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Modelling Mammary Metabolism in the Dairy Cow to Predict Milk Constituent Yield, with Emphasis on Amino Acid Metabolism and Milk Protein Production: Model Evaluation

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A model of mammary metabolism has been constructed and parameterized, with milk protein synthesis represented as a function of five essential amino acids (EAA) (Hanigan *et al*., 2001). Herein the model is evaluated using both the data used to construct the model (reference data) and an independent data set (literature data), and sensitivity to inputs and parameter estimates is assessed. The model predicted metabolite removal well for the reference data with exceptions for glutamate, glucose, and acetate. However, predictions of milk protein synthesis exhibited significant mean positive bias, which apparently was associated with the representation of milk protein synthesis. Adjustment of model parameters removed the mean bias, however, prediction accuracy was still inadequate. Simulation of the single reference experiment containing all critical inputs resulted in predictions of milk protein output that explained 53% of the observed variation, suggesting that the limited accuracy of the model when applied to the entire reference data set was due to assumptions regarding missing inputs. Mammary removal of glutamate, isoleucine, lysine, phenylalanine, tyrosine, valine, glycerol, β-hydroxybutyrate (BHBA), and acetate were predicted less accurately when simulations of the independent data set were conducted. Twenty-five percent of the observed variation in milk protein yields for the independent data set was explained by the model. Refitting parameters for removal of isoleucine, lysine, phenylalanine, tyrosine, valine, glycerol, BHBA, and acetate raised the variation explained to 43%. Sensitivity analysis indicated that milk protein synthesis was responsive to only the five EAA used in its determination, with sensitivity to any single EAA falling to zero as supply of the EAA exceeded protein synthetic needs. Similarly, milk protein synthesis was readily affected by parameters associated with removal and metabolism of the five EAA. Milk lactose was found to be sensitive to glucose input as well as to similar parameters and inputs as milk protein. It is concluded that representation of the milk protein synthesis process as a function of a single limiting EAA may not be adequate and might be better represented by simultaneous consideration of multiple EAA. Additional work on the description of energy metabolism is also suggested.

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Introduction

Identification of amino acids that limit milk protein synthesis has been a focus of ruminant nutrition work for a number of years (Clark, 1975; Fisher, 1972; Fraser *et al.*, 1991; Schwab *et al.*, 1976, 1992a, b). This work has led to prediction of primary limiting amino acids (AA) for a few diets. However, responses to supplementation of AA have been variable (Clark, 1975), presumably due to interactions among diet, stage of lactation, and possibly animal genetics. An integrative model of AA metabolism for the lactating cow could help resolve the variability observed.

A model of AA metabolism in the ruminant was developed by O'Conner *et al.* (1993). However, due to a lack of available information, the representation of post-absorptive AA metabolism in that model required a number of simplifying assumptions that appear to compromise accuracy of predictions (Kohn *et al.*, 1998). A better representation of metabolism by individual tissues should foster progress in the area. A number of models have been developed to examine AA metabolism in the mammary gland (France *et al.*, 1995; Hanigan & Baldwin, 1994; Maas *et al.*, 1997; Waghorn & Baldwin, 1984). Until recently, there has not been a comprehensive representation of AA metabolism in the udder of the lactating dairy cow (Hanigan *et al.*, 2001). The model of Hanigan *et al.* (2001) has been constructed and parameterized, but not tested for sensitivity against parameter estimates and inputs, nor evaluated against an independent data set.

A number of mammary arterio-venous difference studies have been conducted and described in the literature (see Table 1). An objective common to many of these experiments was identification of AA limiting for milk and milk protein synthesis. The intrinsic supposition underlying the majority of hypotheses that were tested was that a single AA was limiting milk protein synthesis, or possibly that two AA were co-limiting protein synthesis, and that provision of additional increments of the limiting AA would result in an increase in milk protein synthesis while provision of additional increments of non-limiting AA would not result in an

TABLE 1
Published experiments used for model evaluation

Study	Abbreviation	Blood flow	Sampling site*
Austin *et al.* (1991)	Austin	eqn (1)	Coccygeal vein
Bickerstaffe & Annison (1974)	Bickerst	Thermodilution and Fick principle†	Carotid artery
Cant *et al.* (1993a,b)	Cant	Fick principle	Coccygeal vein
Casper *et al.* (1987)	Casper	eqn (1)	Coccygeal vein
Choung & Chamberlain (1993)	Choung	eqn (1)	Coccygeal vein
Clark *et al.* (1977)	Clark	eqn (1)	Jugular vein
Derrig *et al.* (1974)	Derrig	eqn (1)	Jugular vein
Drackley & Schingoethe (1986)	Drackley	eqn (1)	Jugular vein
Fisher (1972)	Fisher	eqn (1)	Jugular vein
Griinari *et al.* (1997a,b)	Griinari	eqn (1)	Jugular vein
Guinard *et al.* (1994), Guinard & Rulquin (1994a)	Guinard1	Flow probe	Carotid artery
Guinard & Rulquin (1994b)	Guinard2	Flow probe	Carotid artery
Guinard & Rulquin (1995)	Guinard3	Flow probe	Carotid artery
Illg *et al.* (1987)	Illg	eqn (1)	Coccygeal vein
Karunanandaa *et al.* (1994)	Karunanandaa	eqn (1)	Jugular vein
Lykos *et al.* (1997), Lycos & Varga (1997)	Lykos	Fick principle	Jugular vein
Peeters *et al.* (1979)	Peeters	Flow probe	Carotid artery
Rulquin (1981)	Rulquin	eqn (1)	Carotid artery
Spires *et al.* (1975)	Spires	eqn (1)	Internal iliac artery
Hanigan *et al.* (1992), Hanigan & Baldwin (1995), Miller *et al.* (1991)	UCD	Fick principle	Coccygeal vein
Yang *et al.* (1986)	Yang	eqn (1)	Coccygeal vein

* Site used to obtain arterial samples or samples assumed to represent arterial blood.
† Thermodilution measurements were made in the subcutaneous abdominal vein after exit from the udder.

increase in milk protein synthesis. This theory will be referred to herein as the single limiting AA theory. A similar assumption underlies design of most animal studies (Schwab *et al.*, 1976, 1992a, b; Vanhatalo *et al.*, 1999). These studies have been interpreted collectively as suggesting that methionine and/or lysine are generally limiting milk protein synthesis with histidine, phenylalanine, and threonine occasionally limiting or near limiting. Apparent responses to other EAA have been observed (Schwab *et al.*, 1976; Clark *et al.*, 1978; Vanhatalo *et al.*, 1999).

The model described by Hanigan *et al.* (2001) contains a representation of the single limiting AA theory and provides for possible limitations by methionine, lysine, histidine, phenylalanine, and threonine. Application of the model to experiments designed to manipulate AA supply to the udder should provide a quantitative assessment of the validity of the theory and provide an evaluation of the model. The objectives of the current study were (1) to evaluate the model of Hanigan *et al.* (2001) against experimental data and, thereby, the concepts represented in the model and (2) to test the model for sensitivity to inputs and parameter estimates.

Methods

Evaluation of the model was undertaken in three areas: (1) integration settings required to run stable simulations, (2) evaluation of model accuracy as compared to observed data, and (3) sensitivity analyses. All simulations were run using ACSL® Optimize (Pharsight Corp., Mountain View, CA, U.S.A.) on a Dell Optiplex computer (Dell Computer Corp., Round Rock, TX, U.S.A.) with an Intel P350 processor and 64 Mb of memory. Model parameters used were as defined by Hanigan *et al.* (2001) unless specified otherwise. An overview of the model is depicted in Fig. 1. Arrows represent fluxes and unshaded boxes denote state variables. Abbreviations used are summarized by Hanigan *et al.* (2001).

