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Livestock Production Science 70 (2001) 63–78

LIVESTOCK PRODUCTION SCIENCE

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Modeling mammary amino acid metabolism

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Abstract

Milk pricing schemes place economic importance on milk components. Most current nutrient requirement models do not predict milk component yields accurately. Deaggregation of energy and protein terms in those models may improve prediction accuracy. Descriptions of energy metabolism by the major postabsorptive tissues have progressed over the last 20 years. More recent efforts have been directed at representing amino acid metabolism. Mammary amino acid metabolism appears to be a function of amino acid supply and regulatory elements. Regulation of uptake and blood flow occurs and is represented in some models. Intracellular metabolism of amino acids and possibly energy are determinants of removal. Both the rate of amino acid oxidation and use for protein synthesis appear to be functions of intracellular concentrations. Experimental observations suggest that representation of protein synthesis as a linear function of the first-limiting amino acid is inadequate. A multi-substrate Michaelis–Menten equation form is more consistent with experimental observations and appears to yield better predictions as compared to the single-limiting model. Consideration of energy supply as a driver of milk protein synthesis also appears to be warranted. Additional knowledge of the substrate response surface for protein synthesis and how it is regulated is needed.

Keywords: Model; Mammae; Metabolism; Amino acid; Blood flow

1. Introduction

Nutrient requirement models have been widely used by the US dairy industry for a number of years [National Research Council (NRC), 1989]. The objective of these models is to specify the quantity of nutrients required to maintain a specified level of milk production. If these models accurately reflect requirements, they should be able to be used to predict performance as well. However, maximal responses, i.e., the maximal genetic potential, have not generally been represented. Thus the models predict unlimited milk and milk component production when presented with unlimited intake. This limitation has been overcome by requiring the user to specify the desired level of production. As milk composition has become an important component of pricing schemes, efforts to develop prediction schemes for milk yield and composition have been undertaken.

Considerable effort has been focused on increasing the accuracy of the requirement models by describing the diet in greater detail and by representing some of the interactions among nutrient classes in

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0301-6226/01/$ – see front matter
PII: S0301-6226(01)00198-1

the rumen (O'Connor et al., 1993; Russell et al., 1992). Although an initial evaluation of the rumen model has been conducted (Kohn et al., 1998), subsequent systematic evaluations apparently have not been conducted. Therefore, it is not clear whether these efforts have improved the accuracy of nutrient supply predictions.

Representations of postabsorptive metabolism are much less advanced as evidenced by the highly aggregated representations in use (NRC, 1989). An adequate representation of the affinity of the various postabsorptive tissue beds for metabolites and how that affinity is regulated is required. Such a representation should be able to simulate variable partition of nutrients. For example, infusion of increasing increments of casein into the abomasum of protein deficient cows results in diminishing increments of milk protein output (Guinard et al., 1994; Whitelaw et al., 1986). Similarly, the amino acid composition of absorbed protein can affect milk protein yield and thus partition of dietary protein. Nitrogen balance has been observed to be negative when an amino acid deficiency occurs during lactation and to become positive when that amino acid is supplemented to create a surplus (Iburg and Lebzien, 2000). Obviously neither response can occur indefinitely and thus the temporal aspects of responses need to be considered. Changes in partition can also be elicited by changing dietary energy supply (Rulquin and Delaby, 1997).

Workers at Cornell University have attempted to address the lack of an amino acid description (O'Connor et al., 1993). Although that model contains a relatively complicated description of ruminal metabolism, postabsorptive metabolism is described in aggregated terms (ME and AP) with amino acid supply and requirements described as a function of AP use. Kohn et al. (1998) conducted an evaluation using a mix of measured inputs and feed library values and found the model to be lacking in accuracy with respect to amino acid and absorbed protein predictions. Wu et al. (1997) evaluated the amino acid prediction aspects of the model and found absolute milk protein yields and yield responses to various amino acids or protein supplementation schemes to be overpredicted (Fig. 1). It is likely that a portion of this bias was caused by the underlying NRC absorbed protein system (Hanigan et al., 1998a). For example, the biases evident in Fig. 1 may be related to the cow's ability to mobilize amino acids from body stores during times of deficiency and replenish those stores during times of surplus (Iburg and Lebzien, 2000; Derrig et al., 1974; Whitelaw et al., 1986). Such a mechanism would dampen the response to amino acid supply possibly causing the apparent bias in predictions. Such carryover effects would be extremely difficult to represent in current static models.

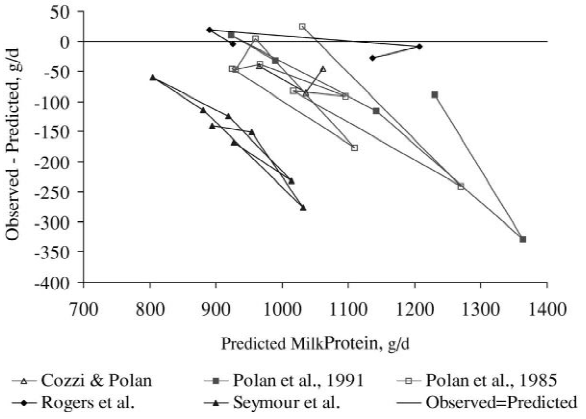

Fig. 1. Residual errors associated with methionine and lysine based predictions of milk protein production. Adapted from an evaluation of the CNCPS model (O'Connor et al., 1993) as reported by Wu et al. (1997).

