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A Model for Quantifying the Contribution of Extracellularly-derived Peptides to Milk Protein Synthesis in the Ruminant Mammary Gland

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A model based on the dual-labelled tracer technique of Backwell and co-workers is proposed which allows detailed resolution of isotope dilution data that can be generated by this technique, provided these data are supplemented with appropriate arterio-venous difference and blood flow measurements. The proposed model is constructed and solved in the steady state. Model solution permits, if assumptions are made, calculation of the uptake of an amino acid Y from the arterial blood supply in both peptide-bound and free form, oxidation of Y, export of Y into the venous drainage, and synthesis and degradation of milk protein and constitutive tissue protein within the gland. The model provides a means of evaluating the role of amino acids of peptide origin in milk and constitutive protein synthesis in the mammary gland of the lactating ruminant.

1. Introduction

It is recognised that mammalian tissues are capable of using small peptides as sources of amino acids for protein synthesis. Studies with monogastrics have shown that the kidney, liver and skeletal muscle can hydrolyse small peptides (Lochs *et al*., 1986, 1988; Roth *et al*., 1988), and positive nitrogen balance can be maintained in baboons by parenteral infusion of a mixture of glycyl peptides (Steinhardt *et al*., 1984). In ruminants a significant proportion of total amino acid in blood is in peptide- and protein-bound forms, and there is now evidence to suggest that sources other than free amino acid in blood or plasma may be required for protein synthesis in the mammary gland (Backwell, 1994; Metcalf *et al*., 1994). The ability of the mammary gland to utilise synthetic dipeptides for milk casein biosynthesis has been demonstrated recently in dairy goats using a dual-labelled tracer technique (Backwell *et al*., 1994). Whether peptides represent a nutritionally important source of amino acids for tissue or milk protein synthesis is unclear, though it has been suggested that a substantial proportion of amino acid uptake across the gastrointestinal tract of ruminants may be in the form of peptide-bound amino acids (Webb *et al*., 1992).

The procedure developed by Backwell *et al*. (1994) was designed to evaluate the ability of the mammary gland of the lactating ruminant to utilise amino acids of peptide origin for milk protein synthesis *in vivo*. It involves infusion into the external pudic artery supplying one half of the udder of a dipeptide XY where Y is a [^{13}C]-labelled amino acid, coupled with a simultaneous peripheral (jugular) infusion of the amino acid Y but with a [^{2}H]-label. In theory, if the half of the udder receiving close-arterial infusion of the dipeptide utilises peptide-bound Y for milk protein synthesis, then the ratio [^{13}C]:[^{2}H] will be greater in milk protein secreted from that half of the udder compared to the other (control) side. This was demonstrated for the infused peptides glycyl-[^{13}C]-L-

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leucine and glycyl-[^{13}C]-L-phenylalanine (Backwell *et al*., 1994).

The model proposed here is based on the dual-labelled tracer procedure outlined above, and allows a detailed kinetic resolution of the isotope dilution data that can be generated by this procedure, if these data are supplemented with appropriate arterio-venous difference and blood flow measurements. It represents a development of the isotope dilution model for partitioning uptake of an amino acid by the mammary gland reported by France *et al*. (1995) by incorporating peptide amino acid into the scheme.

2. The Model

The schema for total (i.e. unlabelled plus labelled) amino acid Y is given in Fig. 1(a). The udder is divided into two halves, the peptide infused half and the control half (i.e. non-infused). Each half (i.e. each mammary gland in the case of the goat) is supplied by a common arterial pool of free Y (pool 2). In addition, there are two intracellular and two extracellular pools in each half udder. The intracellular pools are free Y and Y in milk protein (pools 5 and 4 respectively in the infused half; pools 6 and 7 in the other) and the extracellular ones are arterial peptide-bound Y and venous (free plus peptide-bound) Y (pools 1 and 8; 3 and 9). The fluxes of Y between pools and into and out of the system are represented as arrowed lines. The intracellular free Y pool has four influxes, i.e. from the degradation of constitutive mammary gland protein and undefined sources (F_{50}; F_{60}), from the extracellular arterial peptide-bound (F_{51}; F_{63}) and free (F_{52}; F_{62}) Y pools, and from the degradation of milk protein (F_{54}; F_{67}), and five effluxes, i.e. secretion in milk ($F_{05}^{(m)}$; $F_{06}^{(m)}$), oxidation ($F_{05}^{(o)}$; $F_{06}^{(o)}$), synthesis of constitutive mammary gland protein ($F_{05}^{(s)}$; $F_{06}^{(s)}$), incorporation into milk protein (F_{45}; F_{76}), and outflow into the venous pool (F_{85}; F_{96}). The milk protein-bound Y pool has one influx, from intracellular free Y, (F_{45}; F_{76}), and two effluxes, secretion of protein in milk (F_{04}; F_{07}), and degradation (F_{54}; F_{67}). The extracellular arterial peptide-bound Y pool also has a single influx, entry into the pool (F_{10}; F_{30}), and three effluxes, uptake by the mammary gland (F_{51}; F_{63}), and outflow to the arterial free Y pool (F_{21}; F_{23}) and to the venous Y pool (F_{81}; F_{93}). The venous Y pool has three influxes, entry from the arterial peptide-bound (F_{81}; F_{93}) and free Y (F_{82}; F_{92}) pools and from the mammmary gland (F_{85}; F_{96}), and one efflux (F_{08}; F_{09}). The common arterial free Y pool supplying each half of the udder has three influxes, external entry into the pool, F_{20}, and entry from both the arterial peptide pools, F_{21} and F_{23}, and four effluxes, uptake by each gland, F_{52} and F_{62}, and outflow to the venous pools, F_{82} and F_{92}.