Two separate data sets were used to evaluate the model: (1) the data set used for model parameterization (Hanigan *et al.*, 2001; reference data), and (2) a data set assembled from the literature (literature data). Individual experiments within the reference data set are referred to using the nomenclature described by Hanigan *et al.* (2001; C1, C3, C6, C10). Blood flow values reported for C1 were felt to be erroneous, therefore, blood flow for C1 was predicted using the equation below from Hanigan *et al.* (2001):

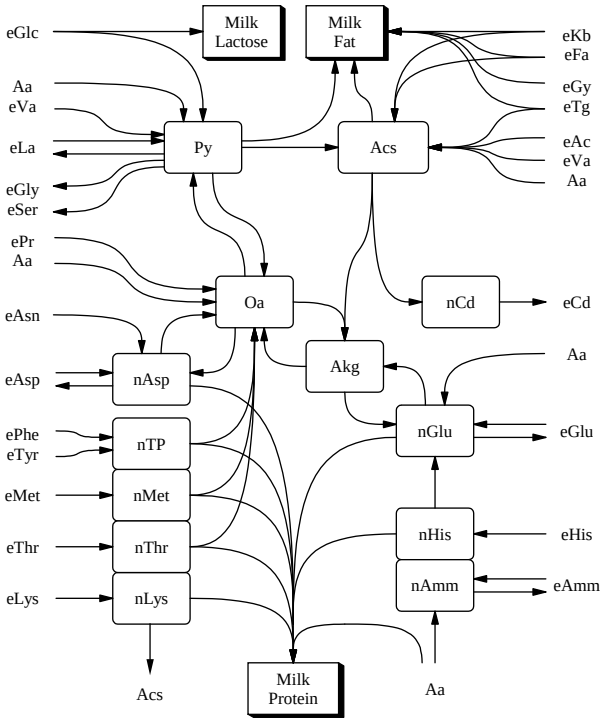

FIG. 1. Flow diagram depicting an overview of metabolism within the udder of a lactating cow. Unshaded boxes denote pools defined in the model, and arrows denote fluxes. Abbreviations are defined in Hanigan *et al.* (2001).

$$Blood\ Flow = 0.365(\pm 0.070)\ Milk\ Yield + 2.78(\pm 1.48). \quad (1)$$

Experimental data available in the literature are listed in Table 1 and summarized in Table 2. These studies are generally referred to individually by the primary authors surname (Table 1) with the exception of work by Baldwin and colleagues (Hanigan & Baldwin, 1995; Hanigan *et al.*, 1992; Miller *et al.*, 1991) which is referred to as UCD. No studies contained complete data sets relative to inputs for the model. However, all of the studies contained AA data. A few studies reported blood flow values (Table 1), although, only the studies of Guinard and colleagues (Guinard *et al.*, 1994; Guinard & Rulquin, 1994a, b, 1995) and Peeters *et al.* (1979) made

TABLE 2
Summary of experiments listed in Table 1*

Metabolite	*N*	Mean	Minimum	Maximum	S.D.
Alanine	108	210	73	328	46
Arginine	108	85	22	203	34
Asparagine	95	53	27	129	19
Aspartate	66	104	27	291	65
Citrulline	106	68	28	146	29
Cysteine	89	35	2	70	12
Glutamate	108	112	37	239	45
Glutamine	95	128	15	277	60
Glycine	108	349	204	596	88
Histidine	107	62	21	99	16
Isoleucine	108	129	30	243	38
Leucine	106	157	42	254	39
Lysine	104	84	49	131	15
Methionine	108	21	9	84	10
Ornithine	106	65	34	116	18
Phenylalanine	108	48	16	71	8
Proline	97	106	52	195	22
Serine	108	91	49	176	20
Threonine	104	89	36	142	20
Tryptophan	79	18	7	44	9
Tyrosine	104	51	33	83	9
Valine	108	282	118	478	81
Urea (mM)	87	3.1	2.0	7.14	1.12
Acetate (mM)	89	1.97	1.02	3.02	0.44
BHBA (mM)	86	1.26	0.47	4.62	0.6
Glucose (mM)	85	2.82	2.28	3.56	0.26
Glycerol	64	43	15	124	21
Lactate	62	778	250	2346	475
NEFA (mM)	82	0.268	0.004	1.353	0.187
O_2 (mM)	8	5.96	5.78	6.12	0.12
TAG (mM)	74	0.095	0.035	0.154	0.029
Mammary artery flow (l/min)	92	14.3	3.7	30.3	5.7
Milk yield (kg/day)	143	27.2	11.6	44.6	7.9
Milk lactose (kg/day)	105	1.22	0.50	1.99	0.37
Milk protein (kg/day)	142	0.84	0.33	1.35	0.23
Milk fat (kg/day)	137	1.09	0.34	2.28	0.32

*Values were derived from samples taken from arterial, jugular, or coccygeal vessels. Units are μM unless specified otherwise.

direct measurements of blood flow, with the others deriving blood flow from milk yield. For studies not reporting blood flow, Eqn (1) was used to estimate it. A number of studies utilized sampling sites other than true arterial blood as a substitute for arterial blood. Venous blood sampled from the coccygeal vein has been found previously to be representative of arterial blood (Emery *et al.*, 1965). Lykos & Varga (1997) examined metabolite concentrations in jugular and coccygeal veins and found them not to differ significantly. Based on these observations, jugular and coccygeal metabolite concentrations were assumed to be equivalent to arterial concentrations and used as such.

For all studies, plasma and whole blood metabolite concentrations were assumed to be equivalent except for glucose which was assumed to be carried in plasma only. Whole blood glucose concentrations were thus derived from plasma values using the observed hematocrit. If hematocrits were not reported, the hematocrit was assumed to be 26% of whole blood (Hanigan *et al.*, 2001). Where both plasma and blood concentrations were reported, a mean of the values was taken. Where model inputs were missing, reference values reported by Hanigan *et al.* (2001) were used.

The analysis and graphical presentation of results and assessment of model performance

followed conventional regression methods as detailed by Draper & Smith (1998). Model accuracy was assessed by evaluation of the proportion of variation observed in an output variable that was explained by the model and was calculated as:

$$\textit{Percent Variation Explained} = \left[1 - \frac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}{\sum_{i=1}^{n}(Y_i - \bar{Y})^2}\right]100,$$

where Y, $\hat{Y}$, and $\bar{Y}$ represent the individual observed, individual predicted, and mean observed values for the output variable, respectively with n representing the number of observations (Draper & Smith, 1998). Although this calculation is analogous to a coefficient of determination in linear regression, the possibility that the mean of $\hat{Y}_i$ is significantly different from $\bar{Y}$ exists, potentially resulting in a negative value for percent variation explained and indicating a mean prediction bias.

Sensitivity analyses were conducted whereby the parameter being varied was perturbed positively and negatively and the average absolute change in the prediction variable was measured and expressed as a percentage of its unperturbed value. A relatively small perturbation was used to avoid underestimates of sensitivity coefficients when nonlinear responses were examined. Results were expressed relative to a 1% change in the varied parameter for ease of interpretation.

During evaluation with the literature data set, it was discovered that several parameters were in need of redefinition. The Nelder–Mead optimization algorithm was used to derive these new estimates (Olsson & Nelson, 1975).

Results

NUMERICAL INTEGRATION

A fourth-order Runge–Kutta integration algorithm with a fixed step size was used for all numerical integration (Conte & de Boor, 1980). A determination of the time required to achieve steady state after inputs were perturbed was undertaken. The perturbation used was a reduction in arterial methionine concentration from 23.3 to 10 μM. This perturbation was chosen as it restricts milk protein synthesis, which subsequently affects all pools in the model. Using this perturbation, the overall time required to reach steady state was determined to be 3 days (Fig. 2). However, EAA pools and the rate of milk protein synthesis [Fig. 2(a)] reached steady state 0.05 days after the perturbation.

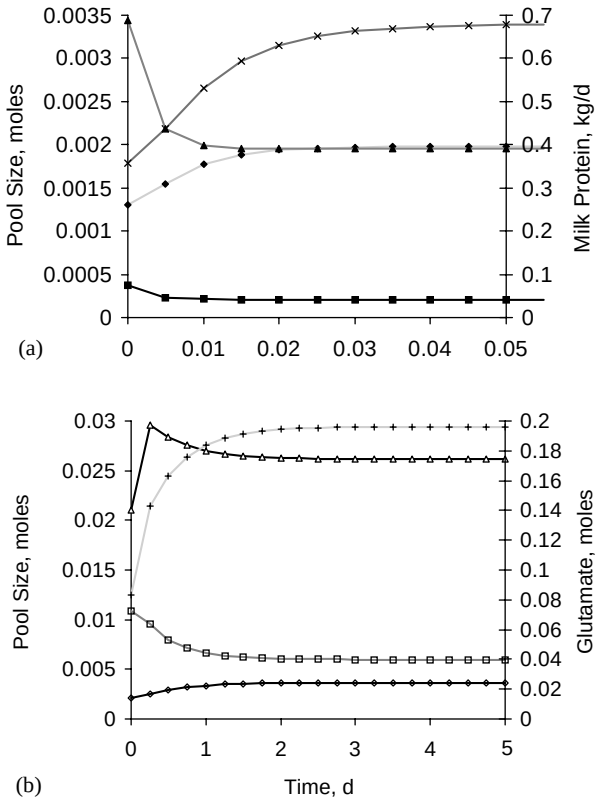

FIG. 2. Model pool sizes and rates of milk protein production (▲, *Pm*) after arterial methionine concentrations were reduced from 23.3 to 10 μM. Methionine concentration was altered at time 0. Pools were intracellular threonine (◆, *nThr*), lysine (×, *nLys*) methionine (■, *nMet*), glutamate (+, *nGlu*), aspartate (△, *nAsp*), oxaloacetate (□, *Oa*), and acetyl coA (◇, *As*).

Four other variable step integration algorithms are available for use in ACSL® Optimize. All four algorithms were tested using the above perturbation of arterial methionine concentration, but none improved the integration interval, time to reach steady state or simulation run time.

