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Modeling portal-drained viscera and liver fluxes of essential amino acids in dairy cows

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ABSTRACT

The objective of this work was to predict essential amino acid (EAA) use and release by the portal-drained viscera (PDV) and liver of dairy cows. Previously derived equations were tested using data assembled from the literature, refit to the data, and modifications were undertaken to determine the best model for each EAA. The refitted model has the same structure as the original equations but is parameterized using a database of group means, as the original equations were derived using a single study with individual cow data and found to be biased. The PDV clearance model predicted portal vein concentrations given inputs of absorbed and arterial fluxes of EAA with root mean squared errors (RMSE) ranging from 3.3 to 12.1% of the observed means, and concordance correlation coefficients (CCC) ranging from 0.86 to 0.99 when using previously reported parameters. The reparameterized model generated from the assembled data set resulted in predictions of EAA portal vein concentrations with RMSE ranging from 3.2 to 8.6% and CCC ranging from 0.93 to 1.00. Slope bias ranged from 12.4 to 55.3% of mean squared errors and was correlated with arterial EAA concentrations. Modifying the model to allow rate constants to vary as a function of arterial EAA concentrations reduced slope bias, resulting in RMSE ranging from 1.9 to 6.5% and CCC from 0.97 to 1.00. Alternatively, splitting the model to account for use of EAA from absorption separately from arterial use resulted in poorer predictions and biologically infeasible parameter estimates. The liver clearance model predicted hepatic vein concentrations from arterial and portal vein input fluxes with RMSE across EAA ranging from 1.9 to 6.8% and CCC ranging from 0.97 to 1.00 when using reported parameters. The reparameterized model generated from the assembled data set resulted in predictions of EAA hepatic vein concentrations with RMSE ranging from 1.9 to 6.7% and CCC ranging from 0.97 to 1.00. Significant slope bias was present for Arg, His, Lys, Phe, Thr, and Val. Altering the model to represent the clearance rate constant as a function of arterial concentrations resulted in RMSE ranging from 1.8 to 6.5% and CCC ranging from 0.97 to 1.00. The combination of PDV and liver clearance models provided predictions of total splanchnic use similar to those of an empirical model representing splanchnic use as a fractional proportion of absorption that had RMSE ranging from 3.0 to 8.6% and CCC ranging from 0.95 to 0.99, with significant slope bias for the majority of EAA.

Key words: post-absorptive, essential amino acid, flow, mechanistic

INTRODUCTION

Milk is an important food source and the primary driver of revenue for dairy farms. Ruminants convert dietary energy into products such as milk more efficiently than they convert dietary N (NRC, 2001; Bequette et al., 2003). Because of their low conversion efficiency in transforming total dietary N, dairy production from ruminants contributes significantly to environmental problems (Tamminga et al., 1995; Howarth et al., 2002). A portion of this inefficiency is due to improperly matching individual AA to animal needs (Arriola Apelo et al., 2014). An accurate representation of AA metabolism in dairy cows will allow construction of diets that more closely match absorbed AA supply to animal needs, thus improving N efficiency and decreasing N excretion.

Models have been developed to evaluate N metabolism in the rumen (Dijkstra et al., 1992; NRC, 2001), liver (Hanigan et al., 2004a), and mammary glands (Hanigan et al., 2000, 2001, 2002). However, to our knowledge, there have been minimal efforts to develop a more mechanistic model of the transfer of AA from the gut lumen to milk protein. Field application models represent the transfer of AA from the gut lumen to net

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protein output as a set of static conversion efficiencies, which lack accuracy and precision (NRC, 2001; White et al., 2017b).

Comparisons of estimated small intestinal disappearance to net portal appearance have shown that the portal-drained viscera (**PDV**) remove approximately 33% of the net AA supply (Hanigan et al., 2004b). However, the majority of this use is from arterial supply after AA have been delivered to general circulation (MacRae et al., 1997a), and thus the fractional use during absorption is considerably less. The liver also uses a significant proportion of the absorbed EAA on a net basis (Hanigan et al., 1998b), again with the arterial supply representing the vast majority of tissue input (Hanigan et al., 2004b). Because the absorbed supply represents a small fraction of the total flux through each tissue, fractional use during first pass is small (Estes, 2016), but overall use is significant due to constant recycling of AA to the splanchnic tissues (Reynolds et al., 1988; Wray-Cahen et al., 1997). Net AA use by splanchnic and mammary tissues has been shown to represent almost the entirety of net body AA use in a lactating, non-pregnant mature cow (Larsen et al., 2015). In addition to the large proportion of AA used by the splanchnic tissues, arterial AA recycling results in variable efficiency of use, which is inconsistent with a fixed transfer efficiency used in field application models (Hanigan et al., 1998a).

A process-based representation of EAA flux through the post-absorptive system may yield benefits in terms of more precise descriptions of the supply of individual AA to the mammary gland and, thus, more accurate and precise representations of milk protein production. From the above, such a representation should consider use during transit by, at minimum, splanchnic and mammary tissues, with the remaining body tissues considered in aggregate. We hypothesized that this process-based model of splanchnic use would provide more accurate and precise predictions of net EAA supplies available for peripheral tissue use than would an empirical representation, which represents tissue use as a fractional proportion of absorption without accounting for arterial supplies. Therefore, the objective of this work was to develop and test the PDV and liver components of such a process-based model.

MATERIALS AND METHODS

Model Description

An overview of a post-absorptive EAA system is depicted in Figure 1. The PDV and liver (**LIV**) models were constructed consistent with the needs of the overall system, considering the effects of blood entry and exit from the system, and evaluated using R version 3.2.2 (R Core Team, 2015). The models represented the flux of 9 EAA through the tissues: Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val. Arterial supplies to the PDV are diffuse, with no single source, and thus denoted in aggregate as **PA**, PDV arterial vessels. Fluxes (moles per day) were denoted as $F_{X(i)}$, where X represented location—absorbed (Abs), PDV arterial vessels (PA), portal vein (PV), hepatic artery (HA), hepatic vein (HV), PDV use (PDV), and hepatic use (LIV)—and i represented each EAA. In the equations below, $C_{X(i)}$ represented EAA concentrations (molar per liter) at location X; $BF_{(X)}$ represented blood flow (liters per day) in vessel X (hence, hepatic arterial, portal vein, and hepatic vein blood flows, $\boldsymbol{BF_{HA}}$, $\boldsymbol{BF_{PV}}$, and $\boldsymbol{BF_{HV}}$); and $K_{T(i)}$ represented the clearance rate constant (liters per day) for the ith EAA by the tissue T (PDV or LIV). Arterial sources for the different locations were assumed to be equal in concentration and thus arterial concentration was denoted as $C_{A(i)}$.

PDV Model

Transfers of EAA from the gut lumen [$F_{Abs(i)}$] to the portal vein [$F_{PV(i)}$], and use by the PDV [$F_{PDV(i)}$] were calculated based on the clearance model of Hanigan et al. (2004b):

$$F_{PV(i)} = C_{PV(i)} \times BF_{PV}, \quad [1]$$

$$C_{PV(i)} = \frac{\left[C_{A(i)} \times BF_{PV}\right] + F_{Abs(i)}}{K_{PDV(i)} + BF_{PV}}, \quad [2]$$

$$F_{PDV(i)} = F_{Abs(i)} + \left[C_{A(i)} - C_{PV(i)}\right] \times BF_{PV}, \quad [3]$$

where $F_{Abs(i)}$, BF_{PV}, and $C_{A(i)}$ were required inputs. For initial evaluation, the clearance rate parameters [$K_{PDV(i)}$] previously derived from Hanigan et al. (2004b) were used. $F_{Abs(i)}$ was the absorbed EAA supply from each diet estimated from digested RUP [grams per day; a summation across feeds (f) within each diet] and microbial protein flows ($\boldsymbol{MiTP}$, grams per day) as described by (Fleming et al., 2019):

$$F_{Abs(i)} = \frac{\left\{\sum_{f=1}^{N_f}\left[DC_{RUP(f,i)} \times F_{RUP(f,i)}\right] + DC_{MiTP(i)} \times F_{MiTP(i)}\right\}}{MW_{(i)}}, \quad [4]$$

in which $DC_{RUP(f,i)}$ and $DC_{MiTP(i)}$ represented the digestibility coefficients (grams per gram) for each AA for RUP and MiTP, respectively, as summarized by White et al. (2017a). $MW_{(i)}$ represents molecular weight (grams per mole) for each AA. The EAA composition of MiTP was assumed to be constant, as described by Sok et al. (2017). Although there is likely diversity in the MiTP digestibility coefficient across EAA, existing data are inadequate to define such variability, and thus the MiTP digestibility coefficient for each EAA was assumed equal to that of the protein (Paz Manzano et al., 2014), which was set to a constant value of 80% (NRC, 2001). N represents the number of feeds (f) within each diet.

Although the total absorbed EAA supply includes EAA derived from reabsorption of endogenous protein, this was not considered in $F_{Abs(i)}$, as it represents a hidden loop within the digestive system. Endogenous protein is synthesized from arterial AA; therefore, the absorbed endogenous AA are simply replacing AA used to secrete more endogenous protein. The portion of endogenous protein that is not digested is represented as net use within $F_{PDV,\ use(i)}$.