The schema for [^{13}C]-labelled Y is given in Fig. 1(b). The [^{13}C]-labelled dipeptide XY is administered to one half of the udder by infusion at a constant rate I_1 into pool 1, and the enrichment of the various pools monitored. The enrichment of the two intracellular pools in each gland cannot be measured directly, therefore the enrichments of the intracellular free Y and milk protein-bound Y pools are assumed equivalent to the enrichments of free and protein-bound Y respectively in secreted milk. The only re-entry of [^{13}C]-label is assumed to occur via the flux I_2 into the common arterial pool 2, following whole-animal hydrolysis of the infused peptide XY. The schema for [^{2}H]-label is given in Fig. 1(c). Following a constant infusion into the jugular vein, ^{2}H-labelled Y enters pool 2 at a rate Φ_2. Φ_2 includes both directly infused and recycled ^{2}H-labelled Y. Each schema for labelled Y assumes that the enrichment of constitutive mammary protein can be regarded as negligible over the period of the infusions.

Conservation of mass principles can be applied to each pool in Fig. 1 to generate differential equations which describe the dynamic behaviour of the system. For total amino acid Y, these differential equations are (mathematical notation is defined in Table 1):

$$dQ_1/dt = F_{10} - F_{21} - F_{51} - F_{81} \quad (1)$$

$$dQ_2/dt = F_{20} + F_{21} + F_{23} - F_{52} - F_{62} - F_{82} - F_{92} \quad (2)$$

$$dQ_3/dt = F_{30} - F_{23} - F_{63} - F_{93} \quad (3)$$

$$dQ_4/dt = F_{45} - F_{04} - F_{54} \quad (4)$$

$$dQ_5/dt = F_{50} + F_{51} + F_{52} + F_{54} - F_{05}^{(m)} - F_{05}^{(o)} - F_{05}^{(s)} - F_{45} - F_{85} \quad (5)$$

$$dQ_6/dt = F_{60} + F_{62} + F_{63} + F_{67} - F_{06}^{(m)} - F_{06}^{(o)} - F_{06}^{(s)} - F_{76} - F_{96} \quad (6)$$

$$dQ_7/dt = F_{76} - F_{07} - F_{67} \quad (7)$$

$$dQ_8/dt = F_{81} + F_{82} + F_{85} - F_{08} \quad (8)$$

$$dQ_9/dt = F_{92} + F_{93} + F_{96} - F_{09} \quad (9)$$

for [^{13}C]-labelled Y:

$$dq_1/dt = I_1 - e_1(F_{21} + F_{51} + F_{81}) \quad (10)$$

$$dq_2/dt = I_2 + e_1F_{21} - e_2(F_{52} + F_{62} + F_{82} + F_{92}) \quad (11)$$

$$dq_4/dt = e_5F_{45} - e_4(F_{04} + F_{54}) \quad (12)$$

$$dq_5/dt = e_1F_{51} + e_2F_{52} + e_4F_{54} - e_5(F_{05}^{(m)} + F_{05}^{(o)} + F_{05}^{(s)} + F_{45} + F_{85}) \quad (13)$$

