

An isotope dilution model for partitioning of phenylalanine and tyrosine uptake by the liver of lactating dairy cows

Article

Accepted Version

Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0

Crompton, L. A., McKnight, L. L., Reynolds, C. K. ORCID: <https://orcid.org/0000-0002-4152-1190>, Mills, J. A. N., Ellis, J. L., Hanigan, M. D., Dijkstra, J., Bequette, B. J., Bannink, A. and France, J. (2018) An isotope dilution model for partitioning of phenylalanine and tyrosine uptake by the liver of lactating dairy cows. *Journal of Theoretical Biology*, 444. pp. 100-107. ISSN 0022-5193 doi: <https://doi.org/10.1016/j.jtbi.2017.12.016> Available at <https://centaur.reading.ac.uk/74567/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1016/j.jtbi.2017.12.016>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1 **An isotope dilution model for partitioning of phenylalanine and tyrosine uptake by the**
2 **liver of lactating dairy cows**

3
4 L.A. Crompton^{a,*}, L.L. McKnight^b, C.K. Reynolds^a, J.A.N. Mills^a, J.L. Ellis^{b,c}, M.D.
5 Hanigan^d, J. Dijkstra^c, B.J. Bequette^{e,1}, A. Bannink^c and J. France^b

6
7 ^a *Sustainable Agriculture and Food Systems Research Division, School of Agriculture, Policy*
8 *and Development, University of Reading, Whiteknights, Reading RG6 6AR, UK*

9 ^b *Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph,*
10 *Ontario N1G 2W1, Canada*

11 ^c *Animal Nutrition Group, Wageningen University & Research, 6700 AH Wageningen, The*
12 *Netherlands*

13 ^d *Department of Dairy Science, Virginia Tech, 2080 Litton Reaves, Blacksburg, VA 24061,*
14 *USA*

15 ^e *Department of Animal & Avian Sciences, University of Maryland, College Park, MD 20742,*
16 *USA*

17
18 ¹Deceased

19
20 ***Corresponding author:** l.a.crompton@reading.ac.uk (L.A. Crompton)

21 **Abstract**

22 An isotope dilution model to describe the partitioning of phenylalanine (PHE) and tyrosine
23 (TYR) in the bovine liver was developed. The model comprises four intracellular and six
24 extracellular pools and various flows connecting these pools and external blood.
25 Conservation of mass principles were applied to generate the fundamental equations
26 describing the behaviour of the system in the steady state. The model was applied to datasets
27 from multi-catheterised dairy cattle during a constant infusion of [1-¹³C] phenylalanine and
28 [2,3,5,6-²H] tyrosine tracers. Model solutions described the extraction of PHE and TYR from
29 the liver via the portal vein and hepatic artery. In addition, the exchange of free PHE and
30 TYR between extracellular and intracellular pools was explained and the hydroxylation of
31 PHE to TYR was estimated. The model was effective in providing information about the
32 fates of PHE and TYR in the liver and could be used as part of a more complex system
33 describing amino acid metabolism in the whole animal.

34

35 **Keywords:**

36 Isotope dilution, Kinetic model, Liver, Phenylalanine, Tyrosine

37

38 **1. Introduction**

39 In ruminant production, the efficiency of milk protein synthesis from absorbed dietary
40 nitrogen is quite low, 25-35 % (Hristov et al., 2004). In addition to the economic burden on
41 the producer, urinary nitrogen losses contribute to greenhouse gas emissions and waterway
42 contamination (Dijkstra et al., 2013). Milk protein synthesis is sensitive to essential amino
43 acid supply, particularly phenylalanine (PHE). *In vivo* studies have demonstrated a reduction
44 in milk protein concentrations when PHE is in low dietary supply (Rulquin and Pisulewski,
45 2000) and/or deficient in infusion mixtures (Doelman et al., 2015; Doepel et al., 2016). In
46 the post-absorptive state, the liver has a key role in regulating PHE homeostasis. The liver
47 can regulate the provision of PHE to peripheral tissues including the mammary gland, and
48 remove PHE not used by the mammary gland or other peripheral tissues.

49 Net flux of PHE across the bovine liver has been examined *in vivo* using the arterio-
50 venous difference technique (Tagari et al., 2004, 2008; Raggio et al., 2007; Berthiaume et al.,
51 2006; Cantalapiedra-Hijar et al., 2014; Larsen et al., 2015). These studies reported a negative
52 net flux of PHE, suggesting the liver is a major site of PHE utilization. In general, PHE has
53 two metabolic fates, incorporation into protein or conversion to tyrosine (TYR) via PHE
54 hydroxylase (oxidation). As a result, PHE catabolism always follows the pathway of TYR
55 catabolism. Therefore, to examine PHE metabolism experimentally the simultaneous
56 infusion of PHE and TYR stable isotope tracers is preferred. To resolve the kinetic data from
57 such isotope infusion studies, mathematical models must be applied.

58 Mathematical models to describe PHE kinetics have varied in complexity (reviewed
59 by Matthews, 2007). Simple models ignore the hydroxylation of PHE to TYR, whereas,
60 more complex models aim to explain PHE conversion to TYR. In our previous publication
61 (Crompton et al., 2014), we presented an eight pool compartmental model describing PHE
62 and TYR metabolism in the mammary gland of lactating dairy cows. The present study is a

63 progression of this work and our previous model of leucine metabolism in the bovine liver
64 (France et al., 1998). The primary objective was to develop a steady state model of hepatic
65 PHE and TYR metabolism. The new model describes the partitioning of the PHE and TYR
66 between constitutive and export protein synthesis and potential other metabolic fates such as
67 hydroxylation of PHE to TYR.