Initial work using the clearance rate constants of Hanigan et al. (2004b) with Equation [2] indicated small but significant slope biases for predictions of portal vein concentrations and fluxes for most EAA. Refitting the model to the current data reduced the problem, but it did not completely resolve the problem, and thus alternative forms of Equation [2] were derived and tested. These included representing the clearance rate constant [$K_{PDV(i)}$, liters per day] as a variable function of arterial blood concentrations:

$$C_{PV(i)} = \frac{C_{A(i)} \times BF_{PV} + F_{Abs(i)}}{\left[K_1 + K_2 \times C_{A(i)}\right] + BF_{PV}}, \qquad [5]$$

and representing EAA during absorption as a separate process from arterial use:

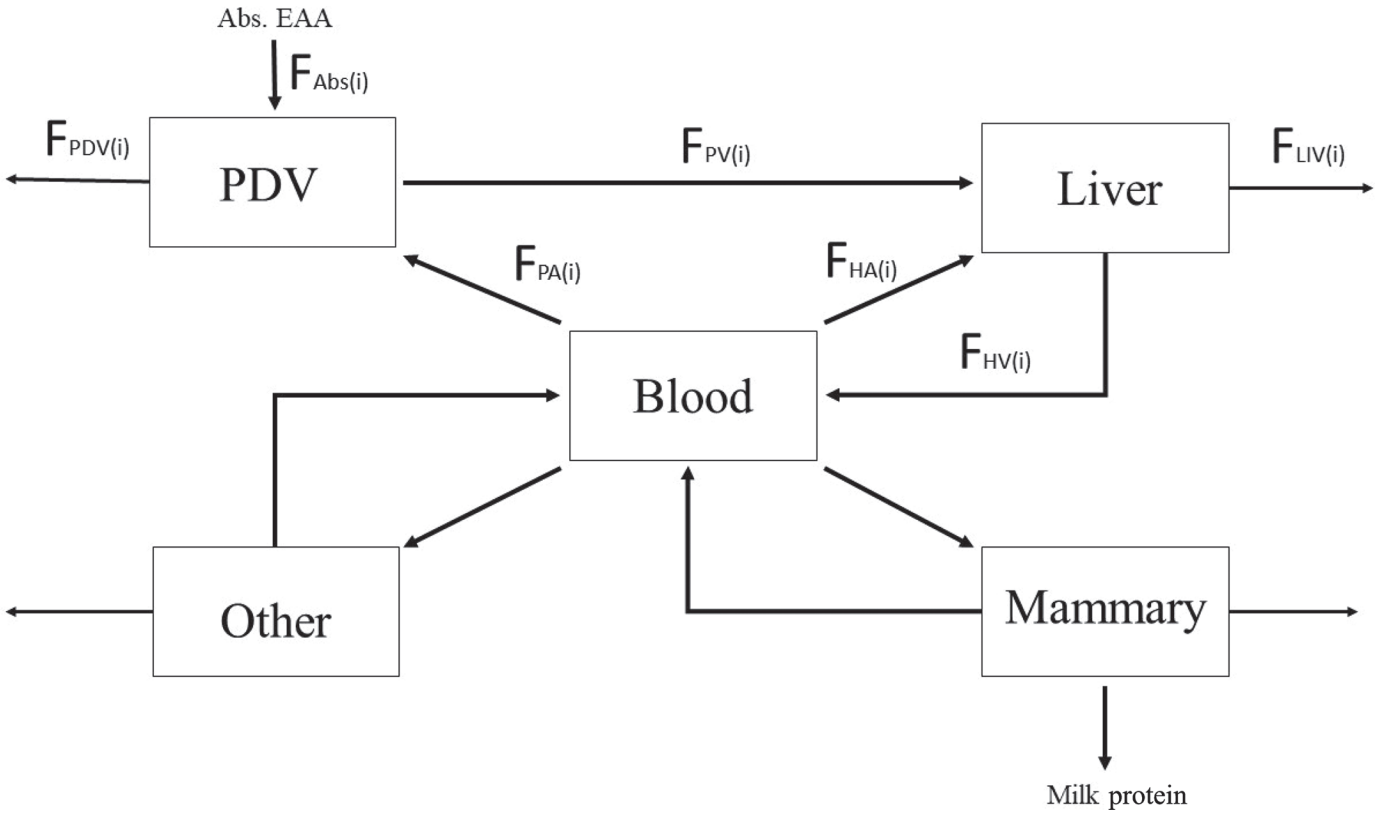

Figure 1. Schematic diagram of a post-absorptive EAA system, patterned after the model laid out in Hanigan et al. (1998b). Arrows indicate fluxes, and solid boxes denote compartments. PDV = portal-drained viscera; (i) denotes each of the EAA considered; $F_{Abs(i)}$ denotes the absorption of each EAA from the gut lumen; $F_{PV(i)}$ denotes the flux of each EAA in the portal vein; $F_{PA(i)}$ denotes the flux of each EAA in arterial blood entering the portal-drained viscera; $F_{PDV(i)}$ denotes the flux of each EAA used by the portal-drained viscera; $F_{HA(i)}$ denotes the flux of each EAA in the hepatic artery; $F_{HV(i)}$ denotes the flux of each EAA in the hepatic vein; $F_{LIV(i)}$ denotes the flux of each EAA used by the liver. Abs. EAA is the absorbed EAA supply to the system.

$$C_{PV(i)} = \frac{C_{A(i)} \times BF_{PV}}{K_1 + BF_{PV}} + \frac{F_{Abs(i)} \times (1 - K_2)_i}{BF_{PV}}, \quad [6]$$

where $(1 - K_2)_i$ represented the fractional use (moles per mole) of each AA during absorption.

We also explored the use of an empirical, fractional use equation to assess the comparative value of the more mechanistic representation. The empirical equation represents tissue use as a fractional proportion of the absorbed supply without consideration of use from arterial supplies:

$$C_{PV(i)} = C_{A(i)} + \frac{F_{Abs(i)} \times (1 - f_{PDV})_i}{BF_{PV}}, \quad [7]$$

where $(1 - f_{PDV})_i$ represents the fractional release (moles per mole) of absorbed AA.

Liver Model. Representation of the transfer of EAA from the portal vein [$F_{PV(i)}$] to the hepatic vein [$F_{HV(i)}$] and hepatic use [$F_{LIV(i)}$] was based on the hepatic clearance model of Hanigan et al. (1998b):

$$F_{HV(i)} = C_{HV(i)} \times BF_{HV}, \quad [8]$$

$$C_{HV(i)} = \frac{\left[C_{A(i)} \times BF_{HA}\right] + \left[C_{PV(i)} \times BF_{PV}\right]}{K_{LIV(i)} + BF_{HV}}, \quad [9]$$

$$F_{LIV(i)} = \left[C_{A(i)} \times BF_{HA}\right] + \left[C_{PV(i)} \times BF_{PV}\right] - \left[C_{HV(i)} \times BF_{HV}\right], \quad [10]$$

where BF_{HV}, BF_{PV}, and $C_{A(i)}$ were required inputs, with BF_{HA} calculated as the difference between hepatic and portal vein flows ($BF_{HA} = BF_{HV} - BF_{PV}$).

As with the PDV model, initial work using the clearance rate parameters of Hanigan et al. (1998b) with Equation [9] indicated small but significant slope biases for most EAA for predictions of hepatic vein concentrations. Refitting the model did not completely remove slope bias, and thus alternative forms of Equation [9] were derived and tested. Similar to the PDV model, an additional equation was derived representing the clearance rate constant as a variable function of $C_{A(i)}$:

$$C_{HV(i)} = \frac{\left[C_{A(i)} \times BF_{HA}\right] + \left[C_{PV(i)} \times BF_{PV}\right]}{\left[K_{1,HV} + K_{2,HV} \times C_{A(i)}\right] + BF_{HV}}. \quad [11]$$

Splanchnic Model. Concentrations of EAA in the hepatic vein were also predicted considering absorbed and arterial supplies, using a combination of Equations [2] and [9]:

$$C_{HV(i)} = \frac{\frac{\left[C_{A(i)} \times BF_{PV}\right] + F_{Abs(i)}}{K_{PDV} + BF_{PV}} \times BF_{PV} + \left[C_{A(i)} \times BF_{HA}\right]}{K_{LIV} + BF_{HA} + BF_{PV}}, \quad [12]$$

where $C_{A(i)}$, BF_{PV}, BF_{HA}, and $F_{Abs(i)}$ were required inputs.

An additional empirical splanchnic model, Equation [13], was explored as a comparison to the more mechanistic approach represented by Equation [12]. As for PDV, the empirical model predicted flux and concentration changes based solely on absorbed EAA supply:

$$C_{SPL(i)} = C_{HA(i)} + \frac{\left\{F_{Abs(i)} \times \left[1 - f_{SPL(i)}\right]\right\}}{BF_{HV}}, \quad [13]$$

where $f_{SPL(i)}$ represented the fractional use (mole per mole) of absorbed EAA by the splanchnic bed.

Data and Statistics. Data used to evaluate and derive parameters for the PDV and LIV models consisted of 196 treatment means from 45 studies (Table 1) published in the literature from 1974 to 2012. All studies were conducted in dairy cows and were considered for inclusion if they reported hepatic arterial, portal vein, and hepatic vein blood flows (BF_{HA}, BF_{PV}, BF_{HV}, liters per day), EAA concentrations, diet composition, and DMI.