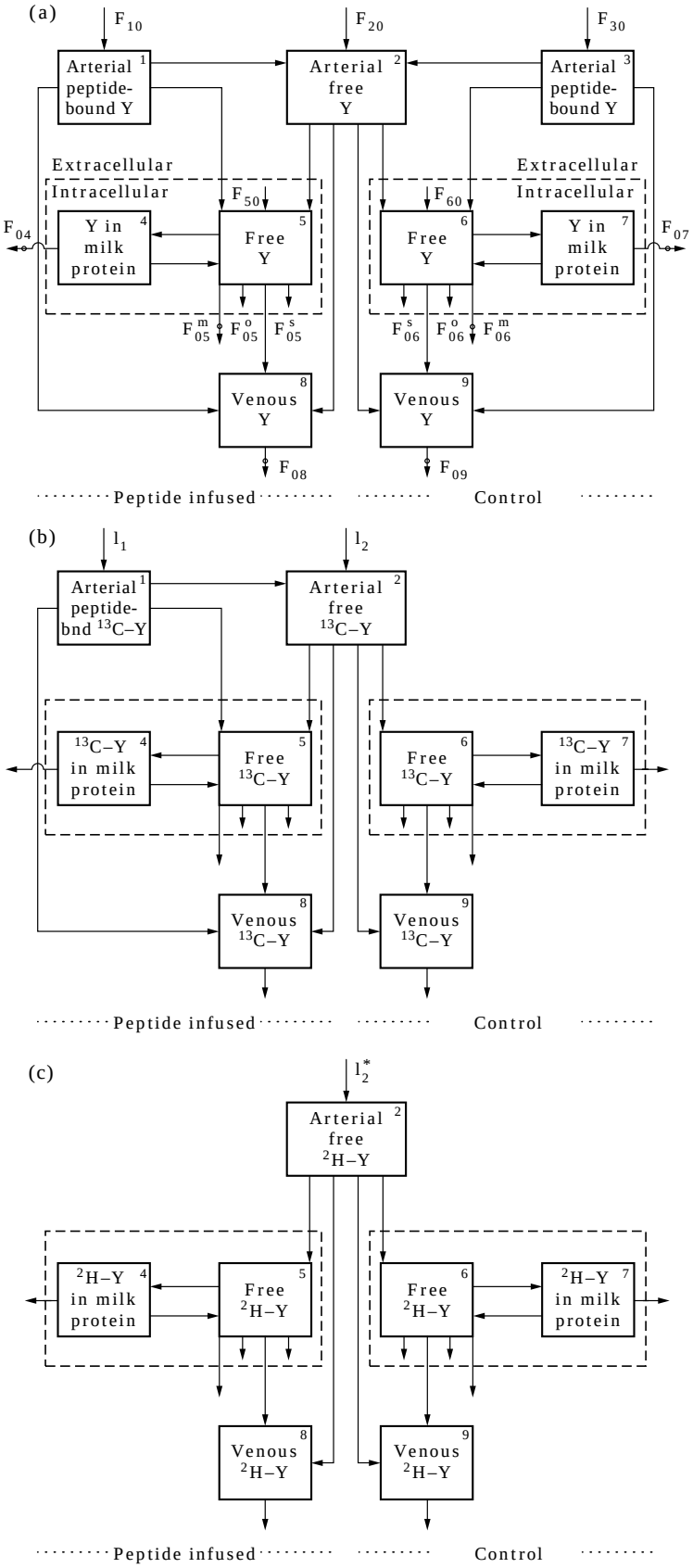

FIG. 1. Scheme for peptide uptake by the udder: (a) total amino acid *Y*, (b) [^{13}C]-labelled *Y* and (c) [^{2}H]-labelled *Y*. The small circles in Fig. 1(a) indicate fluxes which need to be measured experimentally.

TABLE 1
Principal symbols

F_{ij}	Total flux of amino acid Y to pool i from j; F_{i0} denotes an external flux into pool i and F_{0j} a flux from pool j out of the system; a tilde indicates a flux which can be measured experimentally: μmol hr^{-1}
I_i	External entry of [^{13}C]-labelled Y into pool i: μmol hr^{-1}
Φ_i	External entry of [^{2}H]-labelled Y into pool i: μmol hr^{-1}
Q_i	Total quantity of Y in pool i: μmol
q_i	Quantity of [^{13}C]-labelled Y in pool i: μmol
ψ_i	Quantity of [^{2}H]-labelled Y in pool i: μmol
e_i	[^{13}C]-enrichment of pool i ($= q_i/Q_i$): μmol [^{13}C]-labelled Y/(μmol total Y)
ε_i	[^{2}H]-enrichment of pool i ($= \psi_i/Q_i$): μmol [^{2}H]-labelled Y/(μmol total Y)
$R^{(I)}$, $R^{(N)}$	Dilution ratio in the peptide infused and not infused half of the gland respectively: both dimensionless
t	Time: hr

$$\mathrm{d}q_6/\mathrm{d}t = e_2F_{62} + e_7F_{67} - e_6(F_{06}^{(m)} + F_{06}^{(o)} + F_{06}^{(s)} + F_{76} + F_{96}) \quad (14)$$

$$\mathrm{d}q_7/\mathrm{d}t = e_6F_{76} - e_7(F_{07} + F_{67}) \quad (15)$$

$$\mathrm{d}q_8/\mathrm{d}t = e_1F_{81} + e_2F_{82} + e_5F_{85} - e_8F_{08} \quad (16)$$

$$\mathrm{d}q_9/\mathrm{d}t = e_2F_{92} + e_6F_{96} - e_9F_{09} \quad (17)$$

and for [^{2}H]-labelled Y:

$$\mathrm{d}\psi_2/\mathrm{d}t = \Phi_2 - \varepsilon_2(F_{52} + F_{62} + F_{82} + F_{92}) \quad (18)$$

$$\mathrm{d}\psi_4/\mathrm{d}t = \varepsilon_5F_{45} - \varepsilon_4(F_{04} + F_{54}) \quad (19)$$

$$\mathrm{d}\psi_5/\mathrm{d}t = \varepsilon_2F_{52} + \varepsilon_4F_{54} - \varepsilon_5(F_{05}^{(m)} + F_{05}^{(o)} + F_{05}^{(s)} + F_{45} + F_{85}) \quad (20)$$

$$\mathrm{d}\psi_6/\mathrm{d}t = \varepsilon_2F_{62} + \varepsilon_7F_{67} - \varepsilon_6(F_{06}^{(m)} + F_{06}^{(o)} + F_{06}^{(s)} + F_{76} + F_{96}) \quad (21)$$

$$\mathrm{d}\psi_7/\mathrm{d}t = \varepsilon_6F_{76} - \varepsilon_7(F_{07} + F_{67}) \quad (22)$$

$$\mathrm{d}\psi_8/\mathrm{d}t = \varepsilon_2F_{82} + \varepsilon_5F_{85} - \varepsilon_8F_{08} \quad (23)$$

$$\mathrm{d}\psi_9/\mathrm{d}t = \varepsilon_2F_{92} + \varepsilon_6F_{96} - \varepsilon_9F_{09}. \quad (24)$$

When the system is in steady state with respect to total and labelled Y, the derivative terms in eqns (1–24) are zero and, for the representation assumed, the enrichment of the intracellular free Y pool equalises with that of the milk protein-bound Y pool, i.e. $e_5 = e_4$, $\varepsilon_5 = \varepsilon_4$ and $e_6 = e_7$, $\varepsilon_6 = \varepsilon_7$ in steady state, otherwise eqns (4), (12), (19) and eqns (7), (15), (22) respectively are inconsistent. In addition, the ratios $e_2/e_6 = \varepsilon_2/\varepsilon_6$ must be satisfied if eqns (14) and (21) are to be consistent. In steady state, therefore, eqns (1–24) reduce to 19 equations which can be written as:

$$F_{10} - F_{21} - F_{51} - F_{81} = 0 \quad (25)$$

$$F_{20} + F_{21} + F_{23} - F_{52} - F_{62} - F_{82} - F_{92} = 0 \quad (26)$$

$$F_{30} - F_{23} - F_{63} - F_{93} = 0 \quad (27)$$

$$F_{45} - F_{04} - F_{54} = 0 \quad (28)$$

$$F_{50} + F_{51} + F_{52} + F_{54} - F_{05}^{(m)} - F_{05}^{(o)} - F_{05}^{(s)} - F_{45} - F_{85} = 0 \quad (29)$$

