Amino Acid Exchange by the Mammary Gland of Lactating Goats when Histidine Limits Milk Production

B. J. Bequette,* M. D. Hanigan,† A. G. Calder,* C. K. Reynolds,‡ G. E. Lobley,* and J. C. MacRae*
*Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, Scotland
†Dairy Research Group, Purina Mills Inc., St. Louis, MO 63144
‡The University of Reading, Department of Agriculture, Reading RG6 2AT, England

ABSTRACT

The aim of this study was to monitor amino acid (AA) exchange kinetics of the mammary gland in response to an imposed limitation on His supply for milk production. Lactating goats (n = 4, ~120 DIM) were fed a low protein ration that provided only 77% of metabolizable protein and 100% of energy requirements for milk production. The protein deficiency was alleviated by infusion into the abomasum of an AA mixture (67 g/d) including (+H; 4.4 g/d) or excluding (–H) His. Goats were assigned to treatments (6 to 7 d) according to a switchback design. On the last day of the first two periods, [U-^{13}C]AA were continuously infused i.v. for 7 h and arterial and mammary vein blood was withdrawn to determine plasma AA concentration and enrichment. Flow probes monitored mammary blood flow. The secretion and enrichments of AA in milk casein were monitored each hour. A three-pool model of the gland was used to derive bi-directional rates of plasma AA exchange. Arterial plasma His concentration was lower during –H infusion (8 vs. 73 μM), but those of other AA changed little. Responses to low levels of plasma His were: 1) mammary blood flow increased by ~33%; 2) the gland's capacity to remove plasma His increased 43-fold, whereas the gland's capacity for other AA declined by two- to threefold; and 3) influx and efflux of His by the gland decreased. Thus, as the reduction in His efflux was insufficient to offset the reduced influx, milk protein yield decreased from 118 to 97 g/d.

(**Key words:** amino acid, transporter, mammary gland, goats)

Abbreviation key: EPA = external pudic artery, **GC-MS** = gas chromatography-mass spectrometry, **MBF** = mammary blood flow.

INTRODUCTION

The ability to increase milk protein content and yield is a goal of dairy researchers and producers. Achieving that goal requires that researchers identify nutritional regimens to optimize the cow's capacity to synthesize milk protein. Milk protein biosynthesis is a complex process with a number of potential regulatory, or limiting, steps. At the level of the mammary gland the process can be described as involving 1) removal of AA by the mammary cells from the vascular supplies and 2) utilization of those sequestered AA for milk protein synthesis. Removal depends on the supply of AA to and the affinity of the udder for AA. The supply of AA to the mammary gland is a function of the net entry of AA into the postabsorptive blood system, the relative distribution of the blood nutrient supply to the post-absorptive tissues, and the affinity of the individual tissues for those AA (20). This process is collectively termed nutrient partitioning. Currently, our understanding of AA partitioning is limited (6). This is best exemplified by present protein requirement models of the dairy cow, which appear unable to predict responses in milk protein yield from changes in dietary protein or AA availability (19) and where studies designed to identify those AA most likely to be limiting for milk production have proved inconclusive (16).

Further considerations involve mammary blood flow (**MBF**) in the delivery of AA to the gland and the mammary membrane transport systems in facilitating the removal of those AA from the blood supply. Blood flow through the mammary microvasculature appears to be regulated, in part, by locally produced vasoactive compounds (26). Presumably, these local factors are produced in response to changes in mammary intracellular metabolism. Thus, these local control mechanisms may govern the partition of nutrients between the mammary gland itself and other tissues through shifts in the distribution of blood flow to tissues. The mammary cell may also be capable of responding to changes in nutritional adequacy of its external and internal AA environments by altering the activities of the AA transport systems.

Received June 21, 1999.
Accepted November 19, 1999.
Corresponding author: B. J. Bequette; e-mail: bjb@rri.sari.ac.uk.

Indeed, this is the case in other cell types in vitro, where it has been shown that the rate of intracellular utilization of an AA can modulate the affinity of the cell for the extracellular (vascular) supplies of that AA (28). To date, however, these potential mechanisms (blood flow and AA transport capacity) have not been experimentally tested in the lactating ruminant udder in vivo, mainly due to the lack of suitable techniques.

The objective of the present study was to evaluate how the AA supply and exchange kinetics across the udder would respond to the imposition of a single AA limitation. In particular, we were interested in potential changes in the AA removal processes employed by the udder to buffer the AA limitation and potential changes in MBF. To address this objective, MBF, arteriovenous differences of unlabeled (tracee) and labeled (^{13}C tracer) AA across the mammary gland, and the enrichment of AA in secreted milk casein were measured during an intravenous infusion of a mixture of [U-^{13}C]AA. A three-compartment model of mammary gland AA kinetics, based on a previous model applied to the hind-leg (11), was used to derive rates of transfer of AA from plasma to the intracellular pool and from the intracellular pool to the plasma. The limitation imposed was for His. Aspects of this work have been reported elsewhere (4, 6).

MATERIALS AND METHODS

Goats and Diets

Four nonpregnant, multiparous lactating British Saanen goats (59.5 ± 6.4 kg of BW) were used. Under general anesthesia, a catheter was placed into the abomasum (Tygon Lab tubing, 3.5 mm i.d., 6.0 mm o.d., Cole-Parmer Instrument Co., Vernon Hills, IL), the carotid artery was either surrounded in a skin loop or elevated to a subcutaneous position, a mammary vein (superficial epigastric vein) was enclosed in a skin loop, the common collateral vessels between the udder halves were ligated, and an ultrasound flow probe (6 mm; Transonic Systems Inc., Ithaca, NY) was positioned around the external pudic artery (**EPA**) ipsi-lateral to the udder half with the venous loop (7, 8, 9). The Institute's Animal Welfare Committee and the veterinary inspectorate of the Home Office (United Kingdom) approved all surgical procedures and practices.

Following surgery, goats were allowed to recover (~1 mo) and were fed ad libitum a ration comprised (60:40, as-fed basis) of a standard pelleted dairy concentrate (206 g of CP (N × 6.25) and 12.9 MJ of metabolizable energy/kg of DM) and molasses-treated (10%, as-fed basis) hay (56 g of CP and 11.8 MJ of metabolizable energy/kg of DM). Two weeks before the experiment, goats were fed a low protein experimental ration comprised (58:42) of a pelleted concentrate (Table 1, 12.9 MJ of calculated metabolizable energy/kg of DM; 9) and the molasses-treated hay. The total ration (105 g of CP and 12.0 MJ of calculated metabolizable energy/kg of DM) provided sufficient metabolizable energy, but only 77% of the calculated metabolizable protein intake needed for maintenance and milk production requirements (1). The requirements for milk production were based on the amounts of milk produced during the pre-experiment period. Therefore, throughout the experiment (5 wk), this level of feeding was fixed for each goat. The four goats were all born in the same year and were 65, 73, 78, and 265 DIM at the start of the experimental feeding period. Because of damage to the flow probe of one of the original goats, a replacement goat (the one at 265 DIM) was surgically prepared, which meant that the experiment with this goat was conducted towards later lactation, about 4 mo after the other goats.