All models were derived by nonlinear least squares regression using the nls function, which is part of the stats package in R (R Core Team, 2015, version 3.2.2), unless otherwise specified. A mixed model with a random study effect using the nlmer function of the lme4 package was attempted, but it did not converge, indicating that the study effects were represented in blood flow or arterial concentrations. Therefore, random study effects were not included in the model. Resulting models were selected based on parameter significance and root mean squared errors (**RMSE**) and concordance correlation coefficients (**CCC**) associated with model predictions. Agreement between modeled and measured responses was evaluated using RMSE, mean bias, and slope bias as described by Bibby and Toutenburg (1978), and CCC as described by Lin (1989).

RESULTS AND DISCUSSION

A summary of the literature data for arterial, portal, and hepatic concentrations is presented in Table 2, and

Table 1. Studies used for model evaluation of EAA fluxes by portal-drained viscera and liver tissues

Citation	
Bach et al. (2000a)	Lapierre et al. (2004)
Bach et al. (2000b)	Larsen and Kristensen (2009)
Baird et al. (1974)	Larsen and Kristensen (2012)
Baird et al. (1975)	Lomax and Baird (1983)
Benson et al. (2001)	McGuire et al. (1989)
Berthiaume et al. (2001)	Raggio et al. (2004)
Berthiaume et al. (2006)	Reynolds et al. (1988)
Blouin et al. (2002)	Reynolds et al. (1995)
Casse and Rulquin (1993)	Reynolds et al. (1997)
Casse et al. (1994)	Reynolds et al. (1998)
Dalbach et al. (2011)	Reynolds et al. (1999)
Delgado-Elorduy et al. (2002)	Reynolds et al. (2001)
De Visser et al. (1997)	Reynolds et al. (2003a)
Doepel et al. (2007)	Reynolds et al. (2003b)
Doepel et al. (2009)	Reynolds et al. (unpublished data)[1]
Girard and Desrochers (2010)	Røjen et al. (2004)
Hammon et al. (2008)	Røjen et al. (2008)
Hanigan et al. (2004b)	Tagari et al. (2000)
Huntington (1982)	Tagari et al. (2004)
Huntington et al. (1983)	Tagari et al. (2008)
Huntington (1984)	Whitt et al. (1996)
Huntington and Reynolds (1986)	Wray-Cahen et al. (1997)

[1]C. Reynolds (University of Reading, Reading, United Kingdom), L. Crompton (University of Reading, Reading, United Kingdom), D. Beever (University of Reading, Reading, United Kingdom), J. Sutton (Department of Agriculture, Reading, United Kingdom), M. Lomax (University of Reading, Reading, United Kingdom), D. Wray-Cahen (University of Reading, Reading, United Kingdom), J. Metcalf (University of Reading, Reading, United Kingdom), B. Bequette (University of Maryland, College Park), C. Backwell (Rowett Research Institute, Aberdeen, United Kingdom), G. Lobley (Rowett Institute of Nutrition and Health, Aberdeen, United Kingdom), J. MacRae (Rowett Research Institute, Aberdeen, United Kingdom), and M. Hanigan (Virginia Tech, Blacksburg).

a summary of the data for absorbed and predicted AA fluxes and blood flow is presented in Table 3. All studies were conducted with lactating dairy cows; however, some studies also included observations from cows in late gestation. The average observed BW for the late-gestation animals (n = 23) was 570 ± 98 kg, with DMI of 9.0 ± 2.2 kg/d. For the lactating cows (n = 105), the average observed BW was 598 ± 65 kg with DMI, milk production, and milk protein concentration of 17.3 ± 3.53 kg/d, 28.3 ± 9.04 kg/d, and 3.3 ± 0.4%, respectively. The percentage increase in EAA concentrations from arterial to the portal vein ranged from 9 to 27%, whereas the percentage increase in concentrations from arterial to the hepatic vein ranged from 6 to 20%. On average, EAA concentrations in the portal vein were 5% greater than were the EAA concentration within the hepatic vein.

PDV Model

The PDV are a heterogeneous collection of tissues including the total digestive tract as well as the pancreas, spleen, and mesenteric fat (Berthiaume et al., 2001). The implicit assumption for Equation [2] is that the PDV has a fixed activity with respect to EAA concentration and does not discriminate among absorbed and arterial supplies in terms of use. Estimates of these fixed-rate parameters were first derived for the PDV by Hanigan et al. (2004b) based on a single study. Their parameter estimates differed for all EAA, except Leu, in comparison to the parameter estimates from the refit model herein (Table 4). The relative change in mean predicted concentrations using the original parameters, as compared with those from the refit, ranged between −11% and +6.5%. Clearance rate parameters were much greater for His, Leu, Lys, Met, Phe, Thr, and Val than previously reported, suggesting that tissue use is greater on average for those AA. Clearance rates for Arg and Ile were lower than the prior report. Differences are perhaps not surprising, as the original estimates represented a single study with cows (611 kg of BW) eating roughly 16.8 kg of DM per day of a single diet and milking 14.8 kg/d, whereas the average BW, DMI, and milk yield for the studies used in this evaluation were 593 ± 71.2 kg, 15.8 ± 4.62 kg/d, and 23.2 ± 13.6 kg/d, respectively. However, the upper level of production for the studies used in this evaluation were 23.7 kg/d for DMI and 47.7 kg/d for milk yield and included the prior data. Thus, the problem should not be due to use of the models at production levels outside of the data range, which can be problematic. The combination of lower DMI and higher milk production in the current data was counterintuitive, as gut mass can be expected to scale positively with DMI (Reynolds et al., 2004), and we had previously observed lower hepatic clearance rates for nonlactating cows compared with lactating cows, presumably due to smaller liver size. These observations are consistent with reduced arterial concentrations of EAA for the current data, which is indicative of increased demand for AA by the collective tissues relative to the overall supply. Although the Hanigan et al. (2004b) data were present in the current data set, they represented only 4 treatment means and, thus, were a minor influence on the overall solutions.

Using the model and parameter estimates of Hanigan et al. (2004b) to predict C_{PV} given inputs of observed arterial concentrations, estimated absorption, and observed portal blood flows resulted in RMSE ranging from 3.3 to 12.1% of the mean observed and CCC ranging from 0.86 to 0.99 (Table 5; Equation [2]). The predictions had significant mean and slope bias ($P < 0.05$). Derivation of new rate parameters for Equation [2] removed the mean bias and resulted in RMSE ranging from 3.2 to 8.6% of the mean observed and CCC from 0.93 to 0.99. Although the residual variance was small, the residuals displayed significant slope bias (P

Table 2. Observed arterial and venous concentrations (μM) of EAA from the database used for model development and testing

Parameter[1]	EAA	N[2]	Mean	Minimum	Maximum	SD
C_A	Arg	22	73.9	52.0	154.9	23.73
	His	61	42.1	13.0	97.1	15.05
	Ile	61	108.4	36.0	208.1	33.21
	Leu	63	130.4	45.0	233.0	49.23
	Lys	63	67.5	31.0	140.0	21.90
	Met	63	18.6	10.0	49.8	5.67
	Phe	63	45.6	1.9	87.3	11.05
	Thr	61	89.4	42.0	169.9	22.66
	Val	61	200.0	76.0	380.7	67.83
C_{PV}	Arg	22	86.6	60.3	177.7	27.06
	His	61	48.0	16.8	111.2	16.20
	Ile	61	125.9	45.3	238.8	35.62
	Leu	61	156.9	58.6	264.3	55.50
	Lys	61	88.9	41.7	180.9	27.33
	Met	61	25.5	13.2	59.0	6.80
	Phe	61	60.8	19.7	112.5	14.03
	Thr	61	104.4	48.3	164.4	23.29
	Val	61	219.5	86.7	405.7	70.72
C_{HV}	Arg	22	78.7	55.9	154.5	22.02
	His	59	44.2	15.9	96.3	14.82
	Ile	59	121.1	40.3	212.3	33.93
	Leu	59	151.1	52.9	262.2	60.00
	Lys	59	82.2	37.1	162.1	24.51
	Met	59	22.1	10.1	47.0	5.88
	Phe	59	51.9	27.2	87.2	10.28
	Thr	59	97.4	43.0	174.0	24.48
	Val	59	213.3	81.9	401.3	70.15

[1]C_A = arterial concentration; C_{PV} = concentration in the portal vein; C_{HV} = concentration in the hepatic vein.
[2]N = number of observations.

< 0.05; Figure 2) indicating that there might be a better equation form to represent this relationship.

Regression analyses indicated that the residuals were negatively correlated with arterial concentrations, which implies that the tissue has decreasing clearance activity as arterial concentrations increased. This may reflect a need for a specific amount of each EAA, regardless of arterial supply. Assuming that clearance is at least partially driven by needs for tissue maintenance and secretion, such use may be expected to remain constant in the face of changes in absorbed or arterial supplies. Indeed, if endogenous secretions are driven by DMI and body size, which are both independent of EAA supply, variable tissue affinity for arterial EAA could be expected. To reflect this variation, the static clearance rate constant of Equation [2] was replaced with a variable rate function containing a fixed element (K_1, liters per day) and an element driven by arterial concentrations [K_2, $L^2/(mol \times d)$], resulting in Equation [5], which was fitted to the data. The resulting parameter estimates are presented in Table 4. This approach led to reduced RMSE, ranging from 1.9 to 6.5% of the mean observed, and CCC ranging from 0.97 to 1.0, with no significant mean or slope bias present (Table 5).