$$F_{60} + F_{62} + F_{63} + F_{67} - F_{06}^{(m)} - F_{06}^{(o)} - F_{06}^{(s)} - F_{76} - F_{96} = 0 \quad (30)$$

$$F_{76} - F_{07} - F_{67} = 0 \quad (31)$$

$$F_{81} + F_{82} + F_{85} - F_{08} = 0 \quad (32)$$

$$F_{92} + F_{93} + F_{96} - F_{09} = 0 \quad (33)$$

$$I_1 - e_1(F_{21} + F_{51} + F_{81}) = 0 \quad (34)$$

$$I_2 + e_1F_{21} - e_2(F_{52} + F_{62} + F_{82} + F_{92}) = 0 \quad (35)$$

$$e_1F_{51} + e_2F_{52} - e_4(F_{05}^{(m)} + F_{05}^{(o)} + F_{05}^{(s)} + F_{45} + F_{85} - F_{54}) = 0 \quad (36)$$

$$e_2F_{62} - e_7(F_{06}^{(m)} + F_{06}^{(o)} + F_{06}^{(s)} + F_{76} + F_{96} - F_{67}) = 0 \quad (37)$$

$$e_1F_{81} + e_2F_{82} + e_4F_{85} - e_8F_{08} = 0 \quad (38)$$

$$e_2F_{92} + e_7F_{96} - e_9F_{09} = 0 \quad (39)$$

$$\Phi_2 - \varepsilon_2(F_{52} + F_{62} + F_{82} + F_{92}) = 0 \quad (40)$$

$$\varepsilon_2F_{52} - \varepsilon_4(F_{05}^{(m)} + F_{05}^{(o)} + F_{05}^{(s)} + F_{45} + F_{85} - F_{54}) = 0 \quad (41)$$

$$\varepsilon_2F_{82} + \varepsilon_4F_{85} - \varepsilon_8F_{08} = 0 \quad (42)$$

$$\varepsilon_2F_{92} + \varepsilon_7F_{96} - \varepsilon_9F_{09} = 0. \quad (43)$$

Further, we assume that the entries of peptide-bound Y into each half of the udder differ by the rate of infusion of the synthetic dipeptide XY, i.e.

$$F_{10} = F_{30} + I_1 \quad (44)$$

and that the rate of degradation of mammary gland protein is the same in each gland, i.e.

$$F_{50} = F_{60}\,. \quad (45)$$

The fluxes for protein and free Y secretion in milk and Y removal from the venous blood pools, i.e. F_{04}, $F_{05}^{(m)}$,

$F_{06}^{(m)}$, F_{07}, F_{08} and F_{09}, can be measured experimentally (Oddy *et al*., 1988; Bequette *et al*., 1996b). Algebraic manipulation of eqns (25–45) gives:

$$F_{10} = I_1/e_1 \tag{46}$$

$$F_{21} = (e_2\Phi_2 - \varepsilon_2 I_2)/(e_1\varepsilon_2) \tag{47}$$

$$F_{30} = F_{10} - I_1 \tag{48}$$

$$F_{45} - F_{54} = \tilde{F}_{04} \tag{49}$$

$$F_{76} - F_{67} = \tilde{F}_{07} \tag{50}$$

$$F_{96} = (\varepsilon_2 e_9 - e_2\varepsilon_9)\tilde{F}_{09}/(\varepsilon_2 e_7 - e_2\varepsilon_7) \tag{51}$$

$$F_{92} = (e_9\tilde{F}_{09} - e_7 F_{96})/e_2 \tag{52}$$

$$F_{93} = \tilde{F}_{09} - F_{92} - F_{96} \tag{53}$$

$$F_{85} = [\varepsilon_2(e_1 - e_8) - \varepsilon_8(e_1 - e_2)]\tilde{F}_{08}/[\varepsilon_2(e_1 - e_4) - \varepsilon_4(e_1 - e_2)] \tag{54}$$

$$F_{82} = (\varepsilon_8\tilde{F}_{08} - \varepsilon_4 F_{85})/\varepsilon_2 \tag{55}$$

$$F_{81} = \tilde{F}_{08} - F_{82} - F_{85} \tag{56}$$

$$F_{51} = F_{10} - F_{21} - F_{81} \tag{57}$$

$$F_{52} = e_1\varepsilon_4 F_{51}/(e_4\varepsilon_2 - e_2\varepsilon_4) \tag{58}$$

$$F_{50} = [(e_1 - e_4)F_{51} + (e_2 - e_4)F_{52}]/e_4 \tag{59}$$

$$F_{05}^{(o)} + F_{05}^{(s)} = F_{50} + F_{51} + F_{52} - \tilde{F}_{04} - \tilde{F}_{05}^{(m)} - F_{85} \tag{60}$$

$$F_{62} = \Phi_2/\varepsilon_2 - F_{52} - F_{82} - F_{92} \tag{61}$$

$$F_{60} = F_{50} \tag{62}$$

$$F_{63} = (e_2/e_7 - 1)F_{62} - F_{50} \tag{63}$$

$$F_{23} = F_{30} - F_{63} - F_{93} \tag{64}$$

$$F_{20} = \Phi_2/\varepsilon_2 - F_{21} - F_{23} \tag{65}$$

$$F_{06}^{(o)} + F_{06}^{(s)} = e_2 F_{62}/e_7 - \tilde{F}_{06}^{(m)} - \tilde{F}_{07} - F_{96} \tag{66}$$

where the tilde denotes an experimentally-determined flux.