EVALUATION WITH REFERENCE DATA

Initially, treatment means from the reference data set were used to define intracellular fluxes and parameterize the model (Hanigan *et al.*,

2001), however, additional information could be derived from the reference data set by using all the individual observations instead of the means. Results from simulations using the individual data from the reference set are presented in Table 3, Figs 3 and 4. To examine the residuals graphically for any bias, we have plotted individual observed minus individual predicted ($Y - \hat{Y}$) against individual predicted ($\hat{Y}$) which is a standard method of assessment for a linear model, since the residuals and the Y's are usually correlated, but the residuals and the $\hat{Y}$'s are not (Draper & Smith, 1998). Predictions of venous metabolite concentrations were adequate generally as evidenced by percent variation explained (Table 3). Exceptions to this included glutamate, glucose, and acetate. Variation in these predictions may be caused by inadequate descriptions of energy metabolism. Systematic bias was assessed graphically and found to be absent for all metabolites considered. Example plots are presented for histidine, methionine,

TABLE 3
*Percent variation in mammary venous metabolite concentration explained by the model**

Venous metabolite	Reference parameters			Fitted parameters		
	Reference†	Reference‡	C6 only‡	Literature‡	C6 only‡	Literature‡
Alanine	87.99	87.99	66.76	58.9	87.99	58.9
Arginine	78.2	78.2	75.16	46.02	78.2	46.02
Asparagine	89.76	89.76		70.22	89.76	70.22
Aspartate	68.92	71.57	−1750845	72.06	72.11	64.03
Cysteine				69.41		69.41
Glutamine	97.03	97.03	97.07	71.25	97.03	71.25
Glutamate	−24.5	5.84	−4068.35	−442.03	45.44	−1706.66
Glycine	97.56	97.56	97.91	82.82	97.56	82.82
Histidine	93.55	93.55	97.5	38.89	91.08	33.71
Isoleucine	79.37	79.37	80.91	−32.32	79.37	66.87
Leucine	84.16	84.16	83.79	56.47	84.16	63.81
Lysine	89.69	89.69	−16.17	−166.3	92.6	33.71
Methionine	75.59	75.59	77.82	61.13	62.64	66.43
Phenylalanine	77.05	77.05	70.33	−39.8	68.55	40.89
Proline	98.84	98.84	99.29	77.84	98.84	77.84
Serine	83.23	83.23	75.8	−24.8	83.23	−24.8
Threonine	57.93	57.93	57.93	55.32	52.52	60.29
Tryptophan				50.26		50.26
Tyrosine	81.84	81.84	68.25	−20.92	75.6	12.95
Valine	91.24	91.24	94.37	88.51	91.24	88.51
Citrulline	78.33	78.33		85.33	78.33	85.33
Ornithine	90.88	90.88	90.88	75.08	90.88	75.08
Urea	99.67	99.67	99.88	89.87	99.67	89.87
Glucose	33.29	44.58	61.81	61.16	50.54	61.31
Glycerol				1.72		1.72
Lactate	60.69	59.74	66.44	65.62	60.06	64.1
NEFA				93.69		93.69
TAG				57.39		57.39
BHBA	85.22	85.22	86.7	23.99	85.22	35.37
Acetate	28.22	28.22	69.88	3.55	28.22	10.04
Milk components						
Lactose	−181.05	−61.72		26.46	−86.14	37.81
Protein	−88.93	−6.62	53.29	25.27	−17.13	43.78
Fat	8.01	−0.61		−53.94	−11.97	−60.54

*Simulations were compared against reference and literature data sets as described in the Methods section using reference parameters alone, or reference parameters and a subset of parameters derived from the literature data set (fitted parameters).

† Δ set to 0.33.

‡ Δ set to 0.57.

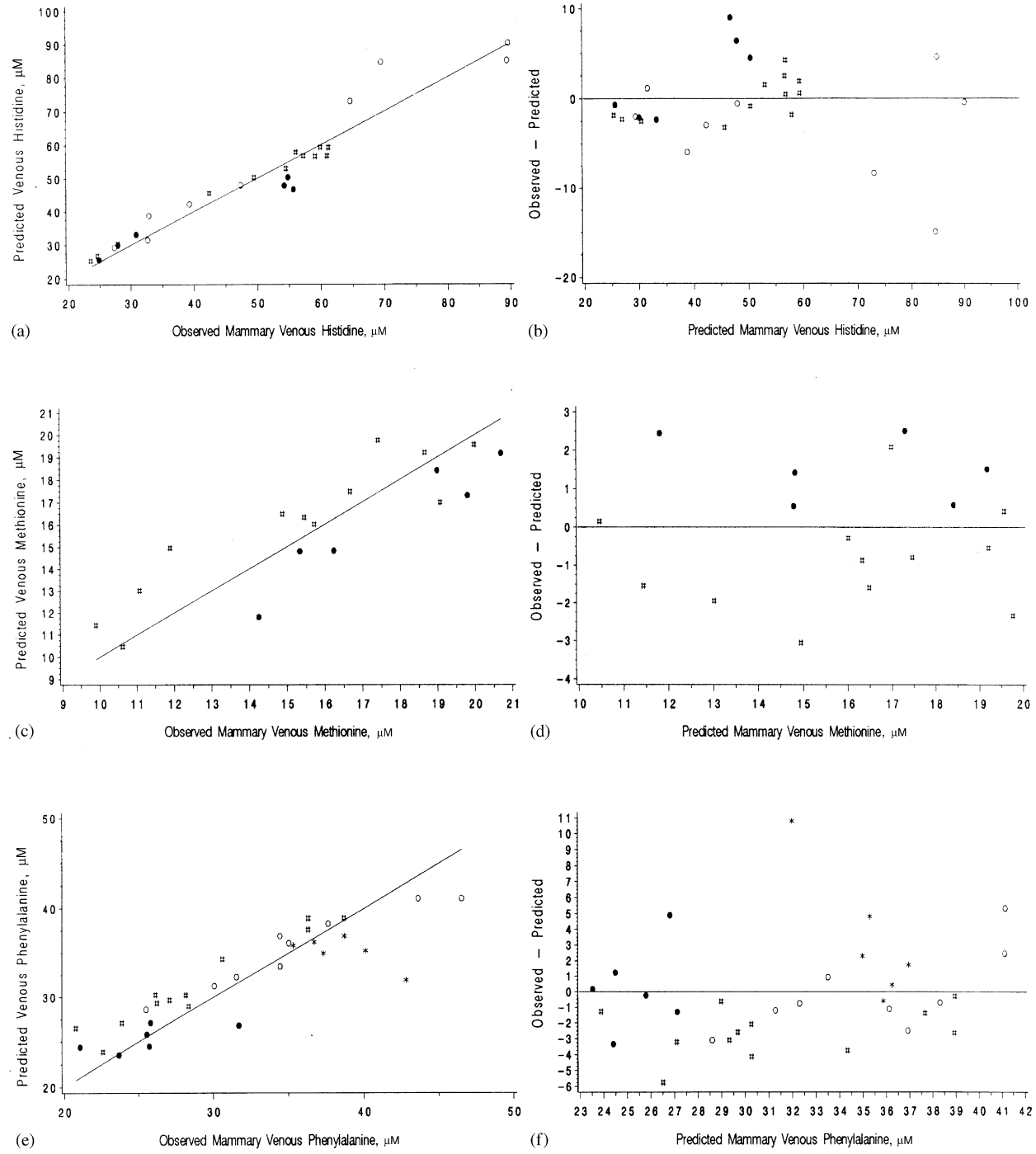

FIG. 3. Predicted and observed mammary venous concentrations and residual errors of predictions for histidine, methionine, and phenylalanine. Simulations were of the reference data set (∗ = C1, ○ = C3, # = C6, ● = C10).

and phenylalanine (Fig. 3). Predictions of milk protein yield were generally less than observed [Fig. 4(a)] with a slight linear bias evident in the predictions [Fig. 4(b)]. Lactose yield was also under predicted on average (Fig. 4), which is not surprising given that milk protein production was a determinate of lactose synthesis, i.e. where milk protein was under predicted, milk lactose would also be under predicted (Fig. 5). Milk fat yield (Fig. 4) was predicted