Several dynamic models of metabolism have been constructed (Baldwin et al., 1987; Smith, 1970; Danfaer, 1990). As these models simulate responses through time, they should accommodate the temporal aspects of previous nutritional status. The metabolic aspects should allow for more robust representations of interactions among metabolites and a framework for application of regulatory aspects. Rates of metabolite utilization in these models were driven primarily by substrate supply and concentration although endocrine regulation was included in each. Based on model analyses, Smith (1970) concluded that more information on the function of liver, mammary, and adipose was required if whole animal metabolism was to be more accurately predicted. Metabolism of mammary, liver, and adipose tissues was further investigated using models of, primarily, energy metabolism as summarized by Baldwin (1995). However, these earlier efforts need to be extended

and complimented with descriptions of amino acid metabolism if accurate whole animal predictions of nitrogen metabolism are to be achieved. Although each of these efforts are important, the rest of this review will focus primarily on representations of mammary amino acid metabolism although some consideration of interactions between energy supply and amino acid metabolism will be included. By focusing on amino acid metabolism, we do not wish to leave the reader with the impression that we feel that energy metabolism is fully understood or that it is unimportant. On the contrary, it is very important and there is still much work to be completed in that area both from an experimental and a modeling standpoint. Similarly many other aspects of whole animal metabolism and regulation thereof are extremely important but will not be considered in this review. Several excellent reviews of these topics have been undertaken.

Modeling amino acid metabolism by the udder has been the focus of a number of recent reports (France et al., 1995; Maas et al., 1997, 1998) including a comprehensive description of amino acid metabolism (Hanigan et al., 2000a,b). This latter effort was intended to complement the earlier work of Waghorn and Baldwin (1984) and Hanigan and Baldwin (1994) which focused on mammary energy metabolism. These works can be loosely categorized as descriptions of metabolite removal and intramammary metabolism. Such a division is useful from a conceptual perspective and thus the remainder of this review will be organized under those broad categories.

2. Metabolite removal

Generally, increased absorption of amino acid from the gut lumen results in an increase in arterial concentrations of those amino acids (Cant et al., 1993; Clark et al., 1977; Guinard and Rulquin, 1994a,b; Miettinen and Huhtanen, 1997; Pacheco-Rios et al., 1999; Seymour et al., 1990; Spires et al., 1975; Whitelaw et al., 1986). Where systemic responses to abomasal infusions of protein or amino acid have not been observed (Griinari et al., 1997; Yang et al., 1986), it seems likely that a change in postabsorptive disposal of amino acid occurred (Whitelaw et al., 1986). Such a change may occur because the maximal milk protein response to amino acid has been achieved and excessive concentrations of some amino acids can be detrimental to long-term survival. Given that systemic concentrations can be affected by absorptive supply, it thus seems logical to pose the following questions: does amino acid supply to the udder affect net removal by the udder? What other factors might affect net removal? How can removal be best represented? The latter question is an important one. In attempting to represent a system, one should consider the objective and then attempt to choose the simplest representation that is capable of achieving the objective. The objective will likely vary depending on the intended use of the model, however, if the goal is to predict milk component production, it seems prudent to set the objective of explaining the observed normal variation in metabolite removal without systematic bias and with minimal residual variation.

A depiction of amino acid removal is presented in Fig. 2. This diagram implies that exchange between capillary and extracellular space is bi-directional, amino acid transport into the cell is bi-directional, uptake is driven by extracellular concentrations, and efflux is driven by intracellular concentrations. It also implies that amino acid concentrations in mammary venous blood are a function of arterial input and cellular net removal. Lastly, it implies that blood

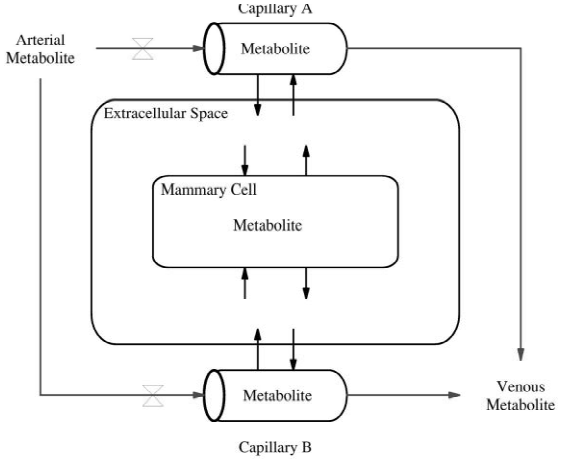

Fig. 2. Flow diagram depicting cell perfusion and metabolite exchange in the udder.

flow both delivers amino acids to the capillary space and removes unused metabolites from that same space. Such a model suggests that A–V may be correlated with arterial concentrations, but that such a correlation would not be perfect. Changes in blood flow as well as regulation of transporter activity would be expected to reduce correlations. Net removal of amino acid by the udder has generally (but not always) been observed to be related to arterial concentrations for essential amino acids (Guinard and Rulquin, 1994a; Linzell and Mepham, 1974; Mepham and Linzell, 1974; Peeters et al., 1979; Rulquin, 1986) and some nonessential amino acids (Guinard and Rulquin, 1994b; Linzell and Mepham, 1974; Mepham and Linzell, 1974; Peeters et al., 1979; Hanigan et al., 1992). Examination of the individual components of the removal system offer insight as to the appropriate representation of the system.