Model solution as given by eqns (46–66) does not allow separation of oxidation of Y and utilisation of Y for constitutive mammary gland protein synthesis [eqns (60) and (66)]. Oxidation, however, can be determined directly by measuring the trans-organ evolution of isotopically labelled CO_2 using a labelled Y infusion (Oddy & Lindsay, 1986; Harris *et al*., 1992; Bequette *et al*., 1996a,b). Furthermore, model solution does not allow separation of the milk protein synthesis and degradation fluxes but merely permits calculation of their difference, i.e. net synthesis [eqns (49) and (50)]. Separation, however, is achieved by assuming that a fixed proportion (≈ 0.1) of the nascent milk protein is cleaved and degraded during the docking and secretory processes (Razooki Hasan *et al*., 1982).

Dilution ratios for identifying whether or not the amino acid Y is limiting protein synthesis in either mammary gland in the steady state can be obtained using the model. Equation (51) gives F_{96}, entry of Y into the venous pool from the mammary gland not infused with XY. This cannot exceed $\tilde{F}_{09}$ [see eqn (33)]. Equation (51) can be written:

$$F_{96} = R^{(N)}\tilde{F}_{09} \tag{67}$$

where $R^{(N)}$, the dilution ratio in the gland not infused, is defined by

$$R^{(N)} = (\varepsilon_2 e_9 - e_2\varepsilon_9)/(\varepsilon_2 e_7 - e_2\varepsilon_7). \tag{68}$$

If Y is limiting protein synthesis, it may be hypothesised that very little will leave the cell and so the flux F_{96} will be negligible. From an inspection of eqn (67), it is apparent that F_{96} is negligible if the dilution ratio $R^{(N)}$ or the flux $\tilde{F}_{09}$ tends to zero. $\tilde{F}_{09}$ is negligible when venous Y concentration in the half-udder not infused is insignificant. Venous concentration and the index $R^{(N)}$ therefore provide, in theory at least, a means of identifying whether Y is limiting. Similarly, a dilution ratio for the infused gland $R^{(I)}$, is obtained from eqn (54), which can be written:

$$F_{85} = R^{(I)}\tilde{F}_{08} \tag{69}$$

where

$$R^{(I)} = [\varepsilon_2(e_1 - e_8) - \varepsilon_8(e_1 - e_2)]/[\varepsilon_2(e_1 - e_4) - \varepsilon_4(e_1 - e_2)]. \tag{70}$$

3. Application

Application of the model is demonstrated using data from the experiment of Backwell *et al*. (1994) with primiparous British Saanen goats (weighing around 65 kg) between day 97 and 117 of lactation in which glycyl-L-[1-^{13}C]-phenylalanine and glycyl-L-[1-^{13}C]-leucine were infused for eight hours into one external pudic artery. Simultaneous infusions of L-[ring-^{2}H]-phenylalanine and L-[5,5,5-^{2}H]-leucine into the jugular vein were conducted to correct for recycling of free amino acid generated by hydrolysis of labelled peptide. Individual halves of the udder were milked hourly, beginning with the start of the infusions, and milk samples were taken on each occasion and analysed. Arterial and mammary venous blood samples were also taken and analysed. The experiment was repeated for five goats but amino acid concentration data are available for only three of the animals. The experimental hypothesis was that

peptides may represent a source of amino acids for milk protein synthesis and was tested by comparison of the [^{13}C]:[^{2}H] enrichment ratios for the infused and non-infused halves of the udder. If peptides were utilized as a significant supply of amino acids for milk protein synthesis, the [^{13}C]:[^{2}H] enrichment ratio of milk protein from the infused mammary gland would be greater than the [^{13}C]:[^{2}H] enrichment ratio from the noninfused mammary gland. Statistical analyses presented by Backwell *et al*. (1994) indicated that both glycyl-L-phenylalanine and glycyl-L-leucine were utilized by the udder for milk protein synthesis.

In the experiment, the enrichments of ^{13}C- and ^{2}H-phenylalanine and leucine were measured in milk casein and in arterial and milk vein plasma, with milk vein sampling from the peptide-infused half of the udder only. The arterial enrichment measurements, however, were systemic not close arterial. Arterio-venous difference for free phenylalanine and leucine prior to infusion, blood flow, the rate of infusion of synthetic dipeptide, and the output of casein in milk were also measured, but not the outputs of free phenylalanine and leucine in milk. Therefore, a number of supplementary calculations and adjustments are needed in order to apply the model to the experiment.

The arterial peptide pool enrichment e_1 was calculated using the formula

$$e_1 = I_1/[I_1 + (0.5 \times f_B \times c_A \times r_A)] \tag{71}$$

where the factor 0.5 reflects division of the udder into two halves, f_B denotes plasma flow (l hr^{-1}), c_A is the concentration of the free unlabelled amino acid Y in arterial plasma (μmol l^{-1}), and r_A denotes the ratio of the unlabelled peptide-bound Y to the unlabelled free Y in arterial plasma. Only small peptides of less than 1.5 kDa are considered in this application and it is assumed that the infused synthetic dipeptide is a representative marker for these.