Experimental Protocol

Goats were placed in individual metabolism crates 10 d prior to experimentation to allow acclimatization to personnel and feeding by automatic feeders (12 equal meals at 2-h intervals). Once a week, for 4 h, each goat was allowed exercise in a floor pen. Milking was performed at 0830 and 1630 h. Milk weights were recorded and subsamples were stored at 4°C in the presence of formaldehyde (50 μl, 40% vol/vol) for milk N analyses. Temporary catheters (polyvinyl chloride, 0.8 mm i.d., 1.2 mm o.d., Critchely Electrical Products, Auburn, New South Wales, Australia) were inserted (10 cm) into the carotid artery, the contra-lateral jugular vein and the

Table 1. Composition of pelleted concentrate[1] fed to goats.

Ingredients	Composition
	(g/kg of DM)
Barley	395
Whole corn	260
Wheatfeed	150
Linseed flakes	50
SUPERSOY[2]	75
Molasses (fluid)	30
Salt	10
Dicalcium phosphate	6
Limestone	16.5
Calcined magnesite	3
Trace mineral and vitamin mix[3]	2.5

[1]The concentrate was 88.0% DM and contained 138 g of CP/kg of DM.

[2]Formaldehyde treated soybean meal containing 67% CP (Norvite Feed Supplies, Insch, United Kingdom).

[3]Contains (per kg of mix) 250 g of Ca (calcium carbonate), 120 g of Na (sodium chloride), 30 mg of Se, 2.0 g of Cu, 400,000 IU of vitamin A, 80,000 IU of vitamin D_3, 600 IU of vitamin E.

mammary vein loop 1 d prior to taking arteriovenous kinetic measurements.

Infusions of AA. The protein deficiency was alleviated by infusion into the abomasum of a mixture of AA (67 g/d) including (4.4 g/d; +H) or excluding (–H) His. Goats were allocated to treatments (+H and –H) according to a balanced, switchback design involving three consecutive periods, each of 6 to 7 d. Daily rates (g/d) of infusion of the AA in the mixture were for Asp 5.8, for Glu 5.8, for Gln 3.8, for Gly 2.4, for Ala 3.8, for Ser 2.2, for Cys 1.4, for Pro 2.4, for Asn 2.7, for Leu 3.8, for Ile 2.6, for Val 2.6, for Phe (including an allowance for conversion of Phe to Tyr) 6.1, for Met 4.4, for Trp 1.9, for Thr 5.4, for Lys 7.7, for Arg 2.4, and, when included, His 4.4. The mixture of AA reflected the pattern of AA in microbial protein (29), except that His was increased 250% and that of Lys, Phe, Met (and Cys), Thr, and Trp increased 20% above their relative proportions found in microbial protein. Thus, His comprised 2.1 and 1.2% (29) of the predicted duodenal supplies of AA in the +H and –H periods, respectively. Mixtures (287 g of AA/4 kg of solution, pH 7.4, equalized Na^+ and Cl^- in all batches) were stored at 4°C and infused (1 kg of solution/d) within 4 d of preparation. Because of limited access to the flow monitor system (scheduling of experiments limited access to flow monitor equipment), for only three of the goats (those at 65, 78, and 265 DIM), MBF was monitored continuously (24 h/d) throughout the three experimental periods. However, MBF was monitored for all four goats during the arteriovenous measurement periods (8 h).

Mammary arteriovenous isotope kinetics. Arteriovenous kinetics of AA by an udder half were monitored on the last day of the first two treatment periods only. Thus, the isotope kinetic measurements were performed on each goat during a +H and –H infusion period, and analyzed according to a crossover design. The third period was added to allow us to establish that the effects of –H and +H treatments on milk production were reversible and to test for carryover effects on milk production. At the morning milking (approx. 0830 h), each goat was given an i.v. dose of oxytocin (2 IU) to induce milk letdown, and machine milking and hand stripping removed milk. Immediately after the milking, a mixture of [U-^{13}C]AA was continuously infused (20 g/h, containing 285 mg of hydrolyzed dried algal powder and 6,500 IU of Na-Heparin) into the jugular vein for 7 h. The mixture of [U-^{13}C]AA was isolated from ^{13}C-enriched algal biomass (Spirulina, delipidated and destarched, 99 atoms % ^{13}C, 450 g of AA/kg of DM, Martek Corp., Columbia, MD) as previously described (5). At 1-h intervals, the glands were milked-out by hand (without oxytocin); the weight of milk recorded for each gland, and subsamples taken for determination of total AA content (8) and the casein isolated (7). During the last 5 h, matched sets of arterial and mammary vein blood samples were continuously (10 ml/h) withdrawn over 1-h intervals as previously described (5).

Blood samples were mixed by gentle hand-rolling and packed cell volume (PCV, %) determined by hematocrit. A blood sample (0.5 g) was accurately weighed, and a known equal weight of hemolyzing solution containing L-norleucine (100 μM) was added. Portions (each 1 g) of blood were then dispensed into tubes and an equal volume of ice-cold distilled water added to disrupt the red blood cells. The remaining blood was centrifuged at 2000 $\times$ *g* for 15 min at 4°C. A plasma sample (0.5 g) was accurately weighed and a known equal weight of the hemolyzing solution added. The remaining plasma was dispensed into tubes (each 1 g). All samples were kept frozen at –20°C until analyzed. Samples of blood, plasma, and casein were obtained on the day before isotope infusion to provide natural abundance "background" samples for gas chromatography-mass spectrometry (**GC-MS**) determinations.

Analytical Procedures

Concentrations of free AA in blood, plasma, and after hydrolysis of whole milk were determined as previously described (8). Free AA in blood and plasma and those obtained following hydrolysis of casein were prepared for GC-MS analyses as previously described (7). Briefly, [^{13}C]AA enrichments were determined under electrical impact or chemical ionization conditions (12, 23, 24; see also 22). D-Amino acids, occurring as a natural product or as consequence of acid hydrolysis of the micro-algae, were detected in blood samples. For most, the D-isomer contribution was small (<2%), but for Lys it represented 22%. Thus, L-Lys enrichments were determined by chiral separation (22). For reasons given previously (30), data for Met, Arg, Trp, Gln, Asn, Asp, and Glu were not obtained or not presented.