Significant metabolite delivery to the udder and endproduct removal cannot occur in the absence of blood flow. Consequently, it is not surprising that blood flow is highly correlated with milk production (Kronfeld et al., 1968; Peeters et al., 1979; Linzell, 1974). However, nutritive flow, i.e., flow through capillaries, is apparently not perfectly correlated with total mammary blood flow. The permeability surface-area product was reduced approximately fourfold in goats during feed withdrawal or extended milking intervals (Prosser et al., 1996). It is not currently clear whether such a reduction in the permeability surface-area has an impact on net nutrient delivery to the udder. The rate of initial loss of a diffusible marker from the capillary is similar to the rate of entry via arterial flow (Fig. 3). Additionally, it has been observed that diffusible markers have longer times to initial appearance in venous samples than nondiffusible markers (Goresky, 1980) suggesting that the initial exchange with interstitial space was so rapid as to completely deplete diffusible marker from the leading edge of the capillary pulse. Such a high rate of exchange implies that equilibrium between capillary and interstitial space would be rapidly achieved. If diffusion through the interstitial space is equally rapid, alterations in the total number of capillaries being perfused may have minimal effects on the concentration of a metabolite at various points on the cell surface. This assumes

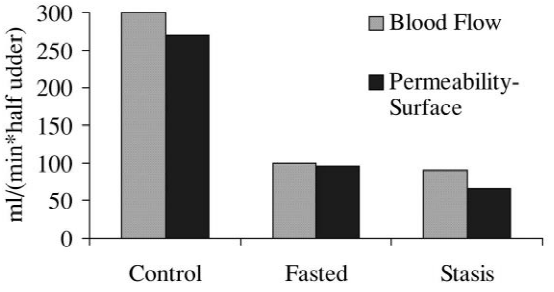

Fig. 3. Responses of mammary blood flow and udder permeability-surface product of goats submitted to an extended milk interval (Stasis) or feed restriction (Fasted). Adapted from Prosser et al. (1996).

that changes in permeability surface-area product are not an indication of blood shunting (Linzell, 1974, see below). If equilibrium between capillary and interstitial compartments is generally achieved, a simplified representation of Fig. 2, where the two compartments are represented as a single entity (Fig. 4), can be adopted.

While changes in the number of capillaries being perfused may not have a dramatic impact on the relationship between arterial input and tissue removal, bypassing capillaries with significant quantities of blood would clearly alter such a relationship. Arteriovenous anastomoses (shunts) and arteriovenular

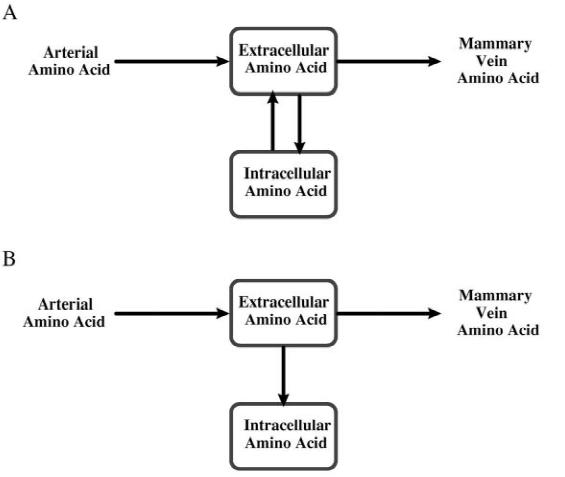

Fig. 4. Flow diagram of mammary amino acid exchange between blood and the intracellular compartment assuming either bi-directional (A) or uni-directional (B) exchange between extracellular and intracellular compartments.

bridges have been observed in mammary tissue (Linzell, 1974); however, it is not clear how prevalent these structures are in the bovine udder. Infusion of adenosine has been found to markedly stimulate blood flow to the udder without a corresponding increase in milk yield (Prosser et al., 1996). As noted by the authors, the incremental increase in flow may not have perfused capillaries which would explain the lack of response. The potential presence of such shunts would require introduction of a bypass flux into models whereby blood is transferred directly from arterial to venous compartments as adopted by France et al. (1995). However, it is not currently clear whether such a mechanism warrants inclusion in other models.

A number of amino acid transporters have been identified as being expressed by mammary tissue (Baumrucker, 1984, 1985). Although not all of these transporters have been completely characterized in bovine mammary tissue, they have been characterized in other species (Calvert and Shennan, 1996; Calvert et al., 1998) and cell types (Bass et al., 1981; Christensen et al., 1967; Oxender and Christensen, 1963). These transporters have been found to exhibit saturation kinetics although a nonsaturable element thought to represent diffusion is generally observed. Maximal velocity of the saturable element is achieved at concentrations that greatly exceed the in vivo range yielding generally linear uptake kinetics within the in vivo concentration range.

Transport of amino acids has been found to be bi-directional (Calvert et al., 1998; Baumrucker, 1984, 1985). There is some support for efflux being driven by intracellular concentrations (Bequette et al., 2001) although it has not been well examined. If influx and efflux are concentration dependent then intracellular and extracellular concentrations are related allowing the representation to be simplified to a uni-directional scheme (Fig. 4b; see Hanigan et al., 1998b for a derivation). Of course, such a simplification would not be adequate for examination of isotope movement.

In general, individual transporters have affinity for more than one amino acids. Such cross-specificity results in potential competition among amino acids. Amino acids have been found to be concentrated within the cell relative to extracellular concentrations (Clark et al., 1980). An energy source is required to establish and maintain this concentration gradient. Several transporters use sodium as an exchange molecule taking advantage of the concentration gradient of sodium maintained by Na/K-ATPase activity (Baumrucker, 1985). However, the L-system exchanges intracellular amino acids for extracellular amino acids using gradients of other amino acids as an energy source. The gradients of these other amino acids are being maintained by sodium dependent transporters (see review by Baumrucker, 1985). To further complicate the matter, most amino acids can be transported by more than one transporter (Calvert and Shennan, 1996, Baumrucker, 1985). This likely helps mitigate negative interactions at a single transporter. Recognition of the potential effects of amino acid competition do not alter the representation in Fig. 4, it simply increases the potential complexity of the representation of uptake by the tissue.

If one accepts that Fig. 4 represents at least a minimal representation of the biology, one can return to our initial question; What are the most appropriate mathematical descriptions of that system? In addressing this question it is useful to keep in mind that models can be used as an aid in the interpretation of data and as prediction tools. The latter generally requires a more robust representation of the biology.