Measurement of venous enrichment was on a free amino acid basis [i.e. in units of mol of [^{13}C]- or [^{2}H]-labelled free Y per μmol of total (i.e. ^{12}C plus ^{13}C and ^{1}H plus ^{2}H) free Y] and did not take account of peptides, so the enrichments e_8 and ε_8 were adjusted to take account of them as follows

$$e_8 = x/(x + y + z) \tag{72}$$

$$\varepsilon_8 = y/(x + y + z) \tag{73}$$

where

$$x = \tilde{e}_8/(1 - \tilde{e}_8 - \tilde{\varepsilon}_8) + r_V e_1/(1 - e_1) \tag{74}$$

$$y = \tilde{\varepsilon}_8/(1 - e_8 - \tilde{\varepsilon}_8) \tag{75}$$

$$z = 1 + r_V \tag{76}$$

where a circumflex denotes the measured enrichment and r_V represents the ratio of the unlabelled peptide-bound Y to the unlabelled free Y in venous plasma. In eqn (74), the first term gives the ratio of [^{13}C]-labelled free Y to unlabelled free Y and the second term gives the ratio of [^{13}C]-labelled peptide-bound Y to unlabelled free Y, with $e_1/(1 - e_1)$ being the ratio of [^{13}C]-labelled peptide-bound Y to unlabelled peptide-bound Y. In eqn (75), there is no second term because all the [^{2}H]-labelled Y is assumed to be in free form. Equation (76) gives the ratio of total unlabelled Y to free unlabelled Y.

The effective infusion rates for pool 2, the close arterial free pool, were calculated from the measured systemic arterial enrichments, denoted $\tilde{e}_2$ and $\tilde{\varepsilon}_2$, as follows

$$I_2 = f_B \times c_A \times \tilde{e}_2/(1 - \tilde{e}_2 - \tilde{\varepsilon}_2) \tag{77}$$

and

$$\Phi_2 = f_B \times c_A \times \tilde{\varepsilon}_2/(1 - \tilde{e}_2 - \tilde{\varepsilon}_2). \tag{78}$$

The denominator is needed to express these measured enrichments in units of μmol [^{13}C]- or [^{2}H]-labelled Y per μmol of unlabelled Y on account of c_A being measured prior to infusion.

The effluxes from the venous pools were determined using the following equations

$$\tilde{F}_{08} = 0.5 \times f_B \times c_V \times (1 + r_V)/(1 - e_8 - \varepsilon_8) \tag{79}$$

and

$$\tilde{F}_{09} = 0.5 \times f_B \times c_V \times (1 + r_V)/(1 - e_9 - \varepsilon_9) \tag{80}$$

where c_V denotes the concentration of the free unlabelled Y in venous plasma (μmol l^{-1}), again measured prior to infusion. The multiplier $(1 + r_V)$ is required to express concentration in terms of total unlabelled Y and the denominators to express it further in terms of total Y. Outputs of free Y in milk, i.e. $\tilde{F}_{05}^{(m)}$ and $\tilde{F}_{06}^{(m)}$, were assumed negligible and set equal to zero.

The measurements from the experiment of Backwell *et al*. (1994) are given in Table 2. In order to apply eqns (71–80) and to solve the model [eqns (46–66), (68), (70)] for these six treatments, values for e_2, ε_2, e_9, ε_9, r_A and r_V which were not measured are needed. Therefore, ranges must be prescribed for these variables so as to undertake a grid search. Ranges of $0 \le e_2 \le \text{maximum } \{e_1, \tilde{e}_2\}$, $0 \le \varepsilon_2 \le \tilde{\varepsilon}_2$, $0 \le e_9 \le \text{maximum } \{e_2, e_7\}$, and $0 \le \varepsilon_9 \le \text{maximum } \{\varepsilon_2, \varepsilon_7\}$ were applied to the enrichments, based on an inspection of Fig. 1(a–c). A range of 0.01–0.5 was adopted for both r_A and r_V (the bound to free ratios); the range encompasses values obtained from other trials using goats in a similar

physiological state (F. R. C. Backwell, B. J. Bequette and co-workers, unpublished data). Increments of approximately 1% of these ranges was chosen for grid search. Such a procedure produces an array of solutions for each treatment, but only solutions in which all the fluxes are non-negative were considered feasible.

The grid search produced feasible solutions for animal 3, 4 and 5 for phenylalanine (PHE), and for animal 4 for leucine (LEU). A feasible solution for a particular treatment (LEU v. PHE) was considered valid if the bound-to-free ratios r_A and r_V were of a similar order of magnitude. Several valid solutions were found for each animal, and representative valid solutions and ranges are given in Table 3. Values for milk protein synthesis and degradation were determined by assuming degradation to be 0.1 of synthesis. The uptake of an amino acid in peptide bound form by the udder is given by F_{51} for the infused half and F_{63} for the control, and that of the free amino acid by F_{52} and F_{62} respectively. Close arterial hydrolysis of peptide is given by F_{21} and F_{23} respectively. The model calculations presented in Table 3 give a peptide bound contribution of 7%, 10% and 4% to total amino acid uptake for animal 3, 4 and 5 respectively for PHE, and $\frac{1}{2}$% for animal 4 for LEU. Correspondingly, the figures for close arterial peptide hydrolysis as a proportion of the free amino acid uptake are 7%, 7% and 4% for PHE, and 9% for LEU. Similar contributions were obtained with the other valid solutions. These results, however, must be treated with circumspection because the model gives a greater peptide uptake for the control side of the gland than it does for the peptide infused side ($F_{63} \geq F_{51}$ in Table 3), perhaps suggesting some of the measured enrichments used in the calculations were not representative of the steady state.

To test the sensitivity of the model, the model was solved for LEU only by in turn (i) increasing the steady-state enrichment values by a standard 10%, and (ii) decreasing the plasma flow values by the same percentage. These two sets of measurements were selected for testing because (i) the previous analysis indicates enrichment might not have plateaued and (ii) blood flow relative to milk yield was perceived to be high in relation to published and other observed values. Model solutions, however, were broadly similar to the original analysis. The peptide contribution to total LEU uptake ranged from negligible to 7%, and close arterial hydrolysis of LEU peptides as a proportion of the free LEU uptake ranged from 1 to 14%. Valid solutions were found for animal 3 when the enrichments were increased by 10% but none were found for animal 5.