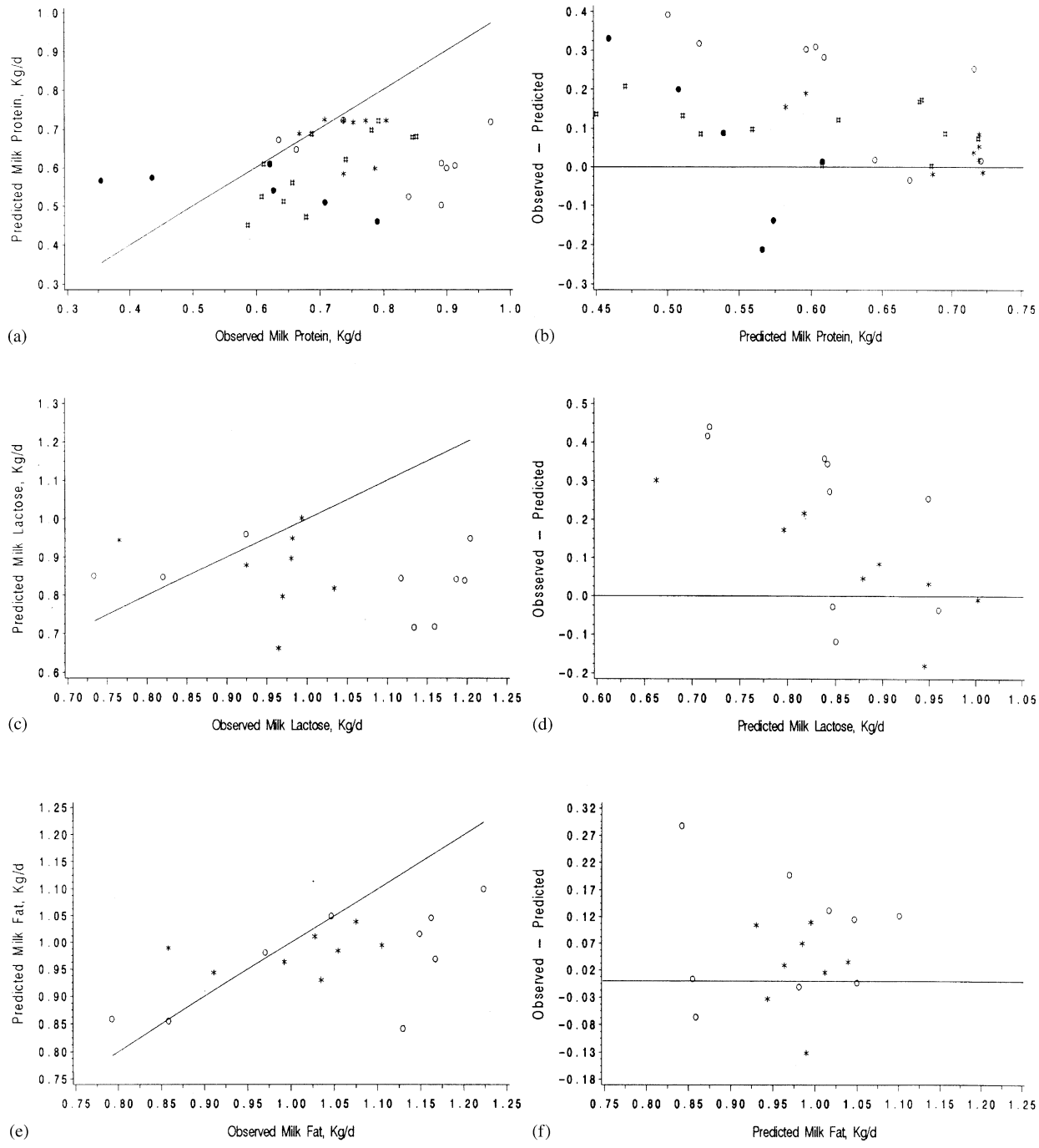

FIG. 4. Predicted and observed milk component yields. Simulations were of the reference data set (∗ = C1, ○ = C3, # = C6, ● = C10).

more accurately than that of milk lactose or milk protein.

SENSITIVITY ANALYSIS

Sensitivity analyses were conducted for predicted outputs of milk lactose, protein, and fat. Sensitivity of milk yield to parameter changes was equal to lactose sensitivity due to the strict dependence of milk volume on lactose yield, and, therefore, is not presented. Sensitivity of milk component predictions to model inputs is given in Table 4. Milk protein predictions were

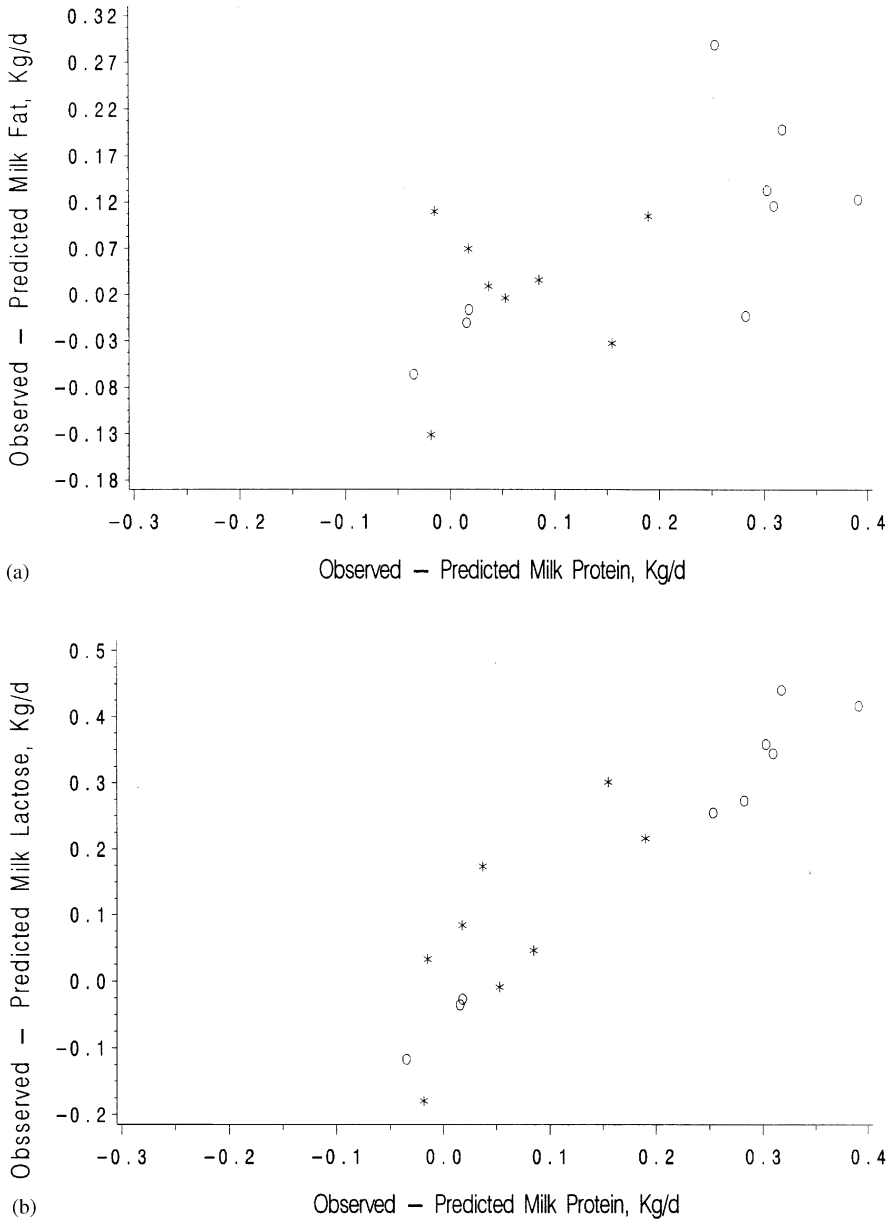

FIG. 5. Associations among residual errors for milk protein, fat, and lactose predictions. Simulations were of the reference data set (∗ = C1, ○ = C3, # = C6, ● = C10).

sensitive to arterial concentrations of phenylalanine, threonine, tyrosine, and blood flow. Among the AA, the model was most sensitive to threonine followed by phenylalanine and tyrosine. The lack of sensitivity of milk protein production to arterial concentrations of histidine, lysine, and methionine (Table 4) was due to the slight oversupply of these three EAA relative to the supply of phenylalanine, threonine, and tyrosine. Small increases in arterial concentrations of sensitive EAA or small reductions in arterial concentrations of non-sensitive EAA resulted in changes in sensitivity. The transition from a responsive concentration region to a non-responsive region is fairly sharp, as would be expected from inspection of the prediction equation (see Fig. 6). Although the slope of responses to increasing arterial concentrations is different by EAA, the general shape of the response surface is similar for all EAA. The model was not sensitive to other AA or to energy yielding substrates due to their

TABLE 4
*Sensitivity of model predictions of milk lactose, milk protein, and milk fat to arterial metabolite concentrations and mammary blood flow**

Arterial amino acids	Milk lactose	Milk protein	Milk fat
Alanine	0	0	0.0010
Arginine	0	0	0.0062
Asparagine	0	0	0.0001
Aspartate	0	0	−0.0111
Cysteine	0	0	0.0006
Glutamine	0	0	0.0039
Glutamate	0	0	0.0063
Glycine	0	0	−0.0002
Histidine	0	0	0.0013
Isoleucine	0	0	0.0097
Leucine	0	0	0.0351
Lysine	0	0	0.0355
Methionine	0	0	−0.0006
Phenylalanine	0.0945	0.1102	−0.0167
Proline	0	0	0.0007
Serine	0	0	0.0008
Threonine	0.3981	0.4644	−0.0880
Tryptophan	0	0	0.0017
Tyrosine	0.0765	0.0892	−0.0119
Valine	0	0	0.0059
Other arterial metabolites and blood flow			
Acetate	0	0	0.2545
BHBA	0	0	0.229
Blood flow	0.376	0.221	0.612
Butyrate	0	0	0.0126
Glucose	1.0001	0	0.0677
Glycerol	0	0	0.0015
Lactate	0	0	0.0079
NEFA	0	0	0.3513
Propionate	0	0	−0.0053
TAG	0	0	0.6324

*Values are expressed as percent change in the response variable associated with a 1% change in the input variable.

non-representation in the milk protein synthesis equation. The lack of sensitivity is consistent with general principles related to regulation of milk protein synthesis as given by NRC (1989).