Historically, evaluation of the metabolite removal process has been by calculation of an extraction percentage or proportion:

$$\text{Extraction} = \frac{C_A - C_V}{C_A} \quad (1)$$

where C_A and C_V represent arterial and mammary venous concentrations (mol/l), respectively. It is not clear when this relationship was initially derived, however Graham et al. (1936) used it to describe mammary metabolite removal, and it has been widely utilized since that time. This model has been used primarily as a data analysis tool. There are two problems with Eq. (1) relative to the implications of Fig. 4. The first is the lack of consideration of blood flow. Such an omission suggests that net uptake can proceed in the absence of blood flow. Although this could occur for a very short period of time (s), it obviously cannot occur for an extended period (min).

The second problem is the assumption that metab-

olite is extracted directly from the arterial supply. If exchange of metabolite between capillary and interstitial compartments is rapid, equilibrium will be achieved and interstitial metabolite concentrations will be equivalent to capillary concentrations and flow limited rather than diffusion limited (Goresky, 1980). Given this exchange, venous and interstitial concentrations should be similar and would both be altered by changes in blood flow (unless extraction is 0) while arterial concentrations would not (Cant and McBride, 1995; Hanigan et al., 1998b).

Consideration of blood flow in Eq. (1) by multiplication of the numerator and denominator terms by blood flow prevents predictions of positive extraction when blood flow is 0, but it does not overcome the errors associated with ignoring the effects of blood flow on venous concentrations. Thus Eq. (1) should not be used to evaluate changes in transporter activity if blood flow is affected by treatment. Similarly, it would seem that this model should not be used for prediction purposes unless use is restricted to situations where blood flow is known to remain constant.

A more robust representation of the effects of blood flow on uptake was derived by Hanigan et al. (1998b). This scheme is depicted in Fig. 4b. Prediction of venous concentrations and thus uptake can be made using the form:

$$C_V = \frac{C_A F_M}{K_M + F_M} \tag{2}$$

where F_M represents mammary blood flow (l/min), C_A represents arterial concentration (mol/l), C_V represents venous concentration (mol/l; equivalent to extracellular), and K_M represents a rate parameter for uni-directional uptake (l/min). Rearrangment allows use of the model for data analysis puroposes:

$$K_M = \frac{C_A F_M}{C_V} - F_M \tag{3}$$

Although such a model provides a representation of blood flow, it does not explicitly define regulation of transporter activity (Clark et al., 1980; Shotwell et al., 1982). Such an omission is often unimportant for data analysis purposes, as it may be the hypothesis under examination. However, use of Eq. (2) as a prediction tool will likely result in significant bias when individual amino acids are manipulated independently (see below).

Neither Eq. (1) nor Eq. (3) address potential competitive interactions among amino acids recognized by a common transporter. Maas et al. (1997) has devised a scheme to represent these various transporters and used the scheme to examine the L-system. The representation reflects not only bi-directional transport but also interactions among amino acids for a common transporter. Therefore, any significant transport interactions among metabolites could be accommodated with this scheme. Although further work is needed on this model, initial observations using the model suggested that competition for common transporters was a minor determinant of supply (Maas et al., 1998).

In deriving Eq (3), it was assumed that instantaneous and complete mixing occurs in the extracellular compartment. As discussed above, it is likely that equilibration occurs between capillary and interstitial compartments. However, it seems unlikely that complete mixing from one end of the capillary to the other would occur. Velocity of flow slows from greater than 40 cm/s in large arteries to 0.07 cm/s in capillaries (Melbin and Detweiler, 1993). This reduction in flow should allow some diffusion axially within the capillary although flow would remain laminar and diffusion and mixing are likely impeded by red blood cells (RBCs; Goresky, 1980; Swenson, 1993; Melbin and Detweiler, 1993). Consequently, diffusion of metabolites out of the capillary while blood traverses the capillary would result in a nonlinear time-dependent fall in concentrations from origin to terminus. Cant and McBride (1995) provided a description of this process for the udder based on previous work:

$$C_{cap} = C_A e^{-kt} \tag{4}$$

where C_{cap} represents capillary concentration at any given point within the capillary, k is a diffusion rate constant, and t represents the transit time from blood entry to the point of interest. This model is depicted in Fig. 5. Using this relationship and substituting a calculation of total capillary transit time for t yields an estimation of venous concentration analagous to Eq. (2):

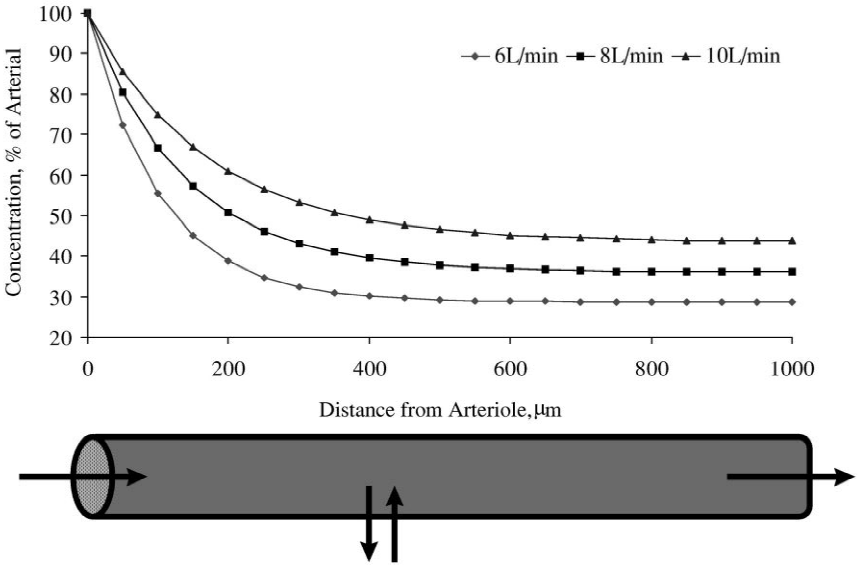

Fig. 5. A representation of the capillary model of Cant and McBride (1995). Blood flow was varied from 6 to 10 l/min.