4. Discussion

Whether peptides can be utilized by peripheral tissues directly as a source of amino acids for protein synthesis remains an important question. Clearly, peptide concentrations in circulation are sufficient to provide a significant source of amino acids (McCormick & Webb, 1982; Seal & Parker, 1991) and numerous studies have demonstrated that peptides can supply essential amino acids and nitrogen in quantities adequate for maintenance functions (Steinhardt *et al*., 1984; Vasquez *et al*., 1986). However, it is not clear whether peripheral tissues such as mammary tissue can utilize circulating peptides directly as a source of amino acids or whether they use

TABLE 2
Experimental measurements

	Goat: 3		4		5	
	LEU	PHE	LEU	PHE	LEU	PHE
Conc. in plasma (μmol l^{-1}): c_A	89.8	18.7	68.7	19.1	60.4	17.2
c_V	53.5	10.7	31.1	10.0	34.9	10.9
Plasma flow (l hr^{-1}): f_B	51.6	51.6	33.6	33.6	32.9*	32.9*
Plateau enrichment (%): $\tilde{e}_2$	3.28	3.71	4.53	4.90	5.70	5.63
e_4	1.50	1.67	2.41	2.63	4.28	4.51
e_7	1.26	1.48	2.63	2.70	4.52	4.66
$\tilde{e}_8$	4.48	4.01	6.93	5.76	5.72	5.35
$\tilde{\varepsilon}_2$	3.47	4.55	5.04	6.24	6.16	7.69
ε_4	1.49	1.05	2.39	2.11	4.62	3.38
ε_7	1.29	1.14	2.70	2.17	4.54	3.60
$\tilde{\varepsilon}_8$	2.98	4.00	4.30	5.67	4.72	6.10
Flux (μmol hr^{-1}): I_1	580	260	580	260	580	260
$\tilde{F}_{04}$	1796	750	646	270	862	361
$\tilde{F}_{07}$	1483	621	675	283	1034	432

*Not measured, therefore calculated from milk yield using an average plasma flow to milk yield ratio obtained from the other two goats.

TABLE 3
Model calculations (ranges for valid solutions are given in parentheses)*

		Goat: 3		4				5	
		PHE		LEU		PHE		PHE	
Bound-to-free ratio:	r_A	0.19	(0.09)	0.16	(0.045)	0.22	(0.09)	0.23	(0.11)
	r_V	0.31	(0.15)	0.34	(0.091)	0.40	(0.165)	0.33	(0.16)
Enrichment (%):	e_1	73.9	(9.25)	75.8	(5.8)	78.6	(7.8)	80.0	(8.15)
	e_2	4.60	(1.05)	4.50	(0.25)	6.10	(0.9)	9.70	(1.6)
	e_8	40.5	(2.4)	45.2	(1.85)	51.2	(3.15)	49.6	(2.9)
	e_9	3.10	(2.1)	3.00	(1.1)	3.70	(0.6)	5.30	(1.0)
	ε_2	4.30	(0.15)	5.00	(0.25)	6.10	(0.15)	7.50	(0.7)
	ε_8	1.91	(0.135)	1.91	(0.075)	2.13	(0.12)	2.48	(0.15)
	ε_9	2.80	(1.65)	3.20	(1.15)	3.40	(0.75)	4.10	(0.8)
Flux (μmol hr^{-1}):	I_2	39	(0)	116	(0)	35	(0)	37	(0)
	Φ_2	48	(0)	129	(0)	45	(0)	50	(0)
	$\tilde{F}_{08}$	628	(45)	1324	(49)	504	(26.5)	498	(29)
	$\tilde{F}_{09}$	288	(39)	560	(44.5)	190	(25)	197	(20.5)
	F_{10}	352	(43.5)	765	(52)	331	(29)	325	(31.5)
	F_{21}	16	(18)	0	(4.5)	12	(10)	35	(7.5)
	F_{30}	92	(43.5)	185	(52)	71	(28.5)	65	(31.5)
	F_{45}	833	(0)	718	(0)	300	(0)	401	(0)
	F_{54}	83	(0)	72	(0)	30	(0)	40	(0)
	F_{76}	690	(0)	750	(0)	314	(0)	480	(0)
	F_{67}	69	(0)	75	(0)	31	(0)	48	(0)
	F_{96}	116	(114)	336	(250.5)	107	(44.5)	100	(36)
	F_{92}	157	(165.5)	177	(268)	68	(47.5)	60	(32)
	F_{93}	15	(18)	47	(47)	15	(28.5)	37	(35)
	F_{85}	29	(16.5)	113	(56.5)	20	(8)	81	(37.5)
	F_{82}	272	(14.5)	452	(55)	169	(7.5)	129	(35)
	F_{81}	327	(45.5)	759	(50)	315	(26)	289	(29.5)
	F_{51}	8	(3)	5	(1.5)	4	(1.5)	1	(1.5)
	F_{52}	270	(129)	768	(106.5)	186	(46)	218	(12)
	F_{50}	827	(336.5)	833	(150)	347	(73.5)	265	(52.5)
	$F_{05}^{(o)} + F_{05}^{(s)}$	326	(472)	847	(225.5)	247	(119)	43	(28.5)
	F_{62}	415	(83.5)	1176	(146)	316	(44.5)	263	(72)
	F_{60}	827	(336.5)	833	(150)	347	(73.5)	265	(52.5)
	F_{63}	45	(27)	6	(83.5)	50	(27)	20	(14.5)
	F_{23}	32	(41)	132	(77)	6	(24.5)	8	(10)
	F_{20}	1066	(83.5)	2441	(217.5)	721	(40.5)	627	(84)
	$F_{06}^{(o)} + F_{06}^{(s)}$	549	(269.5)	1004	(91.5)	323	(48)	16	(10.5)
Dilution ratio:	$R^{(N)}$	0.40	(0.4305)	0.60	(0.4505)	0.57	(0.2635)	0.51	0.246)
	$R^{(I)}$	0.05	(0.025235)	0.09	(0.045725)	0.04	(0.016185)	0.16	(0.0749)