The GC-MS monitors fragment ions containing all the carbon atoms of the AA (22). Because only the m+0 and m+*n* ions, where *n* is the number of carbon atoms in the molecule, were monitored, molar excess cannot be calculated. Therefore, values represented the relative enrichments of $(m + n)/[(m + 0) + (m + n)]$, calculation as

$$(R_s - R_0)/[1 + (R_s - R_0)],$$

where R_s and R_0 are the ratios of the $(m + n)/(m + 0)$ ions in the enriched and natural abundance samples, respectively. In the algal-derived mixture >99% of the molecules were in the $(m + n)$ form (i.e., all carbon atoms fully labeled), therefore, the essential AA enrichment would approximate to molar excess. This may not be the

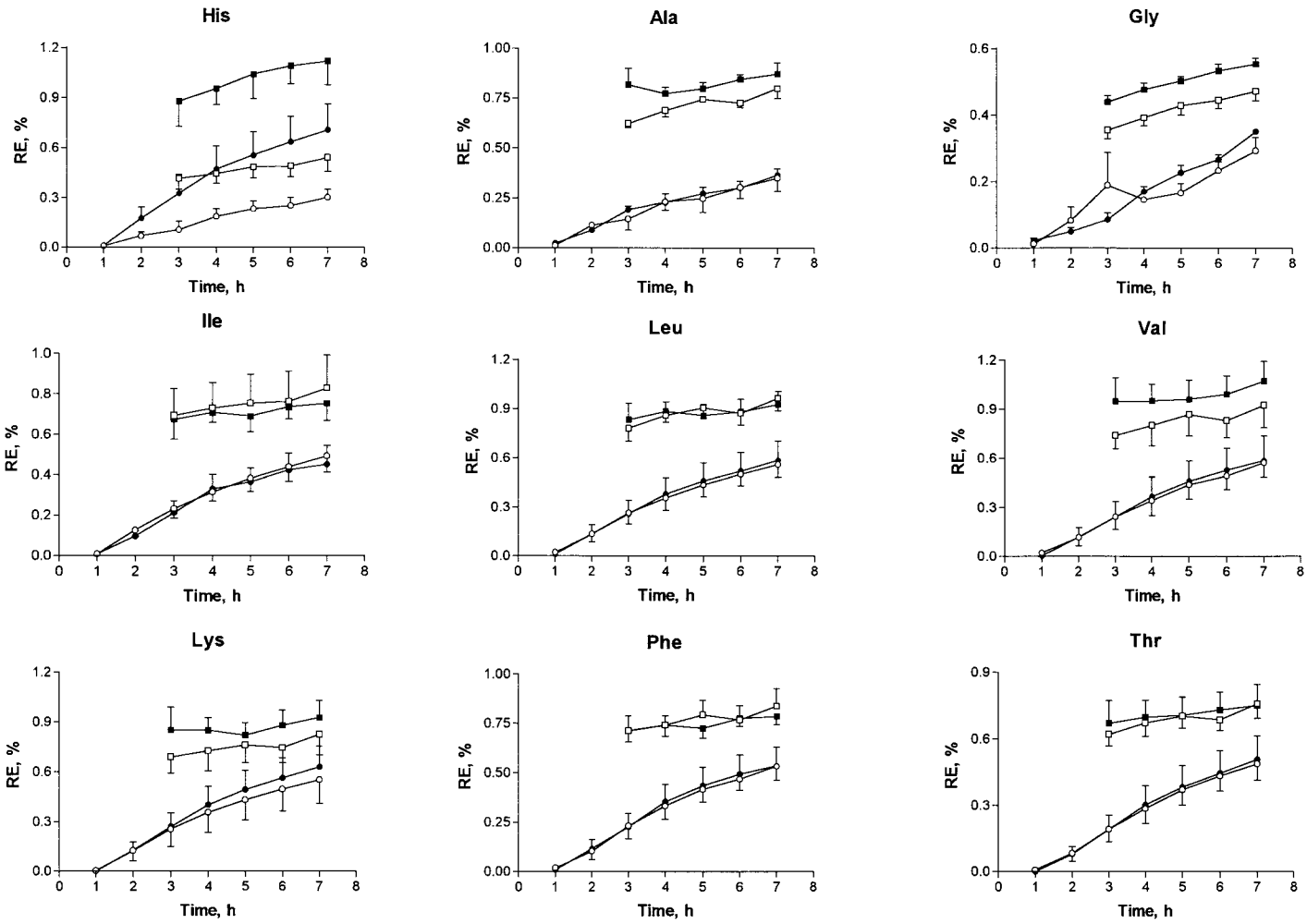

Figure 1. The temporal ^{13}C relative enrichment [RE (%); mean ± SE] of AA in arterial plasma (□, ■; samples from h 3 to 7) and in secreted milk casein (○, ●) at hourly intervals during a 7-h i.v. infusion of a mixture of [U-^{13}C]AA. Lactating goats (n = 4) were each given an infusion into the abomasum of a mixture of AA either including (open symbols) or excluding (closed symbols) His.

case for the nonessential AA because the (m + *n*) species may be formed via de novo synthesis in the body from other AA (or metabolites in the algae) and molecules with less than *n* labeled carbons may also arise via de novo synthesis (10). Caution should be exercised when considering the nonessential AA results.

Calculations

Plasma kinetics. The flux of each AA in plasma (F_{wb}) was calculated from the relative enrichment of the plasma free AA over the last 2 to 3 h of isotope infusion where a quasi-isotopic plateau had been reached (see Figure 1). Flux was calculated as follows,

$$F_{wb}\ (\text{mmol/h}) = [(0.99/E_a) - 1] \times I,$$

where E_a = the relative enrichment of the AA in arterial plasma and I = the rate of infusion (mmol/h) of the L-[U-^{13}C]AA. Lysine was corrected for presence of the D-isomer. Rates of [U-^{13}C]AA infusion (μmol/h) were for His 20, Phe 50, Tyr 43, Leu 105, Ile 44, Val 74, L-Lys 95, Thr 71, Ser 63, Pro 62, Ala 142, and Gly 107.

The unidirectional flux of AA from plasma to the mammary gland (F_{mg}) was calculated from labeled AA transfers; conversion to unlabeled AA flows assumed that arterial plasma represents the primary vascular pool exchanging unlabeled and labeled AA with the mammary gland,

$$F_{mg}\ (\mu\text{mol/h}) = [(C_a \times E_a - C_v \times E_v)/E_a] \times MBF_p,$$

where C = the concentration (μM) of the AA in arterial (a) and mammary vein (v) plasma and MBF_p = the rate of plasma flow (whole blood flow × (100-PCV)/100) to the whole mammary gland. MBF_p was calculated from total milk output × (MPF per udder half/milk output per udder

half). Thus, the proportion of F_{wb} partitioned to the mammary gland (K_{mg}) was calculated as:

$$K_{mg} = F_{mg}/F_{wb}.$$

Mammary AA exchange kinetics. Calculations of free AA kinetics in the mammary gland (μmol/h) were based on a three-compartment model reported by Biolo et al. (11) shown in Figure 2. The mammary model of France et al. (15) was not used because it is not applicable across all AA; therefore, the model of Biolo et al. (11) was employed. However, solutions from the two models were compared for Leu and found to be identical relative to exchange values. Free AA were assumed to have entered and left the mammary gland via the EPA ($F_{a,O}$) and mammary vein ($F_{O,v}$), respectively. Connecting arrows represent unidirectional transfers of AA between arterial (A), venous (V), and mammary gland (MG) free AA pools. Fluxes represented by $F_{mg,a}$ and $F_{v,mg}$ correspond to the net movements of AA from artery to mammary gland and from mammary gland to mammary vein, i.e., transmembrane influx and efflux rates, respectively. $F_{mg,0}$ is the rate of appearance of AA in the intracellular pool arising from protein breakdown and de novo synthesis and $F_{0,mg}$ is the rate of utilization of intracellular AA for protein synthesis and other metabolic fates. $F_{a,O}$ and $F_{O,v}$ for an udder half were computed as:

$$F_{a,O} = C_a \times MBF_p$$
$$F_{O,v} = C_v \times MBF_p,$$

where C = plasma AA concentration. The net balance of AA across the mammary gland (NB) can be calculated as the difference between $F_{a,O}$ and $F_{O,v}$, thus,

$$NB = (C_a - C_v) \times MBF_p.$$

The traditional arteriovenous balance approach can be modified to derive influx and efflux rates if the intracellular enrichment of the free AA is known (11). This measurement is usually obtained by biopsy. In the present study, the enrichment of AA in the secreted milk casein was used as a substitute. Because there is a lag time of only 30 to 60 min between synthesis and secretion of casein into milk (7), a measure of the intracellular free AA pool for casein synthesis can easily be made without recourse to biopsy. The average enrichment in casein (E_c) during the first 4 h (h 2 to 6 of the isotope infusion) of the 5-h arteriovenous blood-sampling period was used in mammary kinetic calculations.

The net balance of unlabeled and labeled AA in plasma across the mammary gland was computed from the difference of influx and efflux rates of unlabeled and labeled AA in the gland as:

$$\text{unlabeled AA: } (C_a - C_v) \times MBF_p = F_{a,O} - F_{O,v}$$
$$\text{labeled AA: } (C_a \times E_a - C_v \times E_v) \times MBF_p = F_{mg,a} \times E_a - F_{v,mg} \times E_c.$$

After substitution and rearrangement, the equations can be solved for $F_{mg,a}$ and $F_{v,mg}$ as demonstrated by Biolo et al. (11), giving the following expressions:

$$F_{mg,a} = [((E_c - E_v)/(E_a - E_c)) \times C_v + C_a] \times MBF_p$$
$$F_{v,mg} = [((E_c - E_v)/(E_a - E_c)) \times C_v + C_v] \times MBF_p$$

The arteriovenous balances of unlabeled and labeled AA across the mammary gland were computed with plasma concentrations, enrichments, and plasma flow because the infused isotope equilibrates with the plasma, but some labeled AA may not equilibrate with the red blood cells (present data not shown, see 5).

A rate parameter for unidirectional removal of AA by the udder (K_{in}) was calculated from the model of Hanigan

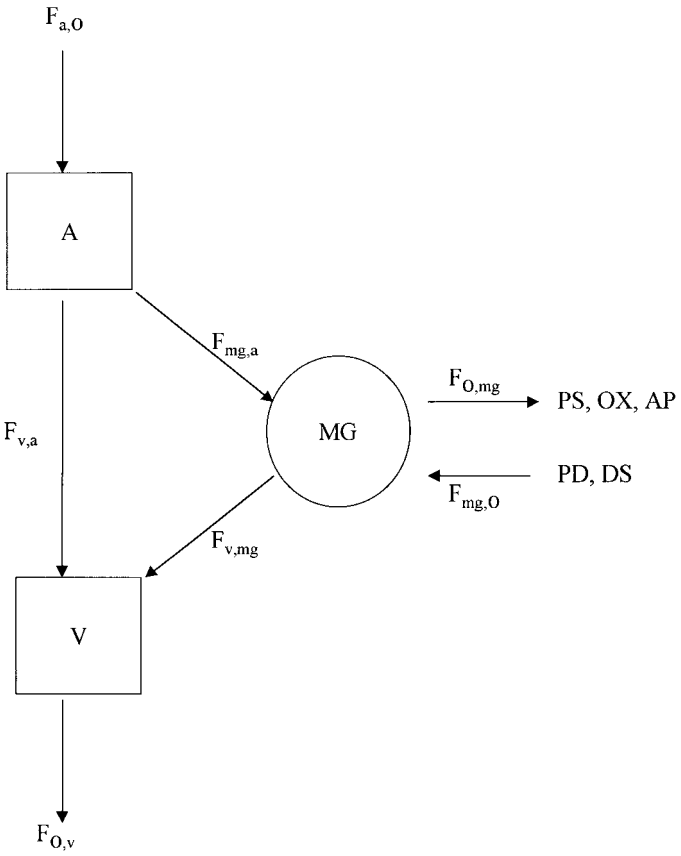

Figure 2. Three-compartment model of the mammary gland to derive transmembrane fluxes ($F_{i,j}$) of plasma AA. See calculations section for descriptions of $F_{i,j}$. A, arterial plasma pool; V, mammary venous plasma pool; MG, mammary intracellular pool; PS, protein synthesis; Ox, oxidation; AP, anabolic processes; PD, protein degradation; DS, de novo synthesis.

et al. (20) by substituting $F_{v,mg}$ for $k_{IM}C_{IM}V_{IM}$ in Eqn. [7]. This substitution replaces the assumption in that earlier work that extracellular and intracellular concentrations are linearly related as defined by Eqn. [9] of that work. Thus,

$$K_{in}\ (l/min) = (F_{a,O} + F_{v,mg} - F_{O,v})/C_v.$$

The term K_{in} is synonymous with clearance, i.e., the ability of the udder to clear plasma AA per unit time. In large part, K_{in} should describe the capacity of the transporter system, that is, the number and affinity of the transporters. However, a change in the effective perfusion of the udder would theoretically expose more transporters to substrate, resulting in an increase in K_{in}, i.e., more transporters exposed to substrate and where the total number and affinity of those transporters may not have changed (26). Consequently, changes in K_{in} may not always be indicative of the capacity of the transporter. However, if a class of transporters is altered in one direction while another class is altered in the opposite direction, then effective perfusion cannot explain both changes.

Model Assumptions

1. For many AA, in both short- (6 to 8 h; 5, 9) and longer- (12 to 30 h; 7, 8) term tracer infusion studies, AA enrichments are higher in plasma than in whole blood. This results from either slow, or the lack of, transfer of tracer AA from plasma to the packed cells (mostly red blood cells). Given the unequal distribution of tracer and tracee (i.e., AA concentrations are often higher in red blood cells than in plasma; 5), in plasma and red blood cells, and the technical difficulties of measuring tracer and tracee exchanges by the plasma and the red blood cells, plasma was the preferred choice for calculating trans-organ exchanges. This choice could, for some AA, lead to a slight underestimation of flux. Furthermore, although indirect evidence does suggest that circulating peptides may also supply certain AA (e.g., Phe, Met, and Lys) for casein synthesis (2, 8), we did not attempt to quantify this contribution because of the technical difficulties associated with this measurement. Thus, the fluxes reported herein represent plasma free AA only. We did observe that the net balances of plasma free His, Phe, and Thr were significantly less then their secretion as milk protein (Table 2), suggesting that peptides may contribute the remainder. However, the uptake to output ratio for those AA was not affected by treatment, and so we assumed that any contribution of peptides to net uptake remained as a fixed proportion of the free AA flux.