Given the link between milk protein production and that of milk lactose, it was not surprising to find lactose yields sensitive to the same inputs as milk protein with similar responses, the exception being blood flow where a 1% change was found to result in a 0.38% change in lactose yield (Table 4). Additionally, lactose yield was sensitive to arterial glucose concentrations, with a 1% change in predicted lactose yield when glucose concentration was altered by 1%. Other energetic substrates had no effect on milk lactose yield as ATP is not used as a substrate or effector of milk lactose synthesis in the model.

As energy drives milk fat synthesis, the predictions of milk fat were found to be sensitive to all inputs, although the coefficients for many were minor (Table 4). Milk fat predictions were most sensitive to arterial concentrations of triacylglycerol (TAG) followed by non-esterified fatty acids (NEFA), acetate and BHBA, all of which are primary substrates for milk fat synthesis. They were also sensitive to blood flow, presumably due to the provision of additional TAG, NEFA, acetate, and BHBA.

Sensitivity of milk component predictions to model parameters is presented in Table 5. As for sensitivity to inputs, milk protein predictions

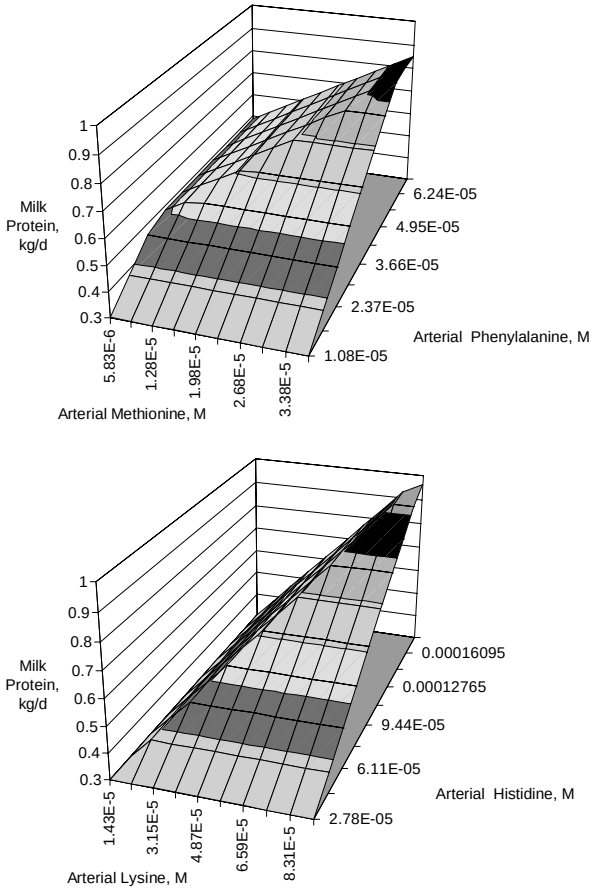

FIG. 6. Relationships between arterial histidine, lysine, methionine, and phenylalanine concentrations and predicted milk protein yields. Concentrations of essential amino acids were varied independently with concentrations of other driving essential amino acids held constant at 2 × reference concentrations.

were only sensitive to parameters associated with removal by the udder of phenylalanine (K_{ePhe}), threonine (K_{eThr}), and tyrosine (K_{eTyr}). Lactose synthesis was affected positively by changes in these same parameters and was affected negatively by changes in the parameter for glycolytic catabolism of glucose ($K_{eGlc,Catab}$). Milk fat synthesis was sensitive to most model parameters, again due to the effects of energetic intermediates on rates of milk fat synthesis.

Sensitivity analysis of milk components to selected parameters at varying concentrations of inputs is presented in Fig. 7. Sensitivity of milk protein production to rate parameters for conversion of the primary limiting EAA to milk protein was significant when arterial concentrations of the respective EAA were low enough to limit milk protein production and was zero when that EAA was no longer limiting. Sensitivity of milk fat synthesis to changes in the rate parameter for conversion of extracellular fatty acids to milk fat ($K_{eFa,Tm}$) increased asymptotically as arterial concentrations of TAG increased and generally increased as arterial concentrations of NEFA increased. However, the reaction became very sensitive to changes in $K_{eFa,Tm}$ when arterial NEFA concentrations were near 0.4 mM. Sensitivity of milk fat synthesis to the rate parameter for conversion of acetyl CoA to milk fat ($K_{Acs,Tm}$) exhibited similar behavior to that of $K_{eFa,Tm}$ with a sensitivity of 1.5% at an arterial acetate concentration of 0.3 mM, 3.5% at 0.45 mM, 0 at 0.75 mM, and returning to slightly greater than 1.5% at concentrations of 0.9 mM and above. Sensitivity of lactose synthesis to changes in $K_{eGlc,Lc}$ was constant with respect to arterial glucose concentrations.

RE-DERIVATION OF THE RATE PARAMETERS FOR MILK PROTEIN SYNTHESIS

In an attempt to address the mean bias observed from milk protein predictions, Δ was re-derived using all the individual observations in the reference data set. The model was fitted to observed milk protein yields in the reference data set allowing Δ to be varied. The optimal value for Δ was found to be 0.57 ± 0.05 as compared to the reference value of 0.33. Utilizing the greater value resulted in a mean predicted milk protein yield that more closely approximated the mean observed yield (Fig. 8) thereby removing the mean bias shown in Fig. 3(a). However, variation in residual errors was apparently unaffected by this adjustment of the intercept. The mean residual error for milk lactose predictions was also reduced, but there was no apparent improvement in the linear bias associated with lactose predictions (Fig. 8).

EVALUATION USING INDEPENDENT DATA

An independent evaluation of the model was conducted using the literature data set. Results of the simulations are presented in Table 3, Figs. 9 and 10. Values for the percent variation explained are presented in Table 3 and should be interpreted with caution as a low value can

TABLE 5
*Sensitivity of milk components to selected rate constants in the model**

Parameter, abbreviation	Milk lactose	Milk protein	Milk fat
Uni- and bi-directional uptakes			
Alanine, K_{eAla}	0.0	0.0	0.001
Arginine, K_{eArg}	0.0	0.0	0.005
Asparagine, K_{eAsn}	0.0	0.0	0.0
Aspartate, $K_{eAsp,nAsp}$	0.0	0.0	−0.011
$K_{nAsp,eAsp}$	0.0	0.0	0.012
Citrulline, K_{eCit}	0.0	0.0	0.0
Cysteine, K_{eCys}	0.0	0.0	0.001
Glutamate, $K_{eGlu,nGlu}$	0.0	0.0	0.005
$K_{nGlu,eGlu}$	0.0	0.0	−0.004
Glutamine, K_{eGln}	0.0	0.0	0.003
Glycine, K_{eGly}	0.0	0.0	0.0
Histidine, K_{eHis}	0.0	0.0	0.001
Isoleucine, K_{eIle}	0.0	0.0	0.008
Leucine, K_{eLeu}	0.0	0.0	0.025
Lysine, K_{eLys}	0.0	0.0	0.026
Methionine, K_{eMet}	0.0	0.0	0.0
Ornithine, K_{eOrn}	0.0	0.0	0.002
Phenylalanine, K_{ePhe}	0.049	0.057	−0.008
Proline, K_{ePro}	0.0	0.0	0.001
Serine, K_{eSer}	0.0	0.0	0.001
Threonine, K_{eThr}	0.309	0.361	−0.068
Trptophan, K_{eTrp}	0.0	0.0	0.002
Tyrosine, K_{eTyr}	0.043	0.050	−0.006
Valine, K_{eVal}	0.0	0.0	0.005
Acetate, K_{eAc}	0.0	0.0	0.083
Ammonia, $K_{eAmm,nAmm}$	0.0	0.0	0.018
$K_{nAmm,eAmm}$	0.0	0.0	−0.017
Fatty acids, K_{eFa}	0.0	0.0	0.091
Glucose, $K_{eGlc,Catab}$	−0.044	0.0	0.079
Glycerol, K_{eGy}	0.0	0.0	0.001
Ketone bodies, K_{eKb}	0.0	0.0	0.083
Propionate, K_{ePr}	0.0	0.0	−0.004
Triacylglycerol, K_{eTg}	0.0	0.0	0.140
Intracellular transactions			
α-Ketoglutarate to glutamate, $K_{Akg,nGlu}$	0.0	0.0	0.009
α-Ketoglutarate to oxaloacetate, $K_{Akg,Oa}$	0.0	0.0	−0.009
Aspartate to oxaloacetate, $K_{nAsp,Oa}$	0.0	0.0	−0.012
Glutamate to α-ketoglutarate, $K_{nGlu,Akg}$	0.0	0.0	−0.008
Histidine to glutamate, $K_{nHis,nGlu}$	0.0	0.0	0.0
Ketone bodies to milk triacylglycerol, $K_{eKb,Tm}$	0.0	0.0	0.034
Lactate to pyruvate, $K_{eLa,Py}$	0.0	0.0	0.009
Lysine to acetyl-co-A, $K_{nLys,Acs}$	0.0	0.0	0.0
Methionine to oxaloacetate, $K_{nMet,Oa}$	0.0	0.0	0.0
Threoinine to oxaloacetate, $K_{nThr,Oa}$	−0.272	−0.317	0.059
Tyrosine/phenylalanine to oxaloacetate, $K_{nTP,Oa}$	0.0	0.0	0.0
Oxaloacetate to α-ketoglutarate, $K_{Oa,Akg}$	0.0	0.0	−0.015
Oxaloacetate to aspartate, $K_{Oa,nAsp}$	0.0	0.0	0.012
Oxaloacetate to pyruvate, $K_{Oa,Py}$	0.0	0.0	0.002
Pyruvate to acetyl-co-A, $K_{Py,Acs}$	0.0	0.0	0.011
Pyruvate to lactate, $K_{Py,eLa}$	0.0	0.0	−0.010
Pyruvate to oxaloacetate, $K_{Py,Oa}$	0.0	0.0	−0.001