In all cases, K_1 increased relative to clearance rate parameters for Equation [2], and K_2 was negative, indicating that the tissue became less active in removing EAA as arterial concentrations increased, consistent with relatively fixed tissue requirements for EAA. There were large correlations among the fixed element parameters (K_1) and the rate parameters driven by arterial concentrations (K_2). However, because the standard errors (SE) were relatively low, the correlations did not appear to contribute to variance inflation. The K_2 values for Met and Phe were the largest, but the magnitude of the change elicited by arterial concentrations in overall activity is perhaps more robustly represented by the ratio of K_2 to K_1. In that case, the change in activity is proportionally large for Met and His and relatively less for branched-chain AA (**BCAA**). Whether the greater sensitivity to arterial His and Met reflects the importance of maintaining their concentrations in blood to avoid metabolic problems is unclear. Based on the relatively smaller K_2 values for the BCAA, PDV removal could be expected to be a relatively constant proportion of supply for those AA.

An alternative hypothesis was that biases for the simple model were associated with differential use of EAA from absorbed and arterial supplies. Absorbed

EAA are not exposed to the activity of the entire PDV (Hanigan, 2005), whereas arterial blood is exposed to the entire tissue bed. Assuming that tissue use is constant per unit of mass, one could expect a smaller fractional extraction from the absorbed supply than from the arterial supply, given that the small intestine represents about 21% of the total gut mass in cattle (Gibb et al., 1992). Equation [6] reflects such a potential case. However, the parameter estimates derived from fitting that model to the data suggest that all tissue use occurs from the absorbed supply, and no use occurs from arterial supplies except for Thr (Table 4). Examination of the correlation matrix indicated high correlations among the 2 sets of parameters (e.g., −0.96

Table 3. Predicted absorbed and observed blood AA fluxes used for model development and testing; predictions of absorbed fluxes calculated as described in Materials and Methods

Parameter[1]	EAA	N[2]	Mean	Minimum	Maximum	SD
BF_{PV}		61	29,610	16,080	43,130	7,289
BF_{HA}		59	6,148	1,152	15,940	3,714
BF_{HV}		59	35,640	18,310	53,060	9,675
F_{Abs}	Arg	22	0.48 (84)	0.33 (57)	0.69 (120)	0.12 (21)
	His	61	0.25 (39)	0.13 (20)	0.35 (54)	0.06 (9)
	Ile	61	0.79 (104)	0.44 (58)	1.09 (143)	0.17 (22)
	Leu	61	1.20 (157)	0.63 (83)	1.81 (237)	0.30 (39)
	Lys	61	0.89 (130)	0.51 (75)	1.31 (192)	0.18 (26)
	Met	61	0.26 (39)	0.15 (22)	0.36 (54)	0.05 (8)
	Phe	61	0.63 (104)	0.34 (56)	0.90 (149)	0.14 (23)
	Thr	61	0.78 (93)	0.44 (52)	1.04 (124)	0.16 (19)
	Val	61	0.93 (109)	0.52 (61)	1.27 (109)	0.20 (23)
F_{PA}	Arg	22	2.38	1.05	4.15	0.60
	His	61	1.21	0.38	2.60	0.46
	Ile	61	3.26	0.68	6.58	1.35
	Leu	61	3.94	0.87	9.28	1.89
	Lys	61	2.00	0.59	3.89	0.80
	Met	61	0.56	0.19	1.33	0.22
	Phe	61	1.35	0.07	2.55	0.48
	Thr	61	2.69	0.80	5.26	1.03
	Val	61	6.01	1.45	12.17	2.56
F_{HA}	Arg	22	0.50	0.14	1.11	0.22
	His	59	0.24	0.04	0.63	0.16
	Ile	59	0.64	0.12	1.62	0.42
	Leu	59	0.76	0.12	2.16	0.49
	Lys	59	0.41	0.07	1.04	0.27
	Met	59	0.12	0.02	0.28	0.08
	Phe	59	0.28	0.05	0.68	0.17
	Thr	59	0.56	0.10	1.51	0.38
	Val	59	1.19	0.21	3.68	0.82
F_{PV}	Arg	22	2.79	1.27	4.76	0.69
	His	61	1.39	0.53	2.98	0.51
	Ile	61	3.77	0.86	7.28	1.46
	Leu	61	4.73	1.11	10.53	2.12
	Lys	61	2.64	0.79	4.85	0.99
	Met	61	0.76	0.25	1.58	0.27
	Phe	61	1.80	0.64	3.15	0.59
	Thr	61	3.14	0.92	5.72	1.12
	Val	61	6.59	1.65	12.82	2.69
F_{HV}	Arg	22	3.09	1.33	4.76	0.71
	His	59	1.53	0.52	2.97	0.56
	Ile	59	4.34	1.12	8.96	1.75
	Leu	59	5.42	1.46	11.52	2.49
	Lys	59	2.94	1.03	5.34	1.15
	Met	59	0.79	0.28	1.61	0.32
	Phe	59	1.85	0.75	3.39	0.61
	Thr	59	3.52	1.19	6.88	1.42
	Val	59	7.66	2.27	16.22	3.29

[1]BF_{PV} = portal vein blood flow (L/d); BF_{HA} = hepatic artery blood flow (L/d); BF_{HV} = hepatic vein blood flow (L/d); F_{Abs} = absorbed EAA flux [mol/d (g/d in parentheses)] as described by Fleming et al. (2019); F_{PA} = portal arterial flux (mol/d); F_{HA} = hepatic arterial flux (mol/d); F_{PV} = portal vein flux (mol/d); F_{HV} = hepatic vein flux (mol/d).

[2]N = number of observations.

Table 4. Clearance rate parameters[1] (K, L/d) derived for several portal-drained viscera models when fitted to observed EAA concentrations

	Eq. [2]	Eq. [2] refit	Eq. [5]		Eq. [6]		Eq. [7]
AA	K_{PDV}	K_{PDV} ± SE	K_1 PDV ± SE	K_2 PDV ± SE	K_1 PDV ± SE	K_2 PDV ± SE	f_{PDV} ± SE
Arg	2,716	523 ± 351	3,674** ± 734	−33.8** ± 7.06	0 ± 940	0.15 ± 0.16	0.15** ± 0.06
His	97	1,368** ± 193	4,752** ± 455	−66.0** ± 8.16	0 ± 471	0.32** ± 0.09	0.32** ± 0.04
Ile	3,338	2,157** ± 170	5,659** ± 392	−28.9** ± 3.01	0 ± 503	0.37** ± 0.07	0.37** ± 0.02
Leu	2,514	2,639** ± 209	6,891** ± 450	−27.9** ± 2.71	0 ± 569	0.38** ± 0.06	0.38** ± 0.02
Lys	1,996	2,710** ± 321	8,830** ± 494	−77.9** ± 5.50	0 ± 949	0.32** ± 0.08	0.32** ± 0.03
Met	1,000	2,431** ± 298	5,599** ± 589	−146** ± 23.2	0 ± 1009	0.26** ± 0.08	0.26** ± 0.03
Phe	1,316	3,137** ± 290	10,530** ± 710	−151** ± 13.4	0 ± 1118	0.32** ± 0.08	0.32** ± 0.03
Thr	286	3,315** ± 188	4,908** ± 726	−16.6** ± 7.23	1,464* ± 819	0.30** ± 0.09	0.46** ± 0.02
Val	1,341	1,567** ± 117	3,802** ± 234	−9.62** ± 0.94	0 ± 299	0.41** ± 0.07	0.41** ± 0.02

[1]K_{PDV} = clearance rate parameter (L/d) used in Equation (Eq.) [2] to evaluate portal vein concentration. K_1 PDV = clearance rate parameter (L/d) used as an intercept term in Eq. [5] to evaluate portal vein concentrations. K_2 PDV = clearance rate parameter [$L^2/(\mu M \times d)$] used as a slope term in Eq. [5] to evaluate portal vein concentrations. Rate parameters were scaled from molar to μM. K_1 PDV = clearance rate parameter (L/d) used in Eq. (6) to represent the rate of EAA coming from the arterial supply. K_2 PDV = clearance rate parameter (mol/mol) used in Eq. [6] to represent the fractional use of EAA from absorption. f_{PDV} = fractional release (mol/mol) of absorbed EAA used in Eq. [7].
*$0.05 < P < 0.1$; **$P < 0.05$.

for Ile and −0.93 for Met), suggesting an identifiability problem that was also reflected in large parameter SE estimates for the arterial use parameter. Such a pattern of use is not biologically feasible, as the rumen and large intestine are not exposed to absorbed supplies that could be used to support tissue function in the absence of arterial uptake. This solution may have been driven by the use of predicted absorbed EAA supplies, which contain no random variance, whereas the measured arterial supplies contained variance. The lack of random variance in the absorbed supplies may have resulted in less prediction error, thus contributing to reduced residual error in the regression analysis when use is weighted toward absorbed.

To determine whether our revised mechanistic models were a more representative approach to evaluating portal vein concentrations than the current fixed transfer efficiency approach, Equation [7] was derived. This equation represented tissue use as a fractional proportion of absorption, ignoring any contribution to the tissue from arterial supplies. However, given the solution for Equation [7] with arterial use solving to 0, there was no functional difference between Equation [7] and Equation [6], and the results from the 2 equations were identical, having RMSE ranging from 2.5 to 8.0% of the mean observed and CCC ranging from 0.94 to 1.00. Both equations had significant slope bias for all EAA except Thr, suggesting that this approach was not as good a representation. This does not come as a surprise, because previous studies have indicated that arterial supply accounts for as much as 80% of AA use by the PDV (MacRae et al., 1997b). The statistical analysis for the predicted concentrations in PDV tissue are presented in Table 5.