2. We selected the enrichment of the secreted milk casein as an indicator of the enrichment of the tissue intracellular free AA pool. In a previous study, we found that the enrichment in casein was similar to that of the milk free AA (15), which probably derives from the intracellular pool. In the current study the enrichment in secreted casein did not reach a plateau by the end of the 7-h infusion (Figure 1). Despite this, rates of influx and efflux of His (Figure 3) during h 3 through 6 of the isotope infusion showed only a slight, but nonsignificant, increase over time. And, there was no significant interaction between treatment and time for any of the flux calculations. This slight rise in the rate of flux may have also been due to the increase (+16% for +H and +40% for –H) in mammary blood flow during the arteriovenous measurement period (data not shown). Nonetheless, the rates of transfer of the unlabeled and labeled AA were, for the most part, in near steady state. In the future it will be necessary to demonstrate that the enrichment of casein reflects that of the tissue's intracellular free pool.

Statistics

Data were analyzed by ANOVA in Genstat 5 (Lawes Agricultural Trust, Rothampstead, United Kingdom). For milk production, only the last 4 d of each of the three infusion periods were used to test for treatment (+H vs. –H) effects. For milk and kinetic data, blocking terms (random effects) were goat, period within goat, and day (d 1 to 21) within period (for milk data only). Treatment terms (fixed effects) were period and treatment. No period effect was found for milk production, so this term was dropped from the model. Although for all goats the kinetic parameter K_{in} responded similarly to treatment, K_{in} for each goat varied in magnitude according to the level of milk production of that goat. Therefore, K_{in} values were log transformed before analyzing by ANOVA.

RESULTS

Milk Production

Given in Table 4 is milk production and MBF. Milk yield ($P = 0.08$), CP (milk N × 6.38) content ($P < 0.05$) and CP yield (118.2 vs. 96.8 g/d; $P < 0.05$) were higher when +H was infused compared with –H.

Plasma Kinetics

Plasma AA kinetics are summarized in Table 3. Arterial plasma concentration of His was considerably lower (8 vs. 73 μM, $P < 0.001$) during infusion of –H. Concentrations of most other AA remained unchanged, except for Phe, Tyr, and Lys, which were slightly higher, and Ala, which was marginally lower with infusion of –H. Plasma

Table 2. Effect of an abomasal infusion of a mixture of AA including (+H) or excluding (–H) His on plasma AA transmembrane exchange kinetics by one udder half of lactating goats.[1]

	NB (μmol/h)			$F_{mg,a}$ (μmol/h)			$F_{v,mg}$ (μmol/h)			Milk secretion (μmol/h)			K_{in} (l/min)		
Transport system:AA	+H	–H	SE	+H	–H	SE	+H	–H	SE	+H	–H	SE	+H	–H	SE[2] (%)
N system															
His	300	261	36	431	287*	25	131	26*	17	452	279†	53	0.14	6.05*	20.4
L system															
Phe	493	352*	28	710	508	101	217	156	90	713	462†	69	0.56	0.25**	2.4
Tyr	440	317*	30	755	449	214	315	133	216	365	241*	21	0.57	0.17	11.5
Leu	1578	1504	66	1766	1689	166	188	185	100	1855	1168*	143	1.63	0.77	1.6
Ile	1190	1119	54	1321	1275	105	131	156	86	937	564†	106	0.76	0.59	5.1
Val	1494	1438	79	1668	1548	122	175	110	84	1448	976†	145	0.69	0.35*	4.9
y^{+}L system															
Lys	1637	1271^{+}	127	2097	1758*	67	461	487	61	1363	876†	143	0.45	0.20*	4.6
ASC system															
Thr	873	665†	60	1357	887	370	482	258	309	1153	735†	117	0.32	0.17*	3.9
Ser	–290	–2158†	263	2244	1866	574	2534	4025	311	1293	804***	63	0.28	0.16	7.4
A system															
Pro	927	693*	56	1323	945	228	396	251	204	2068	1269*	205	0.27	0.16*	4.2
Ala	1209	679	214	2232	2494	400	934	1856†	160	929	595*	80	0.43	0.42*	4.1
Gly	259	613	496	2793	2011	856	2531	1410	1352	802	531***	49	0.05	0.04	9.7

[1]Negative fluxes denote release or production and positive values denote removal. NB, plasma net balance; $F_{mg,a}$, influx from plasma to the mammary gland; $F_{v,mg}$, efflux from mammary gland into plasma; K_{in}, transport system capacity for the AA.

[2]K_{in} data were log transformed for statistical comparisons. The error estimate (SE) is represented as a percentage of the mean.

$^{\dagger}P < 0.10$.

$^{*}P < 0.05$.

$^{**}P < 0.01$.

$^{***}P < 0.001$.

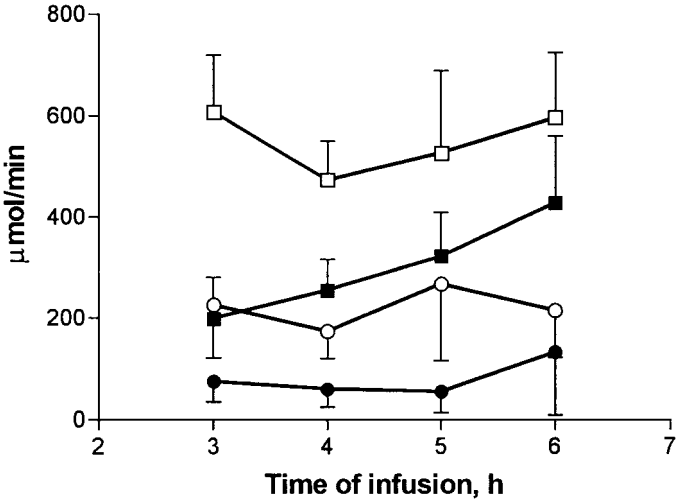

Figure 3. Temporal rates (mean ± SE) of influx ($F_{mg,a}$; □, ■) and efflux ($F_{v,mg}$; ○, ●) of plasma His by half the mammary gland of lactating goats infused into the abomasum with a mixture of AA either including (open symbols) or excluding (closed symbols) His. Flux calculations were performed for h 3 to 6 of the 7-h infusion of isotope (see calculations for explanation). For both influx and efflux rates, there was no significant ($P > 0.20$) interaction between treatment and time of isotope infusion.

flux of His was lower when –H was infused, but those of other AA were not affected by treatment. Although the absorptive supply of His was lower during –H infusion, a greater proportion of plasma His flux was partitioned to the mammary gland. In contrast, less of Phe, Tyr, Lys, Pro, and Gly plasma flux was partitioned to the gland when –H was infused, whereas partition of the remaining AA did not change. Despite the low arterial plasma concentrations of His during –H infusion, the net fractional extraction of plasma His was higher, resulting in extremely low venous concentrations of His (~2 μM). Plasma fractional extraction of nearly all other AA was lower when –H was infused.