* Values are expressed as percent change in the response variable associated with a 1% change in the rate constant.

indicate great variation in residuals (either systematic or random), a large mean residual error, or a combination of the two. Although residual variation generally indicates an inadequate model, particularly if they exhibit a systematic bias, it can also indicate inaccurate

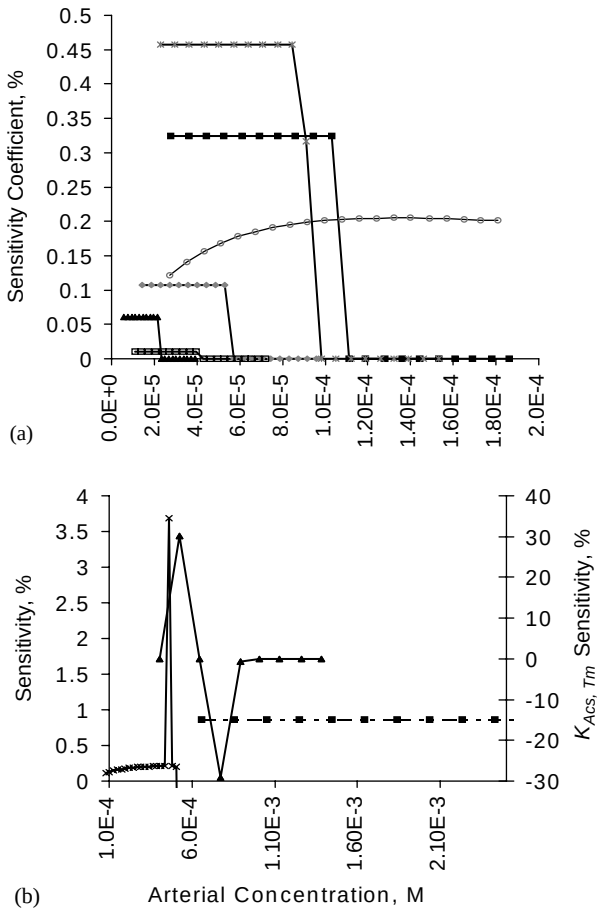

FIG. 7. Sensitivity coefficients for milk protein, lactose, and fat synthesis. Values are expressed as percent change in the response variable associated with a 1% change in the input variable. (a) Sensitivity of milk protein synthesis to changes in $K_{nHis,Pm}$ (◆), $K_{nLys,Pm}$ (■), $K_{nMet,Pm}$ (▲), $K_{nTP,Pm}$ (□), and $K_{nThr,Pm}$ (×) at varying concentrations of arterial histidine, lysine, methionine, phenylalanine, and threonine, respectively. Sensitivity of milk fat synthesis to changes in $K_{eFa,Tm}$ (○) at varying concentrations of arterial triacylglycerol. (b) Sensitivity of lactose synthesis to changes in $K_{eGlc,Lc}$ (■), milk fat synthesis to changes in $K_{eFa,Tm}$ (×) at varying arterial concentrations of non-esterified fatty acids, and milk fat synthesis to changes in $K_{Acs,Tm}$ (▲) at varying arterial concentrations of arterial acetate.

measurements. A significant mean residual error in combination with minimal residual variation indicates that the model is accurate, but improperly parameterized. The values presented in Table 3 must be interpreted in combination with graphical appraisal of the results when the percent variation explained is small. Large values for the percent variation explained suggest that the model is appropriate, is parameterized properly, and that the data examined have reasonably small errors of measurement.

Given that the data contained in the literature data set were published by a number of different laboratories utilizing different analytical techniques on samples drawn from three different sampling sites, uptake of the majority of metabolites was well predicted. Exceptions were glutamate, isoleucine, lysine, phenylalanine, tyrosine, valine, glycerol, BHBA, and acetate. For the AA, mean residuals appeared to be significantly different from zero (Fig. 10) with only glutamate exhibiting a linear bias to the predictions (data not shown). Although the model predicted more of the variation observed in methionine concentrations, the mean residual also appeared to deviate from zero (Fig. 10).

Milk protein yields were generally under predicted (Fig. 9) despite the increase in EAA availability associated with a value of 0.57 for Δ. A portion of the error is likely to be caused by under predictions of lysine, methionine, phenylalanine, and tyrosine removal (Fig. 10). Errors of prediction associated with the apparent differences in the UCD histidine data vs. other data sets (Fig. 10) would not likely be the cause of the under predictions of milk protein, as predictions for the removal of histidine were largely overestimated for the UCD data set, thus rendering histidine a non-limiting EAA. Although there was no evidence of a systematic bias with respect to the milk protein predictions, the model exhibited a lesser range in predicted protein yields (0.5–0.8 kg/day) than the observed range (0.4–1.3 kg/day, Fig. 9).

Milk lactose was predicted with a mean residual error similar to that observed for milk protein (Fig. 9), however, predictions of lactose exhibited a narrower range than the observed range, similar to milk protein predictions. Again, the residual errors for milk lactose predictions correlated well with residual errors for milk protein predictions (data not shown), indicating that the cause of lactose prediction errors was under prediction of milk protein.

Milk fat was predicted without a mean residual error (Fig. 9), but with a slight linear bias. However, the removal of two or three observations would make that conclusion more tenuous. It was surprising, based on observations with the

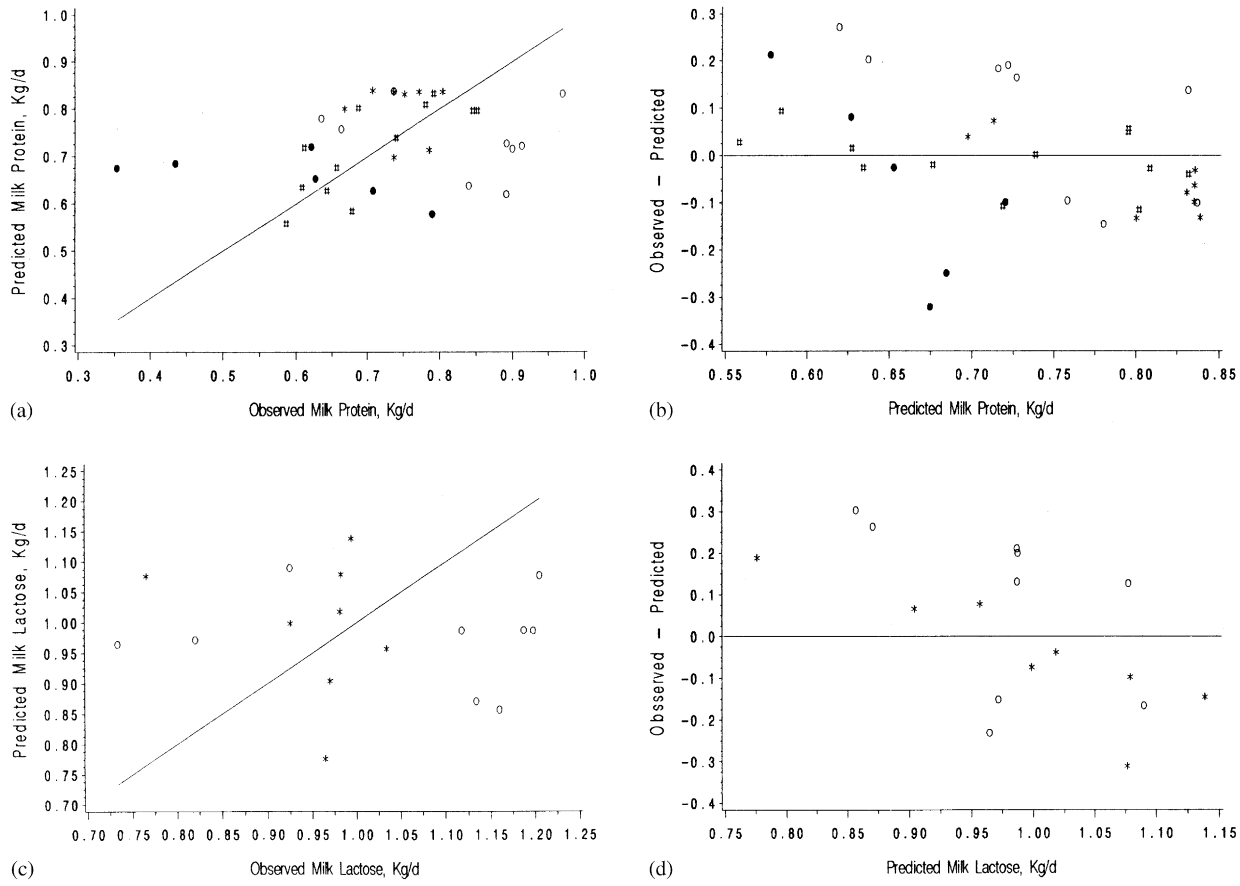

FIG. 8. Results from simulations of milk protein and milk lactose yields. Δ was set to 1.57 as determined by fitting the model to observed milk protein yields. Simulations were of the reference data set (* = C1, ○ = C3, # = C6, ● = C10).