Portal vein fluxes were evaluated using the above models for each EAA. Trends in the flux evaluations were similar to those for predicted concentrations. Evaluation of Equation [2] with the rate parameters derived from Hanigan et al. (2004b) provided RMSE ranging from 3 to 10% of the mean observed and CCC ranging from 0.93 to 0.99. Significant mean bias (>10% MSE; $P < 0.05$) occurred for all EAA except Leu, and significant slope bias (>8% MSE) for all except Thr. Evaluating EAA flux using refit parameters for Equation [2] resulted in RMSE ranging from 2.8 to 7.8% of mean observed and CCC ranging from 0.97 to 0.99. The refit model reduced mean bias for all EAA but still had significant slope bias (>18% MSE). Using Equation [5] to predict EAA fluxes improved overall fit statistics, with RMSE ranging from 1.9 to 6.3% of mean observed and CCC ranging from 0.98 to 0.99. We discovered no significant mean bias with this model form, but a slight slope bias remained (>4% MSE) for Met, and Thr. The empirical model reproduced similar

results to those observed for Equation [5]; however, the empirical model introduced significant slope bias (>8% MSE) for all EAA (Table 6).

Use of each EAA by the PDV was calculated using the refit model and expressed as a fraction only of the absorbed supply or of the total supply feeding the tissue (absorbed plus arterial; Table 7). Use expressed as a fraction of the absorbed supply ranged from 10 to 46%, with Arg being the smallest and Thr the largest. The PDV have been interpreted to represent a barrier to efficient production, but as the majority of the use is from arterial supplies, this is not the case [see discussion by Reynolds (2006)]. Expressing use as a fraction of the total supply yields a range in use of 1.5 to 10.5%, which represents single-pass use. Thus, 90% or more of the absorbed EAA are delivered to the portal vein.

One could assess model performance relative to predictions of EAA clearance by the tissue bed. Because use is small relative to total supply, RMSE would be significantly greater and CCC less. However, the overall objective of this work was to predict transfer of EAA to the mammary glands, and thus we focused on predicting release of EAA from the tissue. The RMSE were generally the greatest for Equation [2] and the least for Equation [5]. Although the overall fit statistics were good for Equation [2] and the refit of Equation [2], both had slope bias for most EAA, whereas Equation [5] exhibited no slope bias.

In conclusion, based on RMSE, MSE partitioning, CCC, and the known biology, Equation [5] best represented transfer of EAA across the PDV. Equation [2] could be used, but it has some slope bias that will feed

Table 5. Statistical summary of predictions of portal vein AA concentrations (μM) by several models

EAA	Eq.[1]	N[2]	Observed mean	Predicted mean	RMSE[3] (%)	CCC[4]	Mean bias (% MSE)	Slope bias (% MSE)
Arg	2	22	86.6	81.6	9.1	0.95	40.4*	38.2*
	2—refit			87.3	5.6	0.98	2.0	30.5*
	5			86.3	3.9	0.99	0.5	0.3
	6 and 7			86.5	5.3	0.98	0.0	24.9*
His	2	61	48.0	50.5	7.6	0.97	46.7*	2.3
	2—refit			48.2	5.7	0.98	0.9	25.2*
	5			47.8	4.0	0.99	0.6	0.0
	6 and 7			47.9	5.3	0.99	0.0	7.5*
Ile	2	61	126	122	6.0	0.97	31.1*	36.7*
	2—refit			126	4.6	0.99	0.7	39.9*
	5			126	2.9	0.99	0.2	0.5
	6 and 7			126	3.9	0.99	0.1	15.2*
Leu	2	61	157	158	5.5	0.99	3.1	44.5*
	2—refit			158	5.5	0.99	1.1	47.5*
	5			157	3.3	1.00	0.3	0.0
	6 and 7			157	4.6	0.99	0.1	25.4*
Lys	2	61	88.9	91.9	9.0	0.95	13.3*	37.8*
	2—refit			89.7	8.6	0.95	1.0	51.4*
	5			88.6	4.3	0.99	0.9	0.1
	6 and 7			88.5	7.3	0.97	0.4	37.5*
Met	2	61	25.5	26.8	9.7	0.92	28.4*	10.2*
	2—refit			25.5	8.1	0.94	0.0	32.1*
	5			25.3	6.5	0.97	0.8	1.1
	6 and 7			25.4	8.0	0.95	0.1	11.2*
Phe	2	61	60.8	64.6	10.0	0.89	38.4*	10.2*
	2—refit			60.8	7.5	0.93	0.0	32.1*
	5			60.5	4.6	0.98	1.5	1.1
	6 and 7			60.5	7.3	0.94	0.4	11.2*
Thr	2	61	104	115	12.1	0.86	75.7*	0.2
	2—refit			104	4.8	0.97	0.1	12.4*
	5			104	4.5	0.98	0.4	1.0
	6 and 7			104	5.0	0.97	0.2	0.0
Val	2	61	219	222	3.3	0.99	13.1*	44.0*
	2—refit			220	3.2	0.99	1.8	55.3*
	5			219	1.9	1.00	0.1	0.2
	6 and 7			219	2.5	1.00	0.0	20.4*

[1]Eq. = equation used to calculate the concentration of the portal vein. Equation [2] was derived by Hanigan et al. (2004b).
[2]N = number of observations.
[3]RMSE = root mean squared error (% of the observed mean).
[4]CCC = concordance correlation coefficient.
*$P \leq 0.05$; otherwise, $P > 0.05$.

forward into the liver model in an integrated prediction system. Equation [6] was not uniquely defined and solved for biologically infeasible parameters, indicating use of AA only from absorbed supply, and had variance inflation due to high correlation among parameters. Thus Equation [6] should not be adopted for further use. From a systems standpoint, use of Equation [5] prevents derivation of an analytical solution for predictions of arterial concentrations, and thus the refit of Equation [2] may represent the best form for such use, despite the small amount of slope bias.

Liver Model

Similar to the PDV, the implicit assumption for Equation [9] was that LIV had a fixed activity with respect to EAA removal and does not discriminate among portal and arterial supplies in terms of use. Us-

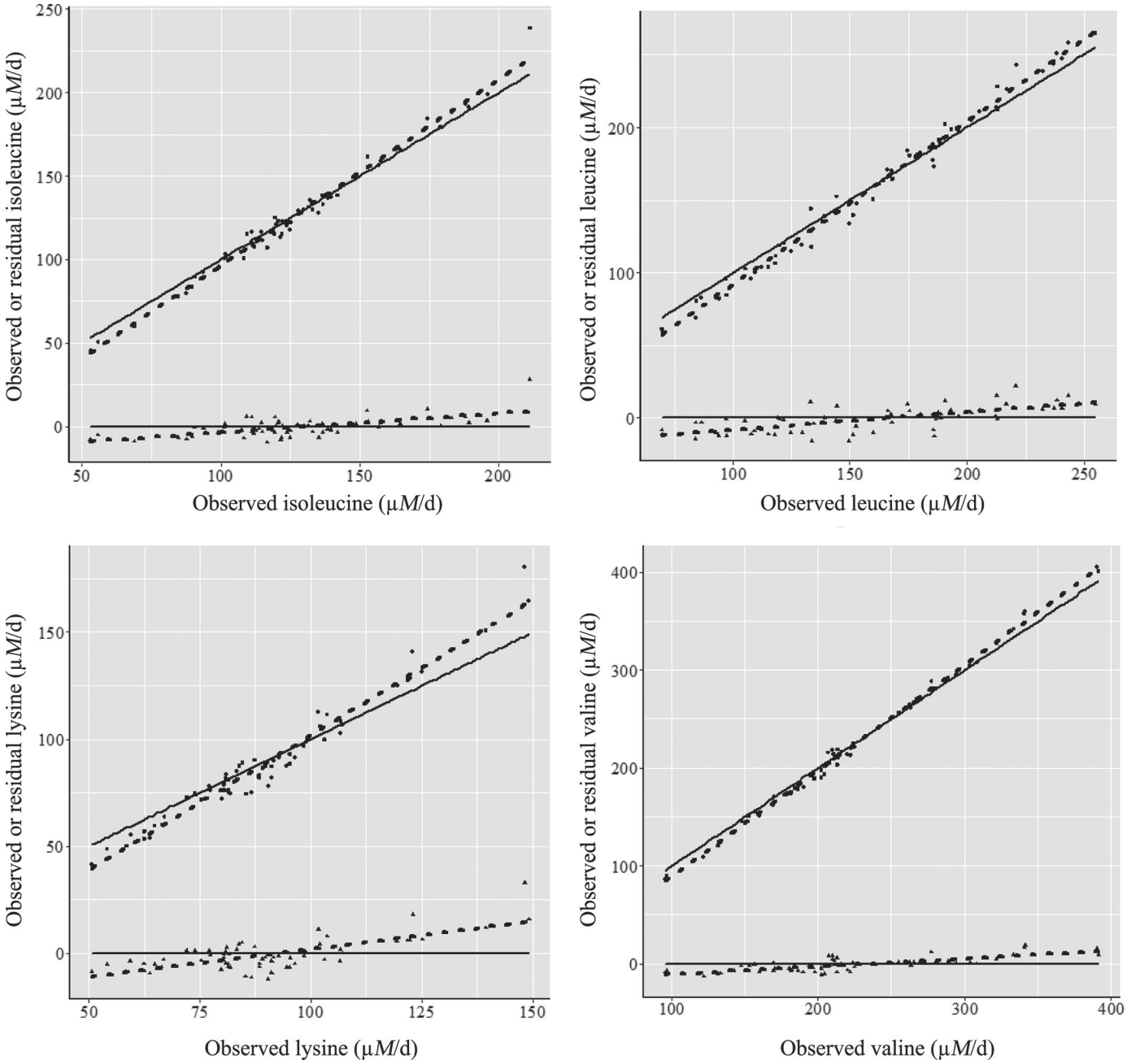

Figure 2. Residual plot of Ile, Leu, Lys, and Val portal-drained viscera concentration (μ*M*/d) predictions using Equation [2] when parameters were refit to the data. The solid black line represents the line of unity, and the dashed line represented the fitted model.