Mammary Kinetics

Mammary blood and plasma flows were ~33% higher during infusion of –H than with +H (Table 4). Table 2 summarizes transmembrane exchange kinetics for plasma AA across one udder half. For the purpose of discussion, and for simplicity, the AA are listed under the transport system that is known to be the main carrier of that AA (3), although most AA are carried by more than one transport system. Compared with infusion of +H, the secretion of AA in milk (measured directly during the blood sampling period) was reduced (~36%) by infu-

Table 3. Effect of an abomasal infusion of a mixture of AA including (+H) or excluding (–H) His on plasma AA concentrations, flux (F_{wb}), partition of F_{wb} to (K_{mg}) and net fractional extraction by the whole udder of lactating goats.

Transport system:AA	Concentration (μM)			F_{wb} (mmol/h)			K_{mg} (/d)			Plasma net extraction (%)		
	+H	–H	SE	+H	–H	SE	+H	–H	SE	+H	–H	SE
N system												
His	73	8***	20	3.29	1.59***	0.03	0.21	0.31†	0.03	17	74*	11
L system												
Phe	40	46*	1	6.79	6.82	0.33	0.18	0.13*	0.01	45	21*	4
Tyr	44	55***	3	4.26	4.04	0.52	0.24	0.18†	0.02	37	15†	7
Leu	79	88	4	11.11	11.16	0.77	0.29	0.27	0.01	72	49**	2
Ile	75	75	2	6.31	5.78	0.31	0.38	0.41	0.04	57	42*	3
Val	108	129	11	6.40	7.40	1.79	0.39	0.41	0.02	52	32†	6
Met[1]	40	46	5	—	—	—	—	—	36	18*	5	
y^+L system												
Lys	147	182†	16	9.72	11.90	1.98	1.36	0.28*	0.02	41	19*	4
Arg[1]	90	98	11	—	—	—	—	—	—	37	20	7
ASC system												
Thr	106	106	5	9.00	9.21	1.07	0.23	0.18	0.03	30	17†	4
Ser	124	152	10	14.53	16.90	1.49	0.15	0.04	0.04	–13	–34	8
A system												
Pro	126	131	11	5.22	6.04	1.02	0.43	0.29†	0.05	27	14	5
Ala	142	115†	8	15.08	15.82	0.57	0.24	0.22	0.03	31	17	7
Gly	897	819	139	20.45	19.18	0.55	0.18	0.04†	0.02	1	–1	2
X^-_{AG} system												
Asp[1]	3	4	7	—	—	—	—	—	—	69	45	19
Glu[1]	98	100	2	—	—		—	—	—	59	56	2

[1]Kinetic fluxes were not computed due to difficulties in GC-MS determinations (see reference 22 for explanations).

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

sion of –H. Except for Ser, which was apparently synthesized by the mammary gland, the rate of influx always exceeded (by 1.1 to 14-fold) that of efflux. For His, the rates of influx and efflux from the udder were both reduced ($P < 0.05$) substantially (–33% and –80%) when -H was infused. For most other AA, the rate of influx was numerically reduced by infusion of –H, but this effect was significant only for Lys. With the exception of Ala, there were only small changes in the efflux of other AA.

In response to the shortage (–85%) of His supply ($C_a \times$ MBF) to the mammary gland, K_{in} for His increased 43-fold ($P < 0.05$). By contrast, K_{in} for most other AA decreased two to threefold due to infusion of –H, and this occurred even though the supplies of these AA to the gland had been increased (>36%) by infusion of –H. The latter effect was only the result of the increase in MBF during infusion of –H.

Table 4. Effect of an abomasal infusion of a mixture of amino acids including (+H) or excluding (–H) His on milk production and blood flows to one udder half of lactating goats.

	+H	–H	SE
Milk yield,[1] kg/d	3.76	3.32†	0.2
Milk CP,[1] g/kg	3.18	2.94*	0.09
Milk CP yield, g/d	118.2	96.8*	7.9
Blood flow,[2] kg/h	34.56	46.84*	3.60
Plasma flow,[2] kg/h	27.68	36.83†	3.57

[1]Values are the means of milk production for the last 4 d of each treatment period. Milk CP was calculated as (milk N × 6.38).

[2]Mammary blood and plasma flows are those monitored during the arteriovenous kinetic measurements taken on the last day of each treatment period.

†$P < 0.10$.

*$P < 0.05$.

DISCUSSION

The experimentation described in this paper was designed to examine the responses of the mammary gland to an artificially induced limitation in the supply of one AA for milk protein synthesis. Excluding His from the AA mixture resulted in plasma arterial His concentrations falling to uncharacteristically low levels (8 vs. 73 μM) and led to a decrease in milk protein synthesis by the mammary gland (96.8 vs. 118.2 g of milk protein/d).

In this His-deficient state, the udder was able to compete well for whole body His supplies, as evidenced by a greater proportion (K_{mg}, 0.31 vs. 0.21) of whole body His flux (an estimation of whole body protein synthesis) being partitioned to the glands while the K_{mg} for most other AA was reduced.

Under typical feeding conditions, the efficiency of extraction of most of the essential AA by the mammary gland rarely exceeds 50% (18). However, we have previously observed that the gland does appear to have a larger capacity to extract AA when the supply of an AA is reduced. Thus, where Leu concentration was reduced, extraction of Leu increased from 52 to 71% (9), suggesting that transporter capacity can be altered in an attempt to maintain intracellular AA concentrations. Leucine is transported by the L system, which can utilize concentration gradients (i.e., high intracellular concentrations) of other AA to drive Leu removal (3, 14). Thus, Leu uptake could also have been facilitated by AA exchange mechanisms, rather than as a response to an increase in the activity of the L system transporter. Similarly, in the present study, we found that the net efficiency of extracting plasma His was enhanced (74 vs. 17%) when arterial concentrations of His fell to low levels on the –H infusion. Changes in MBF and in mammary transmembrane exchange kinetics of AA seemed to suggest that these mechanisms may facilitate AA partition to and uptake by the gland, and thus they may be primary mechanisms involved in supporting, or possibly controlling, milk protein synthesis.

Consideration of MBF

Up-regulation of MBF appeared to be one of the mechanisms by which the gland attempted to maintain AA supply when the –H infusion was given. The increase (36%) in MBF observed here, where MBF increased within 24 h of –H infusion (data not shown) and persisted for the entire –H infusion period, is similar to the response we observed previously (17% increase in MBF; 9) in goats when supply of Leu to the mammary gland was artificially reduced (i.e., relative to other AA). In both studies, the plasma concentrations of the AA being manipulated were decreased by treatment, suggesting that the mammary gland is capable of sensing an AA deficiency, or low concentrations of AA, and that the gland may attempt to overcome these deficiencies by controlling MBF.