$$C_V = C_A e^{-k\frac{V_{cap}}{F_M}} \quad (5)$$

where V_{cap} represents capillary volume and the term V_{cap}/F_M providing an estimate of capillary transit time. An estimation of capillary volume is required to use the equation. Cant and McBride (1995) deduced a single capillary volume of 12 560 μm^3 ($1.26 \cdot 10^{-5}$ μl) based on an average capillary length of 1 mm and diameter of 4 μm. Such an estimate can be calculated (Prosser et al., 1996; Gibb et al., 1992) to equate to a total capillary volume of 0.11 l or 0.5% of bovine mammary volume. Doubling capillary diameter (Detweiler, 1993) results in estimates of 0.44 l or 2% of mammary volume. Either estimate is much less than the 5.7% of wet tissue weight estimated for liver (Goresky, 1980). Using observed fluorescein isothiocyanate-albumin transit times (Prosser et al., 1996) one can calculate a capillary volume of 4.2% which seems more reasonable.

While these calculations are interesting in terms of determining consistency of the minimal observations available, the real question is whether capillary volume changes as a function of nutritional or hormonal status. The results of Prosser et al. (1996) suggest that the number of perfused capillaries does change when intakes are restricted or when animals are milked infrequently. It would be interesting to know whether the number of perfused capillaries also changes during insulin infusion or during a histidine deficiency. If such observations were available then one could determine whether such changes in perfusion had significant effects on nutrient extraction kinetics by fitting Eq. (5) to the data or by calculation of the rate parameter directly using a derivation of Eq. (5):

$$k = -\left(\ln\frac{C_V}{C_A}\right) \cdot \left(\frac{F_M}{V_{cap}}\right) \quad (6)$$

A combination of the representations of Cant and McBride (1995) and Maas et al. (1997) should provide the most descriptive model of amino acid removal, but it is not clear whether such a detailed representation is required. A common mistake made when developing models is to add an undue amount of complexity. Each addition should be carefully evaluated and those additions not providing significant benefit excluded from the model. One should also examine the variation associated with parameter estimates, which provides information relative to the number of parameters that can be supported by a given data set.

The discussion thus far has focused on the phys-

ical system and not considered potential regulation of the system. Eqs. (3) and (6) provide a means of evaluating the constancy of uptake parameters as inputs are altered and animals are exposed to various treatments. Transporter activity was apparently increased when systemic histidine concentrations were reduced (Bequette et al., 2000). Others have observed an apparent increase in transporter activity under hyperinsulinemic conditions (Bequette et al., 2000; Laarveld et al., 1981). Provision of more of a given amino acid often results in a decline in transporter activity (Varvikko et al., 1999) although this is not universal (Clark et al., 1977; Derrig et al., 1974; Guinard and Rulquin, 1994b; Spires et al., 1975).

Assuming that changes in transporter activity are indeed occurring in the above situations, such changes generally seem to reflect the balance of amino acid supply and use for protein synthesis. As intracellular amino acid concentrations likely reflect this balance, it is possible that the observed changes in net transporter activity are mediated by a change in efflux from the cell. However, this does not appear to be the case for the histidine response model used by Bequette et al. (2000). They found that unidirectional uptake explained the majority of the observed change in net uptake suggesting that the number of transporters expressed or the activity of those transporters was altered. These results underscore the need to accommodate regulation of transport activity when attempting to predict amino acid removal (Eqs. (2) and (5)).

If the change in activity results from a true change in transporter activity, the process can be represented as a derivation of Eq. (2):

$$C_V = \frac{C_A F_M}{K_M f(C_I) + F_M} \quad (7)$$

where $f(C_I)$ represents some function of intracellular concentration of any given modulator. The modifying function can be as simple as direct use of the modifier concentration, or a more complex function can be used. We have commonly used a ratio of C_I to some reference intracellular concentration of C_I, typically the mean concentration. An exponent can be applied to the ratio to allow sensitivity changes

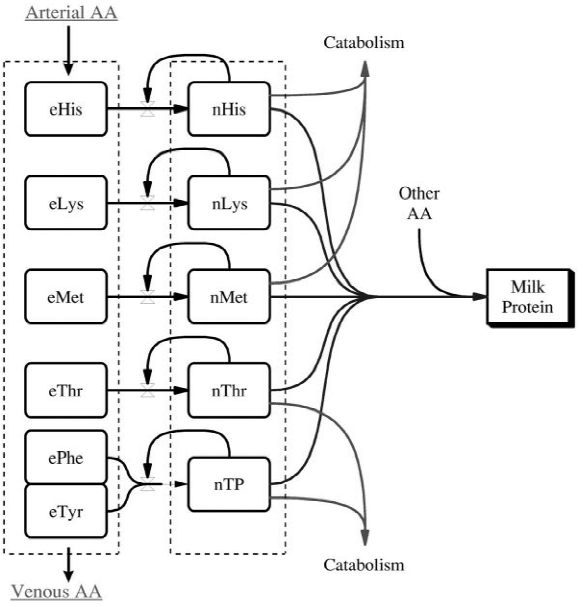

Fig. 6. Flow diagram depicting mammary essential amino acid metabolism. His, Lys, Met, Thr, and TP represent histidine, lysine, methionine, threonine, and tyrosine plus phenylalanine, respectively. Lower case e's and n's preceding amino acid abbreviations designate extracellular and intracellular compartments, respectively.

without affecting the flux rate when concentrations are equal to the reference concentration. The same approach can be used to alter the rate constant in Eq. (5) which is effectively what the term V_{cap}/F_M is accomplishing. Such a representation was used by Hanigan et al. (2000c) (Fig. 6).