Table 6. Statistical summary of portal vein AA fluxes (mol/d) predicted by several models

EAA	Eq.[1]	N[2]	Observed mean	Predicted mean	RMSE[3] (%)	CCC[4]	Mean bias (% MSE)	Slope bias (% MSE)
Arg	2	22	2.79	2.64	8.1	0.93	46.3*	20.2*
	2—refit			2.81	4.9	0.98	2.3	30.3*
	5			2.79	3.7	0.99	0.0	1.8
	7			2.80	4.6	0.98	0.1	29.5*
His	2	61	1.39	1.45	6.8	0.98	48.4*	7.7*
	2—refit			1.40	5.5	0.99	2.3	25.7*
	5			1.39	4.1	0.99	0.0	1.9
	7			1.39	5.0	0.99	0.3	18.8*
Ile	2	61	3.77	3.64	5.8	0.99	35.4*	25.1*
	2—refit			3.79	4.0	0.99	2.4	26.9*
	5			3.77	2.9	0.99	0.0	0.0
	7			3.78	3.4	0.99	0.5	20.7*
Leu	2	61	4.73	4.74	4.7	0.99	0.6	52.2*
	2—refit			4.77	4.6	0.99	4.6	48.0*
	5			4.73	3.0	0.99	0.1	2.7
	7			4.75	3.7	0.99	1.2	42.9*
Lys	2	61	2.64	2.71	7.9	0.97	10.7*	35.5*
	2—refit			2.68	7.8	0.97	2.8	39.8*
	5			2.64	4.3	0.99	0.0	4.0
	7			2.65	6.5	0.98	0.1	53.3*
Met	2	61	0.76	0.79	8.2	0.97	24.8*	29.6*
	2—refit			0.76	7.5	0.97	1.4	41.7*
	5			0.76	6.3	0.98	0.1	16.2*
	7			0.76	7.3	0.98	0.7	39.2*
Phe	2	61	1.80	1.90	8.4	0.96	40.2*	12.8*
	2—refit			1.81	6.9	0.97	0.9	25.9*
	5			1.80	4.3	0.99	0.0	2.3
	7			1.81	6.4	0.98	0.3	33.6*
Thr	2	61	3.14	3.43	10.3	0.96	83.7*	0.2
	2—refit			3.15	4.3	0.99	0.5	17.7*
	5			3.14	4.2	0.99	0.1	7.2*
	7			3.14	4.4	0.99	0.4	8.1*
Val	2	61	6.58	6.64	2.8	0.99	10.0*	37.3*
	2—refit			6.62	2.8	0.99	4.5	41.1*
	5			6.59	1.9	0.99	0.1	2.2
	7			6.60	2.2	0.99	0.9	25.9*

[1]Eq. = equation used to calculate concentration of the portal vein, which could then be used to calculate AA flux using Eq. [1]. Equation [2] was derived by Hanigan et al. (2004b).

[2]N = number of observations.

[3]RMSE = root mean squared error (% of the observed mean).

[4]CCC = concordance correlation coefficient.

*$P \leq 0.05$; otherwise, $P > 0.05$.

Table 7. Proportion of tissue EAA use, expressed as a percentage of total or absorbed EAA supplies

	PDV		Liver	
EAA	Total[1]	Absorbed	Total[2]	Absorbed
Arg	1.6	10.3	7.3	55.2
His	4.7	28.9	7.3	53.2
Ile	7.2	35.5	0.7	3.4
Leu	8.6	35.4	0.7	2.9
Lys	8.8	28.1	3.9	12.8
Met	8.0	25.1	10.4	34.3
Phe	10.1	31.7	12.4	40.5
Thr	10.6	45.8	4.0	18.0
Val	5.3	38.0	−0.1	−0.4

[1]Total = proportion of tissue use coming from both arterial flux and absorption, using the refit of Equation [2].

[2]Total = proportion of tissue use coming from both arterial flux and EAA not used in portal-drained viscera (PDV) flux, using the refit of Equation [9].

Table 8. Hepatic clearance rate parameter estimates[1] (K, L/d) for several models fitted to hepatic vein EAA concentration data

	Eq. [9]	Eq. [9]—refit	Eq. [11]		Eq. [12]	Eq. [13]
EAA	K_{LIV}	K_{LIV} ± SE	K_1 LIV[2] ± SE	K_2 LIV ± SE	K_{LIV} ± SE	f_{SPL} ± SE
Arg	3,776	3,007* ± 359	750 ± 950	23.8* ± 9.64	2,926* ± 378	0.62* ± 0.07
His	2,165	1,979* ± 167	449 ± 520	29.6* ± 9.73	1,950* ± 175	0.66* ± 0.04
Ile	247	217 ± 136	−846 ± 481	8.83* ± 3.87	238 ± 169	0.39* ± 0.03
Leu	215	222* ± 99	821* ± 347	−3.87 ± 2.14	266 ± 229	0.40* ± 0.03
Lys	855	1,343* ± 184	−595 ± 509	24.3* ± 6.16	1,290* ± 296	0.43* ± 0.03
Met	4,280	3,855* ± 311	2,263* ± 809	70.8* ± 34.2	3,727* ± 333	0.58* ± 0.03
Phe	4,971	4,720* ± 218	940 ± 863	75.7* ± 17.2	4,610* ± 239	0.70* ± 0.02
Thr	1,514	1,385* ± 179	4,563* ± 596	−32.5* ± 5.80	1,436* ± 201	0.66* ± 0.02
Val	−166	−17 ± 76	581* ± 245	−2.62* ± 1.02	29 ± 143	0.41* ± 0.03

[1]K_{LIV} = clearance rate parameter (L/d) used in Equation (Eq.) [9] to evaluate hepatic vein concentrations. K_1 LIV = clearance rate parameter (L/d) used as an intercept term in Eq. [11] to evaluate hepatic vein concentrations. K_2 LIV = clearance rate parameter [(L × L)/μM × d] used as a slope term in Eq. [11] to evaluate hepatic vein concentrations. K_{LIV} = clearance rate parameter (L/d) used in Eq. [12] to evaluate EAA concentration in the splanchnic tissue. f_{SPL} = clearance rate parameter (mol/mol) used in Eq. [13] represents the fractional release of absorbed AA in the splanchnic tissue.

*$P \leq 0.05$; otherwise, $P > 0.05$.

ing the model and parameter estimates of Hanigan et al. (1998b) to predict C_{HV} given inputs of arterial and portal fluxes resulted in RMSE ranging from 1.9 to 6.8% of the mean observed and CCC ranging from 0.97 to 1.0. Original parameter estimates resulted in significant slope bias ($P < 0.05$) for several EAA (Arg, Lys, Phe, Thr, Val) when predicting hepatic vein concentrations, thus leading to significant bias in flux predictions for most EAA. Therefore, the model was refit to the data set, and a new set of rate constants were derived (Table 8), which differed for the majority of EAA compared with those initially derived by Hanigan et al. (1998b). The refit model predicted hepatic vein concentrations with RMSE ranging from 1.9 to 6.7% of the mean observed and CCC ranging from 0.97 to 1.00 (Table 9). Although the RMSE and CCC values differed only slightly from those of the original model, the mean bias improved for the majority of the EAA (especially Arg, for which there had been a significant mean bias with the original model).

Although the performance of the refit of Equation [9] appeared good, a slight slope bias remained (Table 9; Figure 3), associated with predictions of hepatic vein concentrations of Arg, His, Lys, Phe, Thr, and Val. As for PDV, the residual errors were correlated with arterial concentrations of the respective EAA. Conversion of the fixed clearance rate constant to a linear function of arterial concentrations (Equation [11]) eliminated slope bias; however, improvements in RMSE and CCC were small, and thus one might question the value of the added complexity.

In most cases, the value of K_2 was positive, indicating that the tissue became more active in removing AA as arterial concentrations increased. This was the opposite of the PDV and provides a mechanism for hepatic maintenance of circulating concentrations for those AA. The base clearance rates were reduced considerably relative to those of Equation [9], indicating that half or more of the clearance activity was variable across AA. In fact, the base rates were essentially 0 for Arg, His, the BCAA, Lys, and Phe. Only Met and Thr had substantial base rates and thus relatively less important variable rates.

It is interesting that hepatic activity for Met and Thr appears to be more consistent regardless of plasma availability, as those 2 AA flow through the lower portion of the TCA cycle via pathways that converge with propionate metabolism (Hanigan et al., 2004a). The nonsignificant or very small variable components for the BCAA would be expected, given the lack of BCAA catabolic activity by the liver (Shimomura et al., 2006). Conversely, Lobley (2003) concluded that enzymes for His, Met, and Phe catabolism are almost exclusively restricted to the hepatic tissues. It was surprising that Arg release was predicted with similar precision to that of the other EAA, as this AA is involved in the urea cycle, with significant cycling and interconversion of ornithine and Arg by the kidney and liver to maintain a supply of Arg for the rest of the body (Newsholme and Leech, 1983). One would have thought this would create more diversity in clearance rates for Arg.