reference data set, that residual errors associated with milk fat predictions apparently were not correlated with residual errors for milk protein predictions (data not shown). A possible explanation is the more complete description of energetic metabolite inputs in the literature data set. Using reference values for inputs of several energetic metabolites may have created the observed relationship among milk fat and milk protein residual errors observed in Fig. 5.

Based on the observation that venous concentrations and uptakes of a number of metabolites were predicted inadequately for the literature data, rate parameters for removal of these metabolites were re-derived by fitting the model to the literature data. After fitting, the proportion of variation explained was significantly improved for all variables (Table 3).

Discussion

Describing AA metabolism by the udder can be divided into two sections: (1) prediction of metabolite removal and (2) prediction of intracellular metabolism. Historically, metabolite removal by the udder has been related to arterial concentrations, i.e. uptake = arterial concentration $\times K_{uptake}$. Such a representation ignores the potential effects of blood flow and suggests that uptake can be significant in the absence of blood flow, which obviously is not possible. Maas *et al.* (1998) have provided a more detailed model for describing the removal of phenylalanine, methionine, and valine by the udder, but did not consider the effects of blood flow. Cant & McBride (1995) presented a more robust model of metabolite removal, however, their model requires an estimate of the number

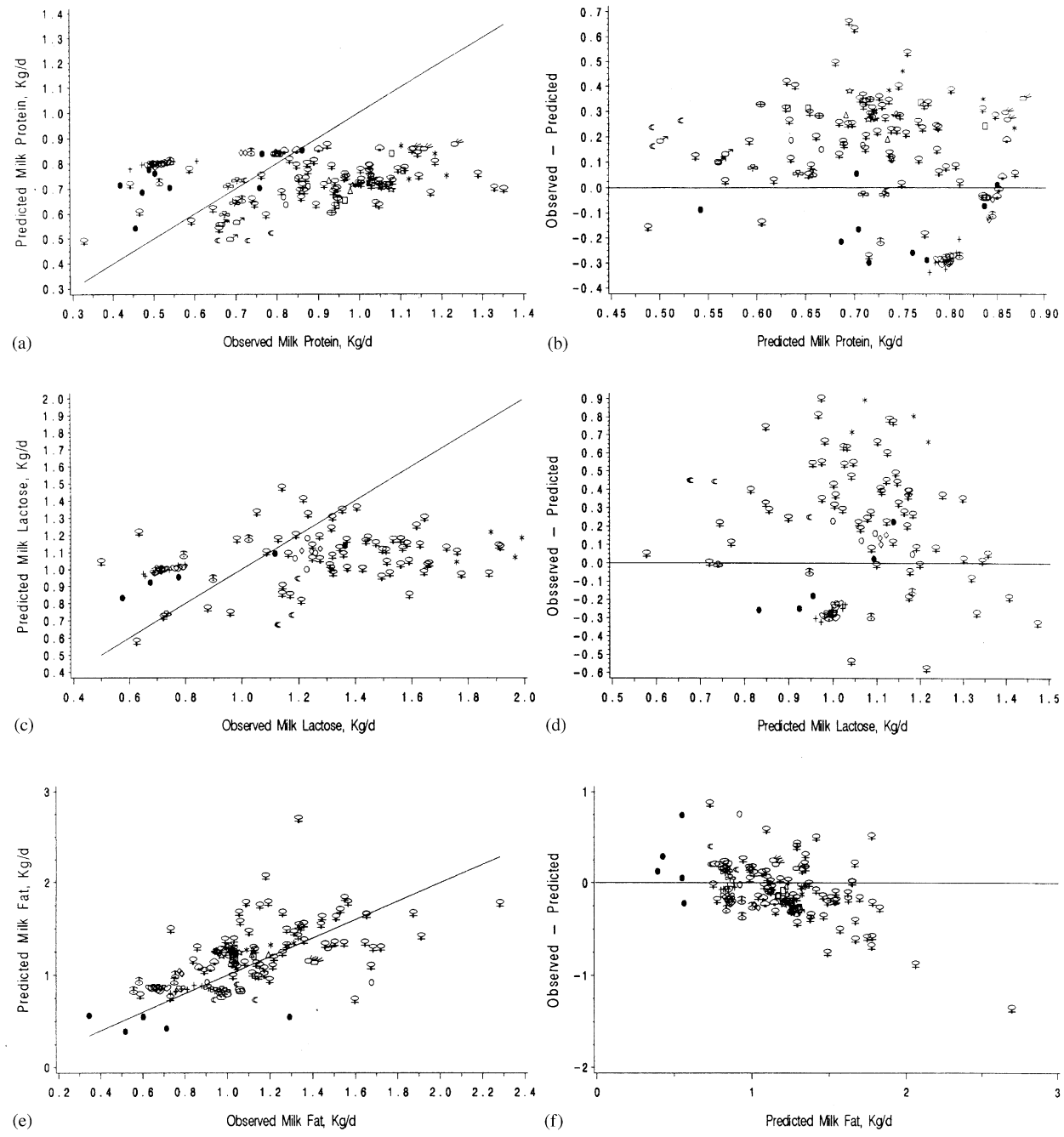

FIG. 9. Results from simulations of the literature data summarized in Table 1 (∗ = Austin, ● = Bickerstaffe, ◯ = Cant, # = Casper, + = Choung, □ = Clark, ◇ = Derrig, △ = Drackley, ♡ = Fisher, ♤ = Griinari, ☾ = Guinard1, ♧ = Guinard2, ♂ = Guinard3, ☆ = Illg, ◎ = Karunanandaa, ♉ = Lykos, ♣ = Peeters, ⊙ = Rulquin, ⊕ = Spires, ♀ = UCD, ⚜ = Yang).

and length of capillaries being perfused and the extracellular compartment must be represented explicitly as a state variable.

Our description of uptake (Hanigan *et al.,* 2001) considered the effects of blood flow, but not potential interactions among AA, and appeared to perform adequately describing a major portion of the observed variation in the experimental data examined (Table 3). However, mean residual errors that deviated from 0 were observed for a number of metabolites when the model was parameterized using the reference