3. Intracellular metabolism

Although mammary amino acid uptake is an interesting topic, knowledge of such is of minimal value if one cannot predict how amino acids that have been taken up will be utilized. As noted above, arterial essential amino acid concentrations and rates of milk protein synthesis generally increase when casein is infused abomasally (Choung and Chamberlain, 1993; Clark et al., 1977; Derrig et al., 1974; Guinard and Rulquin, 1994a; Guinard et al., 1994;

Huhtanen et al., 1997; Schwab et al., 1976; Seymour et al., 1990; Spires et al., 1975). The milk protein response has been observed to be asymptotic (Whitelaw et al., 1986). The asymptote and the variability in responses presumably reflect limitations in other metabolites, such as total energy supply (Hanigan et al., 1998a) or individual metabolites (see Thomas and Chamberlain, 1984), the genetic potential of the animal, the endocrine status of the animal (Peel et al., 1983), and other environmental factors. A multitude of studies designed to examine the relative deficiency of individual amino acids have been conducted. Conclusions that can be collectively drawn are: failure to stimulate arterial amino acid concentrations does not result in a milk protein yield response (Yang et al., 1986). Responses to one or more essential amino acids can be observed (Illg et al., 1987; Metcalf et al., 1996; Rulquin and Delaby, 1997; Schwab et al., 1976). Responses are apparently dependent upon the relative sufficiency of the basal diet with provision of additional amino acids to a sufficient diet not eliciting a response (Guinard and Rulquin, 1994b; Mabjeesh et al., 2000; Pacheco-Rios et al., 1999; Schwab et al., 1976; Vanhatalo et al., 1999; Varvikko et al., 1999). However, cows on diets that are thought to be deficient for a particular amino acid do not always respond to alleviation of this deficiency (Seymour et al., 1990). Failure to respond to an amino acid that is thought to be limiting likely reflects the inadequacy of our understanding of intramammary metabolism. In particular, a description of the response surface with respect to the various amino acids is likely inadequate. Knowledge of nonmammary factors that affect protein synthesis may also be lacking.

It is not clear whether the relationship between amino acid supply and protein synthesis is simply a substrate effect or a reflection of regulatory events. Although tRNA-acylating enzymes have been observed to be saturated at prevailing intracellular amino acid concentrations for nonmammary tissues (Shenoy and Rogers, 1978), such may not be the case for the udder (Elska et al., 1971). If udder tRNA-acylating enzymes are not saturated with amino acids, provision of additional amino acids may result in an increase in acylated-tRNA concentrations. Given that all 20 amino acids are required to synthesize milk protein, limitations may occur simultaneously for any number of amino acylated-tRNAs. Consequently, the relative deficiency and the number of moles of that particular amino acid required to synthesize a mole of milk protein would determine the substrate response when that deficiency is alleviated.

Initiation of translation is regulated by a variety of factors including one or more amino acids (McGown et al., 1973; Perez-Sala et al., 1991; Yokogoshi and Yoshida, 1980) and their unacylated-tRNAs (Iiboshi et al., 1999). Peptide chain elongation and termination and expression and turnover of milk protein mRNAs are also potential points of regulation (see Taylor and Brameld, 1999). These points of control have yet to be examined in mammary epithelial cells. If such a system operates in mammary tissue, then amino acids may act not only as substrates for milk protein synthesis, but also as regulators of milk protein synthesis. Further, multiple amino acids may be rate-limiting simultaneously, thus requiring a shift in our classic definition and use of the term ‘nutritionally limiting amino acid’. Such a concept is consistent with the observations of Clark et al. (1978) wherein responses to three different amino acids were observed under identical culture conditions. If milk protein synthesis is sensitive to multiple amino acids simultaneously, then changes in arterial concentrations of amino acids associated with amino acid (Vanhatalo et al., 1999; Varvikko et al., 1999) or endocrine infusions (Bequette et al., 2001; Griinari et al., 1997) could be very important when predictions of milk protein production are attempted. This multitude of factors may explain some of the difficulty encountered when attempting to define requirements for individual amino acids.

Despite the apparent complexity of protein synthesis and the multiple potential regulation sites, it has typically been represented as a simple linear function of the most limiting amino acid (Hanigan and Baldwin, 1994; Hanigan et al., 2000a; Maas et al., 1997) or of amino acid supply in aggregate (Baldwin et al., 1987; Danfaer, 1990) as has been adopted for monogastric growth models (D’Mello, 1994). However, the work of Clark et al. (1978) suggest that such a representation will not work for mammary tissue. A representation more consistent

with the previous discussion has been used by Hanigan et al. (2000c):

$$U_{AA,Pm} = \frac{V_{AA,Pm}}{1+\left(\frac{k_{I_1,Pm}}{C_{I_1}}\right)^{Exp_{I_1,Pm}}+\left(\frac{k_{I_2,Pm}}{C_{I_2}}\right)^{Exp_{I_1,Pm}}+\ldots\left(\frac{k_{I_n,Pm}}{C_{I_n}}\right)^{Exp_{I_n,Pm}}} \quad (8)$$

where $U_{AA,Pm}$ represents the conversion of amino acid to milk protein, $V_{AA,Pm}$ represents the maximal velocity of the reaction, C_I represents the intracellular concentration of individual amino acids (1 to n), k_I represents the apparent affinity constants for the respective amino acid considered in the reaction, and Exp_I represents an exponent that can be used to adjust sensitivity to concentrations of individual amino acids if needed. Adjustment of the various exponents to values other than 1 should reflect the varied molar proportions of each amino acid in milk protein and the relative importance of the respective amino acid in the regulatory process.