This inverse relationship between milk yield response and MBF is at odds with all previous observations that milk yield and MBF are positively correlated (26). The apparent dissociation of MBF from overall metabolism presumably indicates that the mechanism(s) regulating MBF under these conditions can override those that are responsible for maintaining O_2 delivery or adenylate charge (ATP status) as has been proposed (16). In response to the –H infusion, it is possible that the increase in MBF resulted from a reduction in the synthesis of a metabolite of His. Histamine is synthesized within the gland in a substrate dependent manner via decarboxylation of His, and histamine has been shown to have vasoconstrictive actions on the mammary vasculature (21). Under His-limiting conditions, therefore, one might predict that histamine levels would be lower, thus reducing vascular constriction and causing an increase in MBF. Although histamine is a plausible explanation for the effects of His on MBF, neither Leu nor any of its catabolic end products have been shown to possess vaso-activity, thus precluding histamine action as a common mechanism to explain our present and previous (9) observations in lactating goats.

Consideration of Mammary AA Extraction Processes

Amino acids are taken up from blood and concentrated in the mammary cell (27) via membrane transport systems (A, ASC, GLY, L, N, y^+L, and X^-_{AG}). These systems in vitro are highly efficient, thus, it has often been assumed that uptake of AA by the mammary gland is not limited by the AA transport systems. However, recent studies (e.g., 18) indicate that transport may be limiting under normal conditions, when extraction of AA by the gland is far less than 100%, but where infusion of additional AA increases AA uptake and milk protein yield. In the present study, to determine if the capacity of the gland to extract AA from plasma had been modulated in response to the single AA (His) limitation, rates of transfer of AA from plasma to the intracellular pool and from the intracellular pool to the plasma were derived.

There was considerable influx and efflux of AA across the mammary gland, with rates of influx exceeding those of efflux by 1.1 to 10-fold (Table 2). Under His-limiting conditions, rates of both influx and efflux of His were lower. However, influx appeared to be mostly limited by the arterial plasma concentration of His. The efficiency of single-pass removal of AA by the mammary transport systems can be assessed by $F_{mg,a}/F_{a,O}$ (influx ÷ arterial supply). The efficiency of removal of His increased from 0.24 to 0.83 (SE 0.09, $P < 0.10$) when His supply was limiting. Conversely, the efficiency of removal of other AA, for example Leu (0.82 vs. 0.55 (SE 0.07), $P < 0.05$), was lower when –H was infused. This result suggests that the blood-facing aspect of the N system transporter for His uptake is capable of functioning at a higher rate of efficiency than normal and, thus, may not be the rate-limiting step for milk protein synthesis under normal

conditions. This may not be the case for other AA, however.

The rate parameter K_{in} (Table 2) can be used to assess the capacity of the blood-facing aspect of the AA transport systems and is also analogous to transport activity. A potential limitation of K_{in} is the assumption that exchange kinetics are linear. Herein, plots of model solutions (data not shown) demonstrated linearity over the range of AA concentrations observed, validating the latter assumption. Thus, it appears that when His was limiting, the ability of the gland to remove plasma His was enhanced, as evidenced by a 43-fold increase in the capacity for transfer from plasma to the intracellular pool. Unfortunately, intracellular concentrations of AA could not be measured to determine the capacity for outward transfers. Although the capacity of the His transport system(s) to clear plasma His was undoubtedly enhanced, the aggregated nature of K_{in} does not allow one to distinguish whether changes in capacity resulted from 1) an increase in the affinity of the transport system for substrate, 2) an increase in the number of transporters, or 3) an increase in the capillary surface area being perfused. Given the apparent decline in K_{in} for other AA transport systems (Table 2) when –H was infused, however, a change in capillary perfusion would not appear to be a common explanation for all transport changes. The observed increase in uptake capacity under His-limiting conditions is consistent with observations in rat hepatocytes where transporter activity increased twofold when media was deprived of Gln, an N system substrate (see 17). This response was attributed to an increase in the number of transporters because the affinity of the transporter was not altered. Systems L and A, but not ASC and GLY, are known to be regulated similarly (28).

Net balances of some of the other AA were significantly lower when –H was infused (Table 2). This response involved small decreases (nonsignificant, except for Lys and Ala) in influx or increases in efflux, or both. Although arterial concentrations of these AA remained unchanged or were only slightly altered, uptake capacity declined two- to threefold. This ability of the transport system in vivo to adapt to prevailing substrate supplies relative to requirements confirms many observations in vitro (28). Under His-limiting conditions, where casein synthesis was reduced, the intracellular concentrations of all AA except His were probably increased. In response, the capacity of the blood-facing aspect of the transport systems was attenuated, probably via trans-inhibition mechanisms (i.e., the transport system is locked away on the intracellular side of the membrane where AA concentrations are high) (see review by 17). Such a control mechanism would allow the cell or tissue to detect AA deficiencies or excesses that would otherwise cause the synthetic processes to stop or lead to a build-up of substrates to potentially toxic levels.

Amino acids were categorized by carrier systems that are known to transport these AA predominantly, although many AA may be transported by more than one system (3). Within a treatment period, the absolute rates of influx and efflux varied among the AA. However, K_{in} was not as variable, except for Leu, and, of course, for His. In agreement with observations in vitro (14), capacity of the L system to transport the branched-chain AA (Ile, Leu, and Val) appeared to be greater than for Phe and Tyr. The greater ability of the L system to clear the branched-chain AA may explain why a smaller proportion of the influx of the branched-chain AA (9 to 14%) was transferred back out of the tissues than Phe and Tyr (25 to 28%). The apparent preference for the branched-chain AA, particularly for Leu, could serve to concentrate these AA within the mammary cell. Alternatively, the apparently lower rate of efflux of the branched-chain AA may also reflect that these AA are also disposed of from the intracellular pool via oxidation (9).

CONCLUSIONS

It appears that the cells of the udder can regulate MBF in an attempt to overcome a limitation in a single AA. Additionally, the udder responded to this AA limitation by up-regulating the processes that govern the extraction of that AA while down-regulating those for other AA, i.e., those AA apparently now in excess of requirements. Although the udder responded phenomenally to the –H infusion in terms of its uptake capacity, transport per se cannot be ruled out as the rate-limiting step in milk protein synthesis under conditions of extreme AA limitation. Nonetheless, such potential to respond to this limitation would seem to suggest that the removal process is not the primary limitation under normal conditions, i.e., when more His is required, the udder clearly has a tremendous capacity to extract more. Rather, it would seem that transport capacity is closely linked with utilization to match needs and supply. These adaptive responses serve to reduce the variation in milk protein output that would be caused by short-term (hour-to-hour) perturbations in systemic supplies. Furthermore, this adaptive process would result in relatively flat response curves when relating milk protein synthesis to AA supply. The latter would be consistent with data from casein infusion studies summarized by Hanigan et al. (19) but inconsistent with models containing relatively large coefficients relating the absorption of protein to milk protein yield in the lactating dairy cow (25).

ACKNOWLEDGMENTS

This work is part of a joint project at the Rowett Research Institute (Aberdeen, Scotland) and Reading University (Reading, England) cosponsored by a consortium of the Ministry of Agriculture, Fisheries and Food (England); the Biotechnology and Biological Sciences Research Council (England); the Scottish Office Agriculture Environment and Fisheries Department (Scotland); Purina Mills, Inc. (St. Louis, MO); Hendrix Voeders B.V. (The Netherlands); and the Milk Development Council (England). Gratitude is given to C. Kyle for her special care of the goats and assistance in running the experiments, to G. Horgan for his statistical advice and to V. Buchan for her expertise in AA analyses.