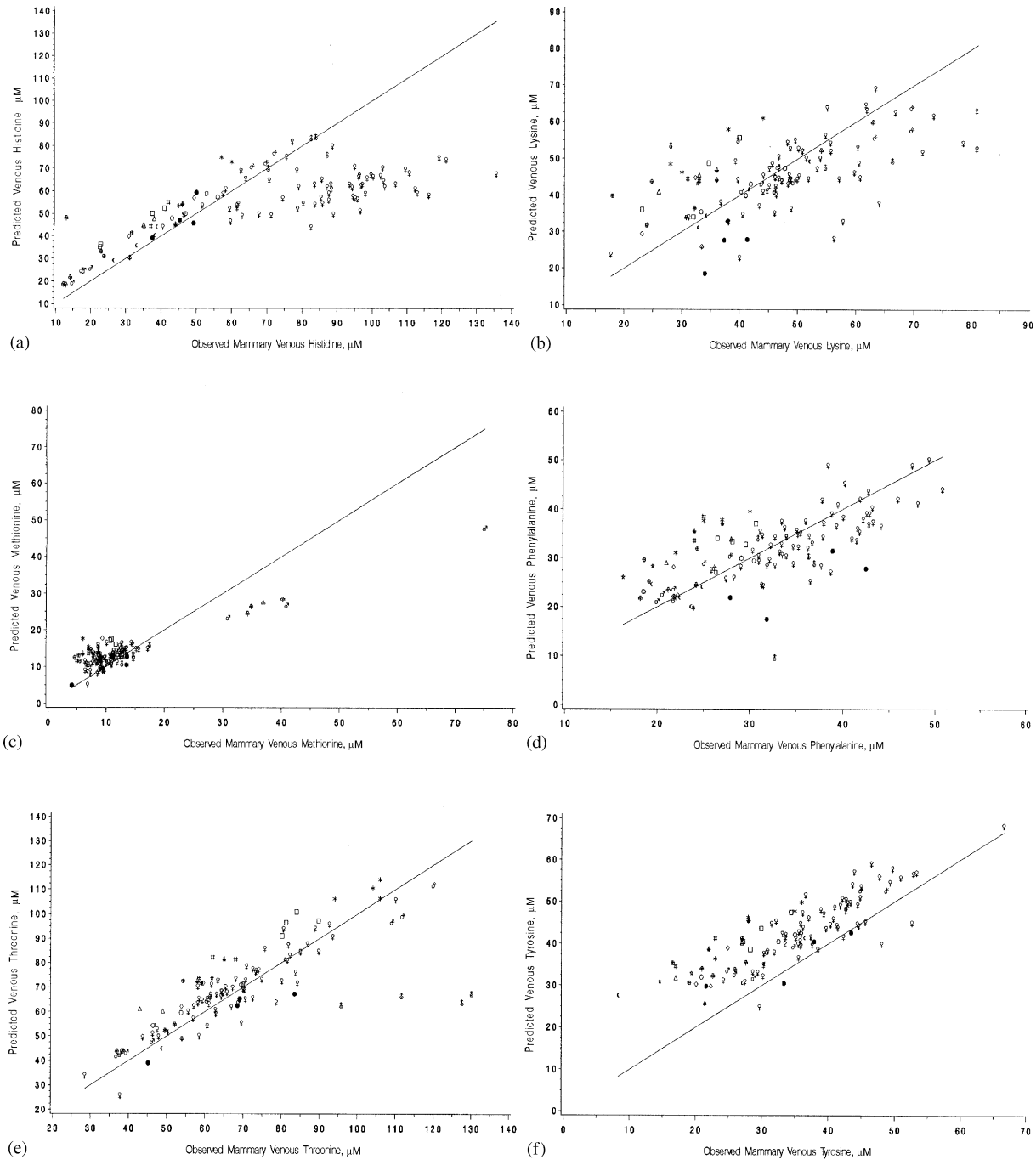

FIG. 10. Results form simulations of mammary amino acid data summarized in Table 1 (✻ = Austin, ● = Bickerstaffe, ◯ = Cant, # = Casper, + = Choung, □ = Clark, ◇ = Derrig, △ = Drackley, ♡ = Fisher, ♤ = Griinari, ☾ = Guinard1, ♧ = Guinard2, ♂ = Guinard3, ☆ = Illg, ◎ = Karunanandaa, ♉ = Lykos, ♧ = Peeters, ☉ = Rulquin, ⊕ = Spires, ♀ = UCD, ⚜ = Yang).

data and applied to independent literature data. There are a number of reasons why this may have occurred, but the most obvious is inter-laboratory differences. Although it is not clear as to why the observations of Metcalf and colleagues (C1, C3, C6, and C10) differed from data collected in other laboratories, it is not surprising given the myriad of variables that are associated with conducting such intensive studies.

Although, the representation of glutamate uptake appears to be inadequate, graphical appraisal of the remaining metabolites indicated that errors of prediction were generally small, being either slightly positive or negative mean residual errors with no bias to the predictions. This supports the conclusion that the model was valid with respect to predictions of removal of extracellular metabolites by the udder. Although inter-laboratory differences may explain most of the prediction errors, it is also possible that the simple model of metabolite removal used by Hanigan *et al.* (2001) failed to capture one or more key physiological differences among the experiments.

The observations of Bequette *et al.* (2000) and Guidotti *et al.* (1978) have suggested that the udder and other tissues can respond to AA deficiencies by increasing their transporter activity. Such a response would tend to dampen the effects of a single EAA limitation on milk protein synthesis and may result in a greater range of predictions, if such a mechanism were included in the model. However, examination of the rate parameters (data not shown) for removal of the various AA by the udder within individual experiments in the reference data, has not shown any obvious differences associated with manipulation of either a single EAA or the total supply of AAs.

It is also possible that interactions among AA for common transporters may play a significant role in regulating the transport activity for one or more AA. The model of Maas *et al.* (1998) contains elements of these interactions for the L system, but the sensitivity of uptake of a single AA was not tested relative to changes in the concentrations of the other AA being transported. Given that AA transported by the L system (isoleucine, leucine, phenylalanine, tyrosine, and valine) were predicted equally well for reference and literature data sets, it seems unlikely that significant transport interactions were occurring.

The equation form chosen to represent milk protein synthesis is a reflection of the commonly accepted hypothesis regarding protein synthesis, i.e. there is a single limiting AA with respect to protein synthesis and alleviation of this limitation is the only action that will result in increased rates of protein synthesis. A rectilinear model describing such a response was proposed by Fisher *et al.* (1973) and has been applied at the whole animal level. The model described by Hanigan *et al.* (2001) reflects that concept, as do previous models (Hanigan & Baldwin, 1994; Maas *et al.*, 1998; Gerritts *et al.*, 1997). Given the consensus that such an equation is appropriate, it was surprising that the mean predicted milk protein yield derived from simulations of the individual observations in the reference data set was less than the observed mean yield.

The model was parameterized using the observed mean yield (0.726 kg/day) and predicted the observed mean yield (0.717 kg/day) when mean inputs were used. As noted above, predictions of EAA removal did not exhibit such bias. As the equation describing milk protein synthesis is a linear function of the single EAA that is most limiting for milk production (Fig. 6), it is not immediately clear how such a bias could occur, although the effects of measurement errors on protein yield predictions may offer an explanation. A portion of the observed variation associated with experimental observations can be attributed to sampling and analytical errors, since errors of measurement would tend to reduce average predicted yields and reduce the overall range of predictions. However, it seems that the under predictions of milk protein yields can be attributed to the representation of milk protein synthesis, and application of the equation leaves the model oversensitive to measurement errors. Model predictions of milk protein yields also exhibited a lesser range than experimental observations (Figs 4 and 9). This lack of responsiveness was probably due to the consideration of a single EAA as the sole driving variable for milk protein synthesis.

Work conducted with other species and *in vitro* appears to support independent and additive effects of provision of additional EAA. Work with hepatic tissue has identified initiation of translation as the step in protein synthesis that appears to be regulated by AA supply (McGown *et al.*, 1973; Perez-Sala *et al.*, 1991; Yokogoshi & Yoshida, 1980) sufficiency. Utilizing cultured mammary cells, Clark *et al.* (1978) demonstrated that increasing the concentration of cystine, threonine, or methionine threefold over basal

levels provided in Eagle's medium resulted in significant increases in milk protein synthesis. As each of these AA stimulated protein synthesis independently and nearly identically, designation of the limiting AA is problematic and calls into question the entire concept. It would appear that none of the AA were truly limiting as milk protein synthesis was stimulated by each of the AA in turn. Clark *et al.* (1978) also observed increases in milk protein synthesis when tryptophan, arginine, lysine, phenylalanine, histidine, or tyrosine concentrations were elevated in the media.

The rate of protein synthesis is probably a function of all EAA, if not all AA. In this manner, sufficiency of several NEAA and deficiency of a single NEAA would result in a greater rate of protein synthesis than would be predicted by consideration of only the most limiting AA, assuming that all of the EAA were available in relative abundance. As oversupply of an EAA has no effect using the current representation of milk protein synthesis, a shift to a representation where oversupply is considered could remove the observed mean bias and result in greater predicted yields.

If milk protein synthesis was represented as an integration of the supplies of several EAA, the response surface with respect to EAA supply would be multidimensional and quite different from that depicted in Fig. 6. Transitions from excess to deficiency for each AA would not be as marked nor would the responses be linear. Such a response surface would suggest that determination of the first- or second-limiting AA would be difficult unless all other AA were controlled. Additionally, the concept of an order to limitations would be called into question. Rather, the marginal response to an AA would be dynamic depending on the relative sufficiency of other AA. Although a rectilinear model has been used in monogastric species to describe productive responses to incremental additions of a limiting AA, observations suggest that a nonlinear model would be a better descriptor (see Fuller, 1994).

It has been hypothesized that the energy status of the animal plays a role in regulating protein synthesis (Balch, 1967) with some evidence in ruminants (Hanigan *et al.*, 1998; Ørskov, 1992). However, attempts to define the interrelationships between energy supply and the response to protein or amino acid supply have apparently been hampered by changes in energy reserves (see Ørskov, 1992). Examination of the relationships among specific energy-providing metabolites and amino acid supply at the tissue level would require at least a minimal description of energy metabolism in the tissue of interest so that energy status could be predicted. Additionally, acetate removal and metabolism must be represented better to achieve any confidence in the energy status predictions.

In summary, metabolite removal by the udder can be predicted utilizing a simple representation of the transport process. Representation of milk protein synthesis as a function of a single limiting EAA does not appear to be adequate and future work should look to represent milk protein synthesis through the simultaneous consideration of multiple EAA. Additional work on the description of energy metabolism is also required.

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