Use of Eq. (8) provided slightly more accurate predictions of milk protein yield when simulating 21 data sets from the literature than an equation based on the single-limiting amino acid theory (143 vs. 153 g/day residual error and 60 vs. 44% variation explained, respectively; Hanigan et al., 2000c). Many of the experiments used for this analysis contained treatments where all amino acid were manipulated simultaneously, i.e., casein infusions. Consequently, the hypothesis that individual amino acids independently affect milk protein synthesis was not particularly well tested other than for lysine and methionine. More recent observations offer a chance to test the model with more independent variation in additional essential amino acid concentrations. Use of a multi-substrate representation of milk protein synthesis may also help explain how the udder is able to minimize a milk protein production drop to 20% when arterial histidine concentrations have declined ninefold (Bequette et al., 2000).

If milk protein synthesis (Eq. (8)) and amino acid removal (Eq. (7)) are both a function of intracellular amino acid concentrations (Fig. 6), an accurate description of those concentrations is required. Assuming milk protein synthesis and amino acid removal can be accurately represented by some combination of the above equations, one need only define the relationship describing amino acid catabolism in order to predict concentrations. This assumes that net endogenous protein synthesis and non-free sources of amino acid are insignificant contributors to amino acid supply which may not be true for some amino acids (Backwell et al., 1994). The various mammary models that have attempted to describe intracellular amino acid concentrations (Hanigan et al., 2000a; Hanigan and Baldwin, 1994; Maas et al., 1997, 1998; Waghorn and Baldwin, 1984) have generally incorporated the assumption that amino acid catabolism is a mass action function of amino acid concentration. Provision of additional amino acid to the udder results in an increase in oxidation of that amino acid (Bequette et al., 1996; Mabjeesh et al., 2000) supporting such a concept. However, it has been observed that mammary oxidative enzymes for some amino acids are regulated (DeSantiago et al., 1998) which may require a more complicated representation.

Fig. 6 represents an integration of the above concepts. In this scheme, the effects of factors other than amino acids can easily be accommodated, e.g., $V_{AA,Pm}$ or k_I (Eq. (8)) can be made a function of the appropriate stimulator or inhibitor. Using the effects of insulin as an example, stimulation of protein synthesis would result in additional intracellular amino acid use leading to a reduction in intracellular concentrations. This fall in concentrations would result in both a reduction in oxidation and an increase in transport activity in the above model. Conversely, if insulin stimulates amino acid transport, intracellular concentrations would increase which should result in an increase in milk protein synthesis (provided the system is not saturated or limited by other substrates) and an increase in amino acid oxidation (Bequette et al., 2001).

The concept of other metabolites possibly limiting the milk protein response to amino acids is an important one as it is an energetically demanding process. Milk protein synthesis appears to consume 10 to 12% of the total ATP supply in the udder (calculated from Hanigan and Baldwin, 1994). Such an estimate does not consider any turnover of milk or mammary constitutive protein (Bequette et al., 1998; Razooki-Hasan et al., 1982). It has been observed that milk protein synthesis and endogenous protein

turnover vary in concert at a ratio of 1:2.5 (Bequette et al., 1998). If that is the case, then a minimum of 35% of the available ATP supply may be utilized in support of protein synthesis. Energy supply to the animal and protein yield are correlated (Hanigan et al., 1998a; Rulquin and Delaby, 1997; see Thomas and Chamberlain, 1984) suggesting that energy status plays a role in determining protein synthesis rates. Milk protein yield generally responds positively to infusions of glucose, acetate, and casein and negatively to infusions of butyrate with mixed responses to propionate (Maas et al., 1995; Thomas and Chamberlain, 1984). Milk protein yield has also been observed to increase when cows are fed high fat diets (DePeters and Cant, 1992; unpublished observations, Purina Mills, Inc.). In the case of glucose and propionate infusions, it is not clear whether the effects are direct substrate effects, a reflection of endocrine status, or amino acid sparing. High starch diets and glucose infusions should result in a shift in insulin status. Additionally those diets may spare essential amino acids by reducing amino acid catabolism. Endocrine and amino acid sparing effects seem less likely to be the mechanism of action for acetate infusions and fat feeding. It seems more likely that they exert their effects by altering the energy status of the udder. The model of Hanigan et al. (2000a) did not consider energy status as a driving variable for milk protein synthesis. Analysis of residual errors from simulations of literature data (Hanigan et al., 2000c) indicated that acetate supply was correlated with milk protein yield prediction errors. Such a residual pattern suggests that acetate supply or some factor associated with acetate supply should be considered as a driver of milk protein synthesis.

4. Metabolic regulation

A number of factors likely regulate the rate of mammary metabolism including endocrines and substrates. As blood flow plays a role in determining substrate delivery to the udder and uptake by the udder, it is possible that the rate of metabolism may be regulated to some extent by blood flow. The observation that the udder has an intrinsic ability to regulate its own flow (Linzell, 1974) has been construed to indicate that no external factors regulate flow. Although a correlation between CO_2 production by the udder and mammary blood flow has been observed, variation explained was moderate (Nielsen et al., 1995) suggesting that multiple factors are involved. The difficulty lies in determining the effects of these other factors as most also affect milk yield. A summary of some of those factors is provided by Knight et al. (1994). However, limitations in a single amino acid have recently been associated with increases in blood flow (Bequette et al., 1996, 2000) despite significant declines in milk protein output (and presumably CO_2 production) supporting the hypothesis that factors external to the secretory cell are involved in regulating flow. Similarly, Rulquin and Verite (1993) observed that mammary blood flow increased by an equal amount in dry and lactating cows injected with bovine somatotropin (bST). As milk production obviously was not stimulated in the dry cows, changes in metabolic activity of the udder would not appear to be the driving force for increased flow during bST treatment in those animals. If flow were a driving variable, it would help explain the lack of a milk protein response to IGF-1 and insulin in vitro (Hanigan et al., 2000d).