REFERENCES

1 Agricultural and Food Research Council. 1993. Energy and Protein Requirements of Ruminants. An Advisory Manual Prepared by the AFRC Technical Committee of Responses to Nutrients. CAB Int., Wallingford, United Kingdom.
2 Backwell, F.R.C., B. J. Bequette, D. Wilson, A. G. Calder, J. A. Metcalf, D. Wray-Cahen, J. C. MacRae, D. E. Beever, and G. E. Lobley. 1994. Utilization of dipeptides by the caprine mammary gland for milk protein synthesis. Am. J. Physiol. 267:R1–R6.
3 Baumrucker, C. R. 1985. Amino acid transport systems in bovine mammary tissue. J. Dairy Sci. 68:2436–2451.
4 Bequette, B. J., and F.R.C. Backwell. 1997. Amino acid supply and metabolism by the ruminant mammary gland. Proc. Nutr. Soc. 56:593–605.
5 Bequette, B. J., F.R.C. Backwell, A. G. Calder, J. A. Metcalf, D. E. Beever, J. C. MacRae, and G. E. Lobley. 1997. Application of a U-carbon-13-labelled amino acid tracer in lactating dairy goats for simultaneous measurements of the flux of amino acids in plasma and the partition of amino acids to the mammary gland. J. Dairy Sci. 80:2842–2853.
6 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. 81:2540–2559.
7 Bequette, B. J., F.R.C. Backwell, M. S. Dhanoa, A. Walker, A. G. Calder, D. Wray-Cahen, J. A. Metcalf, J. D. Sutton, D. E. Beever, G. E. Lobley, and J. C. MacRae. 1994. Kinetics of blood free and milk casein-amino acid labeling in the dairy goat at two stages of lactation. Br. J. Nutr. 72:211–220.
8 Bequette, B. J., F.R.C. Backwell, C. E. Kyle, L. A. Crompton, J. France, and J. C. MacRae. 1999. Vascular sources of phenylalanine, tyrosine, lysine, and methionine for casein synthesis in lactating goats. J. Dairy Sci. 82:362–377.
9 Bequette, B. J., F.R.C. Backwell, J. C. MacRae, G. E. Lobley, L. A. Crompton, J. A. Metcalf, and J. D. Sutton. 1996. Effect of intravenous amino acid infusion on leucine oxidation across the mammary gland of the lactating goat. J. Dairy Sci. 79:2217–2224.
10 Berthold, H. K., D. L. Hachey, P. J. Reeds, O. P. Thomas, S. Hoeksema, and P. D. Klein. 1991. Uniformly ^{13}C-labeled algal protein used to determine amino acid essentiality in vivo. Proc. Natl. Acad. Sci. USA 88:8091–8095.
11 Biolo, G., D. Chinkes, X.-J., Zhang, and R. R. Wolfe. 1992. A new model to determine in vivo the relationship between amino acid transmembrane transport and protein kinetics in muscle. J. Parenter. Enter. Nutr. 16:305–315.
12 Calder, A. G., and A. Smith. 1988. Stable isotope ratio analysis of leucine and ketoisocaproic acid in blood plasma by gas chromatography/mass spectrometry. Use of tertiary butyldimethylsilyl derivatives. Rapid Commun. Mass Spectrom. 2:14–16.
13 Cant, J. P., and B. W. McBride. 1995. Mathematical analysis of the relationship between blood flow and uptake of nutrients in the mammary glands of a lactating cow. J. Dairy Res. 62:405–422.
14 Christensen, H. N. 1990. Role of amino acid transport and counter transport in nutrition and metabolism. Physiol. Rev. 70:43–77.
15 France, J., B. J. Bequette, G. E. Lobley, J. A. Metcalf, D. Wray-Cahen, M. S. Dhanoa, F.R.C. Backwell, M. D. Hanigan, J. C. MacRae, and D. E. Beever. 1995. An isotope dilution model for partitioning leucine uptake by the bovine mammary gland. J. Theor. Biol. 172:369–377.
16 Fraser, D. L., E. R. Ørskov, F. G. Whitelaw, and M. F. Franklin. 1991. Limiting amino acids in dairy cows given casein as the sole source of protein. Livest. Prod. Sci. 28:235–252.
17 Guidotti, G. G., A. F. Borghetti, and G. C. Gazzola. 1978. The regulation of amino acid transport in animal cells. Biochim. Biophys. Acta 515:329–366.
18 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. 77:3304–3315.
19 Hanigan, M. D., J. P. Cant, D. C. Weakley, and J. L. Beckett. 1998. An evaluation of postabsorptive protein and amino acid metabolism in the lactating cow. J. Dairy Sci. 81:3385–3401.
20 Hanigan, M. D., J. France, D. Wray-Cahen, D. E. Beever, G. E. Lobley, L. Reutzel, and N. E. Smith. 1998. Alternative models for analyses of liver and mammary transorgan metabolite extraction data. Br. J. Nutr. 79:63–78.
21 Jakobsen, K., E. O. Mikkelsen, and M. O. Nielsen. 1994. Studies on responses to potassium, noradrenaline, serotonin, histamine and prostaglandin-F_2-alpha of isolated pudendal arteries from nonlactating goats. Comp. Biochem. Physiol. 109 (C Comp. Pharmacol.):167–172.
22 Lobley, G. E., A. Connell, D. K. Revell, B. J. Bequette, D. S. Brown, and A. G. Calder. 1996. Splanchnic-bed transfers of amino acids in sheep blood and plasma, as monitored through the use of a multiple U-^{13}C-labeled amino acid mixture. Br. J. Nutr. 75:217–235.
23 MacKenzie, S. L., and D. Tenaschuk. 1979. Quantitative formation of N(O,S)-heptafluorobutyryl isobutyl amino acids for gas chromatographic analysis. I. Esterification. J. Chromatogr. 171:195–208.
24 MacKenzie, S. L., and D. Tenaschuk. 1979. Quantitative formation of N(O,S)-heptafluorobutyryl isobutyl amino acids for gas chromatographic analysis. II. Acylation. J. Chromatogr. 173:53–63.
25 National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.
26 Prosser, C. G., S. R. Davis, V. C. Farr, and P. Lacasse. 1996. Regulation of blood flow in the mammary microvasculature. J. Dairy Sci. 79:1184–1197.
27 Shennan, D. B., I. D. Millar, and D. T. Calvert. 1997. Mammary-tissue amino acid transport systems. Proc. Nutr. Soc. 56:177–191.
28 Shotwell, M. A., P. M. Mattes, D. W. Jayme, and D. L. Oxender. 1982. Regulation of amino acid transport system L in Chinese hamster ovary cells. J. Biol. Chem. 257:2974–2980.
29 Ulyatt, M. J., J. C. MacRae, R.T.J. Clarke, and P. D. Pearce. 1975. Quantitative digestion of fresh herbage by sheep. IV. Protein synthesis in the stomach. J. Agric. Sci. (Camb.) 84:453–458.