In addition to arterial concentrations, residuals were regressed on intake of digestible energy (**DEIn**) to determine whether variation in DEIn was driving the changes in extraction kinetics. Results indicated that DEIn was not contributing significant bias to the overall EAA concentration predictions.

For the refit LIV model, the fraction of total supply of each EAA used by the tissue bed ranged from −0.1 to 12.4%, with Val being the smallest and Phe the larg-

est (Table 7). For Val, the predicted rate parameter was not significantly different from zero; therefore, the slightly negative use estimate is also not meaningful. It makes less sense to express use relative to absorbed supplies for the liver; however, doing so results in fractional use ranging from −0.4 to 55%.

In addition to assessing predictions of EAA concentrations, we also evaluated predictions of EAA fluxes for each of the models. These results are presented in Table 10. Flux predictions using Equation [9] with rate parameters reported by Hanigan et al. (1998b) resulted in RMSE ranging from 2 to 7% of observed mean and CCC ranging from 0.97 to 1.0. With the original parameter estimates, significant mean bias occurred for Arg (30% MSE), and slope bias (>5% MSE) was present for Arg, His, Lys, Phe, and Thr. This was expected based on observed bias when evaluating EAA concentrations. Using the rederived rate parameters resulted in similar RMSE and CCC values, reduced mean bias for Arg, but slightly increased proportions of MSE associated with slope bias (>7% MSE) for all EAA excluding Ile, Leu, and Met. Evaluating EAA fluxes using Equation [11] resulted in similar RMSE and CCC but reduced both mean and slope bias compared with the other LIV models.

In conclusion, based on the evaluation criteria, the best model for representing hepatic release was generally Equation [11], although all of the models adequately represented venous concentrations of BCAA (Ile, Leu, and Val) because the use of these AA by the liver is essentially 0. In a sense, we can predict use of these AA by the tissue as 0 and assume that tissue release is equal to the inputs.

Splanchnic Model

An evaluation of predictions of hepatic vein concentrations was conducted using inputs of F_{Abs}, C_{PA}, C_{HA}, BF_{PA}, and BF_{HA} and the combined PDV (Equation [2]) and LIV (Equation [9]) models (Equation [12]) or an empirical equation using only F_{Abs} as an input (Equation [13]). Results are presented in Table 11. Using Equation [12] yielded RMSE ranging from 3.5 to 7.3% of observed mean and a CCC ranging from 0.95 to 0.99. Slope bias was significant ($P \leq 0.05$; >16% MSE) for predictions of EAA concentrations for all EAA except

Table 9. Statistical summary of hepatic vein AA concentrations (μM) predicted by several hepatic models fitted to the data

EAA	Eq.[1]	N[2]	Observed mean	Predicted mean	RMSE[3]	CCC[4]	Mean bias (% MSE)	Slope bias (% MSE)
Arg	9	22	78.7	76.6	4.9	0.98	29.7*	14.1*
	9—refit			78.1	4.4	0.99	3.3	33.6*
	11			78.6	3.9	0.99	0.1	3.9
His	9	59	44.2	43.8	4.1	0.99	3.4	5.1
	9—refit			44.1	4.0	0.99	0.3	7.6*
	11			44.2	3.7	0.99	0.2	0.0
Ile	9	59	121	121	3.3	0.99	2.0	2.1
	9—refit			121	3.3	0.99	1.3	2.3
	11			121	3.2	0.99	0.4	1.1
Leu	9	59	151	151	2.4	1.00	0.1	2.2
	9—refit			151	2.4	1.00	0.2	2.2
	11			151	2.3	1.00	0.9	0.3
Lys	9	59	82.2	82.8	4.6	0.99	2.4	22.5*
	9—refit			81.6	4.4	0.99	2.6	16.1*
	11			82.0	3.8	0.99	0.3	0.0
Met	9	59	22.1	21.8	6.8	0.97	4.1	1.8
	9—refit			22.0	6.7	0.97	0.2	3.1
	11			22.1	6.5	0.97	0.1	0.2
Phe	9	59	51.9	51.4	4.6	0.98	4.2	25.6*
	9—refit			51.7	4.5	0.98	0.4	28.8*
	11			51.9	3.8	0.98	0.1	1.5
Thr	9	59	97.4	97.5	4.1	0.99	0.1	13.4*
	9—refit			97.9	4.1	0.99	1.3	12.5*
	11			97.5	3.3	0.99	0.1	2.5
Val	9	59	213	214	1.9	1.00	4.6	3.4
	9—refit			213	1.9	1.00	0.0	6.5*
	11			213	1.7	1.00	0.7	0.1

[1]Eq. = equation used to calculate concentration of the hepatic vein. Equation [10] was derived by Hanigan et al. (1998b).
[2]N = number of observations.
[3]RMSE = root mean squared error (% of the observed mean).
[4]CCC = concordance correlation coefficient.
*$P \leq 0.05$; otherwise, $P > 0.05$.

Arg, which likely propagated from the slope bias of the underlying models. However, the slope was less than 0.2 μmol/L and contributed very little to the MSE.

Equation [13] had a similar range in CCC, with RMSE ranging from 3.0 to 8.6%, with slope bias for Arg, His, Leu, Thr, and Val. The bias associated with these 3 EAA across the splanchnic tissue was less than 30% MSE (<0.1 μmol/L) and thus made a minor contribution to overall bias.

In addition to assessing predictions of hepatic vein concentrations, we also evaluated predictions of hepatic vein fluxes for each of the 2 models. These results are presented in Table 12. Using Equation [12] yielded RMSE ranging from 2.6 to 7.9% of the observed mean

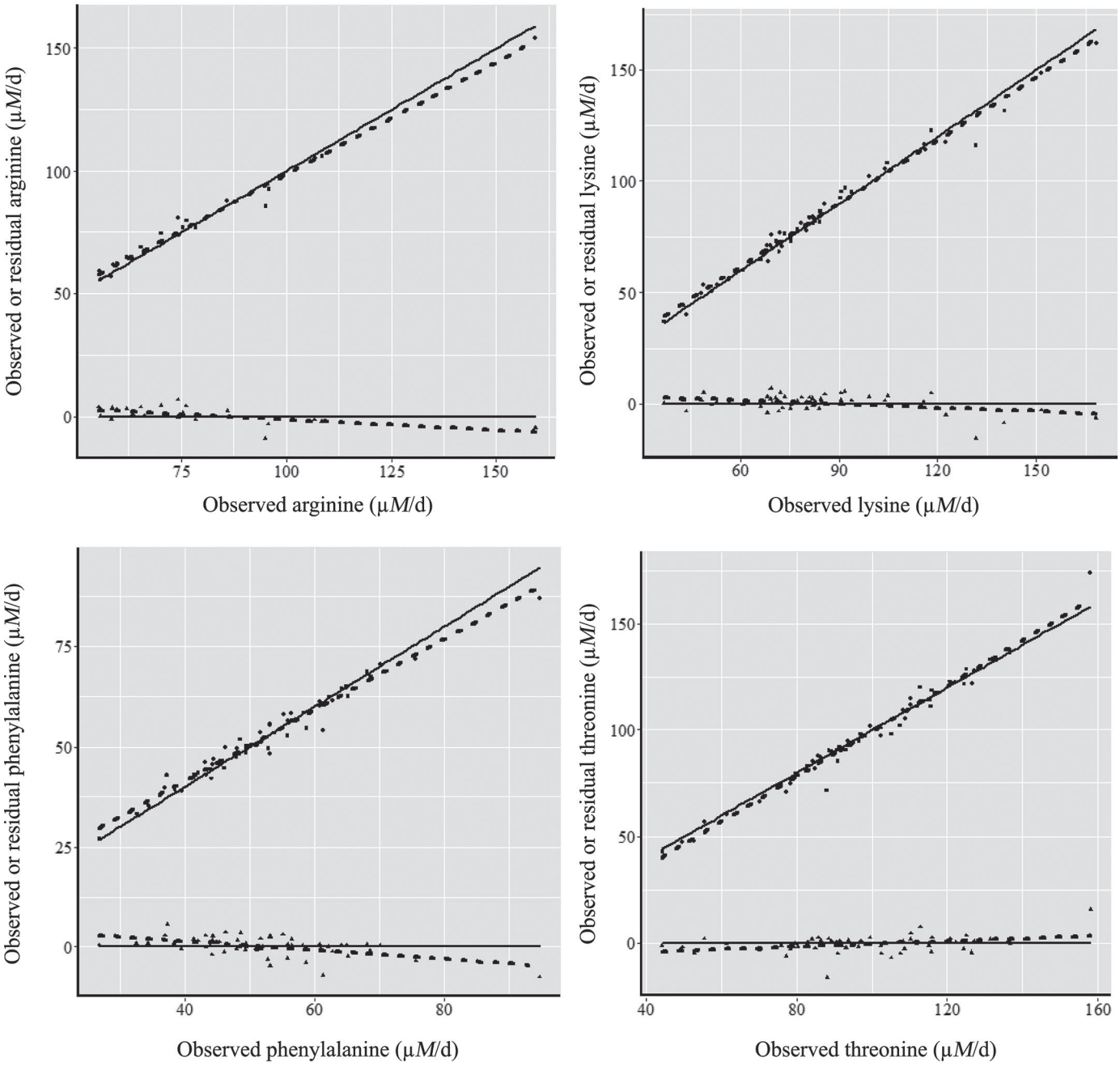

Figure 3. Residual plot of Arg, Lys, Phe, and Thr liver concentration (μ*M*/d) predictions using Equation [9] when rate parameters were refit to the data. The solid black line represents the line of unity, and the dashed line represented the fitted model.