Although the above evidence suggests that blood flow may partially drive milk protein yield, several reports where blood flow changes have not affected protein yield exist (Prosser et al., 1996). Similarly, blood flow increases during insulin infusions precede increases in milk protein yield (Bequette et al., 2001), suggesting that blood flow is not the sole driving variable. Cant and McBride (1995) have provided a framework for representing both the physical and the regulatory aspects of the system.

Where increased blood flows have failed to stimulate milk protein yields, mammary enzymatic capacity may be the limiting factor. Stage of lactation, management practices (times milked per day), and genetic potential play a role in determining enzymatic capacity. Although stage of lactation effects on milk production have been modeled (Dijkstra et al., 1997; Friggens et al., 1999; Neal and Thornley, 1983; Wood, 1967) and extensive studies of enzyme levels in rodents have been undertaken (reviewed by Baldwin and Yang, 1974), stage of lactation effects on mammary metabolism have not been well examined in ruminants. Udder protein mass appears to

decline as lactation progresses and the decline appears to be greater for animals on a low energy diet than for those on a higher energy diet (Gibb et al., 1992). This may suggest that enzymatic capacity is affected similarly. Clearly it declines as lactation progresses (Knight and Wilde, 1993; Knight and Peaker, 1984). The relationship between component yields and various enzyme levels has been examined (Currie (1972; Knight and Peaker, 1984; Wilde et al., 1986). Others have looked at A–V at four time points during the first 118 d of lactation (Hanigan et al., 1992; Miller et al., 1991b). In this latter study, calculated uptake parameters for only arginine, lysine, and methionine were negatively associated with week of lactation ($P<0.05$) although the general trend for most essential amino acids was negative. Although these observations are not comprehensive, it would seem that the effects of stage of lactation are mediated by changes in the total quantity of enzyme present in the udder and thus $V_{AA,Pm}$ in Eq. (8) should be represented as a function of time and initial cell numbers rather than as a constant.

Management techniques such as thrice-daily milking or once-daily milking also exert effects on productivity. Wilde et al. (1987) observed an increase in gland mass, DNA, RNA, and, enzyme activity for most key energy and milk fat related enzymes in glands milked 3× versus those milked 2×. A water soluble inhibitor secreted with milk (Rennison et al., 1993) is thought to cause inhibition of enzymatic activity and increased degradation of synthesized milk proteins prior to secretion (Wilde et al., 1989). The effect of this factor on mammary enzyme activity has been represented in the model of Baldwin et al. (1987). However, it generally has not been considered in models of mammary metabolism. Such consideration may be pertinent given that the milking interval is not consistent across experiments. Clearly it is pertinent for field application of models.

The maximal response to metabolites would ultimately be genetically determined. Considerable information pertaining to the effects of genetic selection on milk yield and composition exist. However, it is not clear what metabolic pathways are affected. Milk output at peak lactation has been found to be correlated with udder size (Linzell, 1974) and parenchymal tissue mass (Fowler et al., 1990). This is an important parameter as the response to additional amino acids would depend on how close to the process is to V_{max} with excess amino acid used to support tissue accretion or oxidized (Iburg and Lebzien, 2000; Derrig et al., 1974; Hanigan et al., 1998a; Whitelaw et al., 1986). Some work using cows with varying milk potential has been conducted (Hanigan et al., 1992; Miller et al., 1991a,b). Using this latter work and Eq. (3), there appears to be little relationship among essential amino acid uptake parameters and genetic potential (data not shown). With current progress in genomics, it would seem likely that a wealth of information will become available on, at least, gene expression over the coming years. Such information can be used to construct representations of rate parameters in the models that are a function of genetic potential, stage of lactation, management, environment, etc.

5. Summary

The modeling work reviewed herein provides a framework for implementation of basic mammary biological information in that the physical systems have been described or a framework for their description has been outlined. However, much work remains in that these descriptions are juvenile particularly those of intracellular metabolism and regulation thereof. A tremendous amount of information exists in the literature that can be used to test and extend these models. These extensions can take the form of submodels within the existing framework wherein more detail is provided for a specific function (e.g., the rate constant for protein synthesis is determined from a protein synthesis and export submodel) or as complete replacements of one or more functions. The intended use of the model, field application or research, would dictate which approach is taken. Such a task is not trivial and will require the efforts of both biologists and modelers. Use of these models for analyses of future experiments should provide advantages as compared to classical statistical approaches in that changes in substrate concentrations other than the independent variable can be accommodated in the analysis whereas such changes are generally ignored or treated in a less satisfactory manner in classical statistical models. Future progress relative to regulation of the

system will require that relationships among various treatment factors and changes in rate parameters in the models be examined. Simply describing changes in A–V, although providing useful information, will not, in and of itself, provide insight as to how the system is regulated. Regulation of intracellular metabolism must be further evaluated. We have little knowledge of control of protein synthesis and it has been assumed that catabolism is a function of intracellular amino acid concentrations. Data adequate to better define and parameterize those equations are needed. If protein synthesis responds independently to a number of amino acids and energy supply, the experimental work required to define such a response surface would be prodigious, but must be completed if further progress is to be made.

Acknowledgements

This work was supported as part of a consortium of government and industrial entities including the Ministry of Agriculture Fisheries and Food, the Biotechnology and Biological Science Research Council, Purina Mills, Inc., the Milk Development Council of England and Wales, and NUTRECO, Inc.

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