*Range expressed as (maximum − minimum)/2.

the amino acids released by hydrolysis of the circulating peptides, i.e. are peptides hydrolyzed in intracellular space (transcellular hydrolysis concurrent with transport cannot be discriminated from intracellular hydrolysis) or extracellularly (in plasma, erythrocytes or plasma membrane). Significant peptide hydrolytic activity has been demonstrated in plasma (Piez *et al*., 1960; Adibi & Johns, 1984), erythrocytes (Krzysik & Adibi, 1977; Backwell *et al*., 1994), by plasma membrane (Stehle *et al*., 1988) and in various tissue cytosolic pools (Krzysik & Adibi, 1977).

The current model defines the data required to derive various fluxes pertinent to resolving the above question. However, the fluxes of isotope (and total metabolite) must be in steady state. The data of Backwell *et al*. (1994) should be adequate to discriminate between intra-/trans-cellular hydrolysis and extracellular hydrolysis of peptides, provided that steady-state conditions were satisfied. However, it cannot be ascertained from these data whether the administered isotopes were infused long enough to achieve steady state. Previous observations in goats (Bequette *et al*., 1994) and cows (France *et al*., 1995) indicate that steady state conditions can be reached by 8 hr after commencement of infusion. Therefore, we have attempted to calculate fluxes based upon the data collected by Backwell *et al*. (1994) using an 8 hr infusion. Since our calculations are based upon single observations (the 8 hr time point) without extrapolation, the error in the fluxes could be quite large and the results should perhaps be viewed more as a demonstration of the model than as a definite application pertaining to the lactating goat.

The model does predict that extracellular hydrolysis of both glycyl-leucine and glycyl-phenylalanine occurs whilst transcellular hydrolytic activity appears to be limited (Table 3). It cannot be determined from this analysis whether the hydrolysis occurs in plasma or erythrocyte pools. Although peptides are hydrolyzed in the extracellular space, little leucine or phenylalanine derived from the hydrolytic process was determined to enter the milk protein precursor pools directly. This prediction is somewhat surprising in that the statistical analyses undertaken by Backwell *et al*. (1994) concluded that milk proteins were being labelled by both leucine and phenylalanine that were derived from peptides. One explanation that can be offered is that the statistical analyses undertaken by these workers utilized all the time points obtained during the infusion while results presented herein are based on a single time point (the last). Given the small differences in labelling patterns of milk protein obtained from the control and the treated gland and the inherent variability in those measurements, the results of this model are probably consistent with those presented by Backwell *et al*. (1994). In order to utilize all of the data collected by these workers, a dynamic simulation model would need to be constructed as opposed to the steady state analysis undertaken herein.

Rapid rates of peptide hydrolysis in the extracellular space intimated by this analysis could compromise the results of previous work in which the Fick principle was used to derive blood flow rates (Zierler, 1961) from arterio-venous differences of amino acids (e.g. Hanigan & Baldwin, 1994). If significant quantities of peptide or, indeed, plasma proteins are hydrolyzed between the arterial and venous sampling sites, blood flow will be overestimated proportional to the quantity of the amino acid of interest that is liberated. As the majority of ruminant mammary metabolism studies have used the Fick principle to derive blood flows and models of mammary metabolism have been constructed based upon these values (Waghorn & Baldwin, 1984; Hanigan & Baldwin, 1994), data relevant to deriving the rate and extent of peptide and protein hydrolysis is crucial to making further progress in the area.

The assumption that peptides available for hydrolysis were derived solely from the population of molecules with molecular weights less than 1.5 kDa should not have a major bearing on the solutions to the model presented herein (Table 3). This is because, as arterio-venous measurements of peptides were not made, ranges rather than unique values were ascribed to the bound-to-free amino acid ratios r_A and r_V for the purposes of undertaking a grid search. However, if such measurements are made and these are subsequently utilized to obtain solutions to the model, the choice of a molecular weight cut-off is important. If the contribution of peptides to amino acid supply is largely determined by the concentration of large molecular weight peptides while only peptides of 1.5 kDa or less are measured then this omission of substrate will be reflected in the solutions obtained.

In addition to elucidating the role of extracellular peptides in amino acid delivery for milk protein synthesis, the model provides a means of elucidating the turnover of constitutive mammary protein. The results presented in Table 3 suggest a much larger contribution from net mammary protein degradation to amino acid delivery than that from extracellular peptides. The calculation of constitutive protein degradation [eqn (59)] is sensitive to the value used for milk protein enrichment, with an underestimate of enrichment resulting in an overestimate of degradation. Thus steady-state conditions are essential if overestimates of constitutive protein degradation are to be avoided.

As previously suggested, a dynamic model of mammary peptide utilization would be required to make full use of the data of Backwell *et al*. (1994). However, construction of such a model would require additional information to that reported by these workers, including extracellular and intracellular volumes, intracellular milk protein mass, and constitutive protein mass. While such a model could circumvent the requirement that isotope be infused long enough to reach plateau enrichments, the collection of additional data required to parameterize and solve the model would not be an easy endeavour, and the model would have to be solved numerically as opposed to the algebraic solution presented here.

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