Table 10. Statistical summary of hepatic vein fluxes (mol/d) predicted by the hepatic model[1]

EAA	Eq.[2]	N[3]	Observed mean	Predicted mean	RMSE[4]	CCC[5]	Mean bias (% MSE)	Slope bias (% MSE)
Arg	9	22	3.09	3.01	4.9	0.98	30.4*	13.2*
	9—refit			3.07	4.5	0.98	2.9	24.1*
	11			3.08	3.9	0.99	0.3	6.3
His	9	59	1.53	1.52	3.8	0.99	2.6	8.2*
	9—refit			1.52	3.8	0.99	0.9	9.3*
	11			1.53	3.6	0.99	0.0	1.4
Ile	9	59	4.34	4.31	3.2	0.99	4.5	1.1
	9—refit			4.34	3.1	0.99	0.3	0.5
	11			4.34	3.0	0.99	0.0	7.1*
Leu	9	59	5.42	5.40	2.2	0.99	1.5	6.9*
	9—refit			5.42	2.2	0.99	0.1	4.7
	11			5.40	2.2	0.99	0.1	0.6
Lys	9	59	2.94	2.95	4.7	0.99	0.8	2.5
	9—refit			2.92	4.6	0.99	1.4	1.2
	11			2.94	4.0	0.99	0.0	5.0
Met	9	59	0.79	0.78	6.7	0.99	4.0	0.7
	9—refit			0.79	6.6	0.99	0.1	0.2
	11			0.79	6.4	0.99	0.0	2.0
Phe	9	59	1.85	1.84	4.3	0.99	2.6	10.1*
	9—refit			1.84	4.3	0.99	1.2	10.8*
	11			1.85	3.9	0.99	0.1	1.2
Thr	9	59	3.52	3.53	4.1	0.99	0.4	0.9
	9—refit			3.53	4.1	0.99	0.5	0.9
	11			3.52	3.5	0.99	0.2	6.6*
Val	9	59	7.66	7.68	1.7	0.99	2.0	6.1
	9—refit			7.67	1.7	0.99	0.4	7.2*
	11			7.66	1.7	0.99	0.0	0.6

[1]Inputs were arterial and portal vein EAA concentrations and observed splanchnic blood flows.
[2]Eq. = equation used to calculate concentration of the hepatic vein. Equation [9] was derived by Hanigan et al. (1998b).
[3]N = number of observations.
[4]RMSE = root mean squared error (% of the observed mean).
[5]CCC = concordance correlation coefficient.
*$P \leq 0.05$; otherwise, $P > 0.05$.

and a CCC ranging from 0.97 to 0.99. Significant slope bias ($P \leq 0.05$; > 16% MSE) occurred for all EAA except Arg and His. Equation [13] had similar values, with RMSE ranging from 1.3 to 7.4% MSE and CCC ranging from 0.98 to 0.99. Similarly, significant slope bias ($P \leq 0.05$; > 10% MSE) occurred for all EAA except Arg. Given the prior demonstration of better performance for the underlying PDV and hepatic clearance models when clearance rates were expressed as a function of arterial AA concentrations, one should use those more complicated models for integrated splanchnic representation, but that does add complexity to the model.

We found no clear advantage to using the more mechanistic representation of the effects of blood flow and recycled arterial EAA over the simple fractional use of EAA from the absorbed stream, as there was no significant correlation between arterial EAA concentration and the predicted absorbed EAA supply. The simpler empirical representation provides an advantage, in that it does not require predictions of splanchnic blood flow (Ellis et al., 2016) or arterial concentrations. The mechanistic approach may prove to be superior when inputs other than EAA are manipulated separately from EAA. For example, BF has been observed to be related to energy intake (Ellis et al., 2016); thus, it is possible that attempts to reduce EAA supply and increase DEIn will result in aberrant predictions of the responses. The empirical model also cannot accommodate increased fractional recycling, as the post-splanchnic EAA supply approaches saturation of milk protein production. In such a case, the marginal uptake and deposition of EAA in milk protein will decline, resulting in marginal increases in arterial concentrations and recycling. The mechanistic model will respond to such changes through increased removal and disposal of the excess, whereas the empirical model will not. Thus, the mechanistic representation may provide more robust predictions over a broader range of input conditions than can be expected with the empirical representation. Hence, given the near equality of accuracy and precision of predictions, it seems prudent to select the more mechanistic representation where it can be supported.

Table 11. Statistical summary of predicted hepatic vein EAA concentrations (μM) using arterial and absorbed fluxes and the combined PDV and LIV models

EAA	Eq.[1]	N[2]	Observed mean	Predicted mean	RMSE[3]	CCC[4]	Mean bias (% MSE)	Slope (μM/L)/(μM/L)	Slope bias (% MSE)
Arg	12	22	78.7	78.7	4.7	0.98	0.0	0.0	0.2
	13			78.5	5.1	0.98	0.2	−0.1	29.6*
His	12	59	44.2	44.4	4.2	0.99	0.9	0.1	15.7*
	13			44.3	4.9	0.99	0.2	−0.0	9.2*
Ile	12	59	121.0	121.0	4.1	0.99	0.0	0.0	24.2*
	13			121.0	4.1	0.99	0.2	0.1	3.0
Leu	12	59	151.0	152.0	5.6	0.99	0.3	0.1	41.0*
	13			151.0	5.0	0.99	0.2	0.1	18.2*
Lys	12	59	82.2	82.4	7.1	0.97	0.1	0.1	28.5*
	13			81.7	6.4	0.98	0.8	0.0	4.1
Met	12	59	22.1	22.1	7.3	0.95	0.1	0.2	26.9*
	13			22.1	8.6	0.95	0.0	−0.0	1.0
Phe	12	59	51.9	51.9	5.0	0.96	0.0	0.1	16.0*
	13			51.9	5.9	0.95	0.0	−0.0	0.4
Thr	12	59	97.4	97.8	4.6	0.98	0.6	0.2	50.6*
	13			97.3	3.6	0.99	0.1	0.1	10.7*
Val	12	59	213.0	214.0	3.5	0.99	0.6	0.1	49.7*
	13			213.0	3.0	0.99	0.2	0.0	23.3*

[1]Equation [12] used the derived models for the portal-drained viscera (PDV) (Eq. [2]) and for the liver (LIV) (Eq. [9]) with the specified rate parameters from Table 4 and Table 8, respectively.

[2]N = number of observations.

[3]RMSE = root mean squared error (% of the observed mean).

[4]CCC = concordance correlation coefficient.

*$P \leq 0.05$; otherwise, $P > 0.05$.

CONCLUSIONS

Both PDV and LIV release of EAA can be accurately and precisely predicted based on absorbed EAA supplies given knowledge of arterial concentrations and blood flow. A combination of the 2 models predicted splanchnic release with high accuracy and precision, and can be used to predict total splanchnic EAA re-

Table 12. Statistical summary of splanchnic EAA fluxes (mol/d) predicted using the derived parameters from the combined portal-drained viscera (PDV) and liver (LIV) models

EAA	Eq.[1]	N[2]	Observed mean	Predicted mean	RMSE[3]	CCC[4]	Mean bias (% MSE)	Slope (mol/mol)	Slope bias (% MSE)
Arg	12	22	3.1	3.1	5.3	0.97	0.3	−0.1	5.6
	13			3.1	4.5	0.98	0.0	0.0	0.0
His	12	59	1.5	1.5	4.7	0.99	1.3	0.0	1.1
	13			1.5	4.1	0.99	1.4	0.0	10.3*
Ile	12	59	4.3	4.4	3.7	0.99	0.5	0.0	18.4*
	13			4.4	3.8	0.99	1.2	0.1	29.0*
Leu	12	59	5.4	5.4	4.1	0.99	1.1	0.1	41.6*
	13			5.5	4.8	0.99	3.9	0.1	50.4*
Lys	12	59	2.9	2.9	6.2	0.99	0.2	0.1	25.4*
	13			3.0	1.3	0.98	1.3	0.1	29.7*
Met	12	59	0.8	0.8	7.9	0.98	1.4	0.1	19.2*
	13			0.8	7.4	0.98	1.3	0.1	42.4*
Phe	12	59	1.9	1.9	5.4	0.99	1.4	0.1	16.1*
	13			1.9	5.1	0.99	0.5	0.1	16.9*
Thr	12	59	3.5	3.5	3.3	0.99	0.6	0.0	20.7*
	13			3.5	4.5	0.99	2.0	0.1	26.1*
Val	12	59	7.7	7.7	2.6	0.99	0.5	0.0	37.4*
	13			7.7	3.2	0.99	4.0	0.1	44.2*

[1]Equation [12] was using the derived models from Hanigan et al. (2004b) for the PDV and Hanigan et al. (1998b) for the LIV.

[2]N = number of observations.

[3]RMSE = root mean squared error (% of the observed mean).

[4]CCC = concordance correlation coefficient.

*$P \leq 0.05$; otherwise, $P > 0.05$.

lease, but with some linear bias. However, the models were not clearly superior to more empirical representations driven solely by absorbed EAA supply.

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