, M. Womack and W.C. Rose. 1946. The role of arginine in growth with some observations on the effects of argininic acid. J. Biol. Chem. 166: 585.

Bruckental, I., I. Ascarelli, B. Yosif and E. Alumot. 1991. Effect of duodenal proline infusion on milk production and composition in dairy cows. Anim. Prod. 53: 299.

Clark, R.M., P.T. Chandler and C.S. Park. 1978. Limiting amino acids for milk protein synthesis by bovine mammary cells in culture. J. Dairy Sci. 61: 408.

DePeters, E.J. and J.P. Cant. 1992. Nutritional factors influencing the nitrogen composition of bovine milk: A review. J. Dairy Sci. 75: 2043.

El-Kadi, S.W., R.L. Baldwin VI., N.E. Sunny, S.L. Owens and B.J. Bequette. 2006. Intestinal protein supply alters amino acid, but not glucose, metabolism by the sheep gastrointestinal tract. J. Nutr. (accepted).

Elska, A., G. Matsuka, U. Matiash, I. Nasarenko and N. Semenova. 1971. tRNA and aminoacyl-tRNA synthetases during differentiation and various functional states of the mammary gland. Biochim. Biophys. Acta 247: 430

Emmanuel, B. and J.J. Kelly. 1984. Kinetics of methionine and choline and their incorporation into plasma lipids and milk components in lactating goats. J. Dairy Sci. 67: 1912.

Fuller, M.F., R. McWilliam, T.C. Wang and L.R. Giles. 1989. The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. Br. J. Nutr. 62: 255.

Gate, J.J., D.S. Parker and G.E. Lobley. 1999. The metabolic fate of the amido-N group of glutamine in the tissues of the gastrointestinal tract in 24 h-fasted sheep. Br. J. Nutr. 81: 297.

Guinard, J. and H. Rulquin. 1994. Effect of graded levels of duodenal infusions of casein on mammary uptake in lactating cows. 2. Individual amino acids. J. Dairy Sci. 77: 3304.

Heitmann, R.N. and E.N. Bergman. 1981. Glutamate interconversions and glucogenicity in the sheep. Am. J. Physiol. 241: E465.

Jenkinson, C.P. and M.R. Grigor. 1996. Rat mammary arginase-isolation and characterization. Biochem. Med. Metab. Biol. 51: 156.

Lacasse, P., V.C. Farr, S.R. Davis and C.G. Prosser. 1996. Local secretion of nitric oxide and the control of mammary blood flow. J. Dairy Sci. 79: 1369.

Lapierre, H., J.J. Dibner, M. Vazquez-Anon, D. Parker, P. Dubreuil, M. Babkine, G. Zuur and G.E. Lobley. 2002. Use of 2-hydroxy-4-[methylthio]-butanoic acid by lactating dairy cows. J. Dairy Sci. 85(Suppl. 1): 286 (Abstr.)

Lee, J., B.P. Treloar, B.R. Sinclair, C.P. Prosser, S.R. Davis and P.M. Harris. 1996 Utilisation of methionine by the mammary gland of the lactating goat. Proc. NZ Soc. Anim. Prod. 56: 53.

Lobley, G.E. and H. Lapierre. 2001. HMB metabolism in ruminants. Proc.16th Ann. Southwest Nutr. Mgt. Conf. Arizona, USA, pp. 15-23.

Mabjeesh, S.J., C.E. Kyle, J.C. MacRae and B.J. Bequette. 2000. Lysine metabolism by the mammary gland of lactating goats at two stages of lactation. J. Dairy Sci. 83: 996.

MacRae, J.C., L.A. Bruce, D.S. Brown, D.A.H. Farningham and M. Franklin. 1997. Absorption of amino acids from the intestine and their net flux across the mesenteric- and portal-drained viscera of lambs. J. Anim. Sci. 75, 3307.

NRC. 2000. National Research Council. Nutrient Requirements of Beef Cattle: Seventh Revised Edition, National Academy of Sciences, Washington, DC.
NRC. 2001. National Research Council. Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, National Academy of Sciences, Washington, DC.
Oba, M., R.L. Baldwin VI. and B.J. Bequette. 2004. Oxidation of glucose, glutamate, and glutamine by isolated ovine enterocytes in vitro is decreased by the presence of other metabolic fuels. J. Anim. Sci. 82: 479.
Reeds, P.J. 2000. Dispensable and indispensable amino acids for humans. J. Nutr. 130: 1835S.
Reeds, P.J., D.G. Burrin, B. Stoll, F. Jahoor, L. Wykes, J. Henry and M.E. Frazer. 1997. Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. Am. J. Physiol. 36: E408.
Reeds, P.J., D.G. Burrin, T.A. Davis and B. Stoll. 1998. Amino acid metabolism and the energetics of growth. Arch. Anim. Nutr. 51,187.
Rogers, Q.R. 1976. The nutritional and metabolic effects of amino acid imbalances. In: Cole, D.J.A., K. N. Boormann, P. J. Buttery, D. Lewis, R. J. Neale and H. Swan (eds.) *Protein Metabolism and Nutrition.* Europ. Assoc. Anim. Prod., Publ. no. 16, Butterworths, London, UK, pp. 279-299.
Rose, W.C., M.J. Oesterling and M. Womack. 1948. Comparative growth on diets containing ten and nineteen amino acids, with further observations upon the role of glutamic and aspartic acids. J. Biol. Chem. 176: 753.
Shenoy, S. and Q.R. Rogers. 1978. Effect of dietary amino acids on transfer ribonucleic acid charging levels in rat liver. J. Nutr. 108: 1412.
Sunehag, A., S. Tigas and M.W. Haymond. 2003. Contribution of plasma galactose and glucose to milk lactose synthesis during galactose ingestion. J. Clin. Endocrinol. Metab. 88: 225.
Verbeke, R., G. Peeters, A-M. Massart-Leën and G. Cocquyt. 1968. Incorporation of DL-[2-$^{14}$C]ornithine and DL-[5-$^{14}$C]arginine in milk constituents by the isolated lactating sheep udder. Biochem. J. 106: 719.
Wester, T.J., M. Vasquez-Anon, D.S. Parker, J.J. Dibner, A.G. Calder and G.E. Lobley. 2000. Synthesis of methionine from 2-hydroxy-4-methylthio butanoic acid in growing lambs. J. Anim. Sci. 78 (Suppl. 1): 269.
Wu, G. 1998. Intestinal amino acid catabolism. J. Nutr. 128: 1249.