68

69 **2. The model**

70 The scheme adopted is shown in Figure 1a. It contains four intracellular and six
71 extracellular pools. The intracellular pools are free PHE (pool 6), PHE in export protein
72 (pool 5), free TYR (pool 7) and TYR in export protein (pool 8), while the extracellular ones
73 represent portal vein PHE and TYR (pools 1 and 3), hepatic artery PHE and TYR (pools 2
74 and 4) and venous PHE and TYR (pools 9 and X). Pools external to the model and therefore
75 not specifically represented are indicated by the digit zero. The flows of PHE and TYR
76 between pools and into and out of the system are shown as arrowed lines.

77 The export protein-bound PHE pool has a single inflow: from free PHE, F_{56} , and two
78 effflows: secretion of export protein, F_{05} , and degradation, F_{65} . The intracellular free PHE
79 pool has four inflows: from the degradation of constitutive liver protein, F_{60} , from the
80 extracellular portal vein pool, F_{61} , from the hepatic artery pool, F_{62} , and from degradation of
81 export protein, F_{65} . The pool has four effflows: synthesis of constitutive liver protein, F_{06} ,
82 incorporation into export protein, F_{56} , hydroxylation to the intracellular free TYR pool, F_{76} ,
83 and outflow to the extracellular hepatic vein PHE pool, F_{96} . The intracellular free TYR pool
84 has five inflows: from the degradation of constitutive liver protein, F_{70} , from the extracellular
85 portal vein TYR pool, F_{73} , from the hepatic artery TYR pool, F_{74} , from the intracellular PHE
86 pool, F_{76} , and from the degradation of export protein, F_{78} . The pool has four effflows:
87 oxidation and TYR degradation products, $F_{07}^{(o)}$, synthesis of constitutive liver protein, $F_{07}^{(s)}$,

88 incorporation into export protein, F_{87} , and outflow to the extracellular hepatic vein TYR pool,
89 F_{X7} . The export protein-bound TYR pool has one inflow: from the intracellular free TYR
90 pool, F_{87} , and two efflows: secretion of export protein, F_{08} , and degradation, F_{78} .

91 The extracellular portal vein PHE pool has a single inflow: entry into the pool, F_{10} , and
92 two efflows: uptake by the liver, F_{61} , and bypass to the extracellular hepatic vein PHE pool,
93 F_{91} . The extracellular hepatic artery PHE pool has a single inflow: entry into the pool, F_{20} ,
94 and two efflows: uptake by the liver, F_{62} , and bypass to the extracellular hepatic vein PHE
95 pool, F_{92} . The same description applies to the corresponding TYR pools, i.e. pools 3 and 4
96 with flows F_{30} , F_{73} , F_{X3} , F_{40} , F_{74} , and F_{X4} , respectively. The extracellular hepatic vein PHE
97 pool has three inflows: bypass from the portal vein PHE pool, F_{91} , bypass from the hepatic
98 artery PHE pool, F_{92} , and release from the intracellular PHE pool, F_{96} , and one efflow from
99 the system, F_{09} . The same description applies to the corresponding TYR pool with flows F_{X3} ,
100 F_{X4} , F_{X7} , and F_{0X} respectively.

101 The schemes adopted for the movement of label are shown in Figures 1b and 1c.
102 Labelled [$1-^{13}\text{C}$]PHE and [$2,3,5,6-^2\text{H}$]TYR were infused into the jugular vein at a constant
103 rate for 8 h.. The enrichments of the extracellular pools are measured directly by taking
104 blood samples from the portal vein, hepatic artery and hepatic vein during the isotope
105 infusion. The enrichments of the intracellular pools can only be measured directly using
106 invasive procedures and to avoid such procedures, the enrichments of the intracellular pools
107 were set as a prescribed fraction of the corresponding arterial enrichment in this study. Blood
108 flow rate across the liver is measured by downstream dye dilution using *para*-amino hippuric
109 acid (PAH), but uncorrected for any N-acetylation that occurs across the liver (Katz and
110 Bergman, 1969). The scheme assumes that the only entry of label into the system is into the
111 PHE and TYR portal vein and hepatic artery pools via the effective infusion rates, flows I_1 ,

112 $I_2, I_3, \Phi_3, I_4,$ and Φ_4 and that the duration of the infusion is such that no label arises from the
 113 breakdown of constitutive protein.

114 Conservation of mass principles can be applied to each pool in Figure 1a, b, c to generate
 115 differential equations that describe the dynamic behaviour of the system. For total (isotopic
 116 plus non-isotopic) PHE and TYR, these fundamental equations are (mathematical notation is
 117 defined in Table 1):

$$118 \quad \frac{dQ_1}{dt} = F_{10} - F_{61} - F_{91} \quad (1)$$

$$119 \quad \frac{dQ_2}{dt} = F_{20} - F_{62} - F_{92} \quad (2)$$

$$120 \quad \frac{dQ_3}{dt} = F_{30} - F_{73} - F_{X3} \quad (3)$$

$$121 \quad \frac{dQ_4}{dt} = F_{40} - F_{74} - F_{X4} \quad (4)$$

$$122 \quad \frac{dQ_5}{dt} = F_{56} - F_{05} - F_{65} \quad (5)$$

$$123 \quad \frac{dQ_6}{dt} = F_{60} + F_{61} + F_{62} + F_{65} - F_{06} - F_{56} - F_{76} - F_{96} \quad (6)$$

$$124 \quad \frac{dQ_7}{dt} = F_{70} + F_{73} + F_{74} + F_{76} + F_{78} - F_{07}^{(a)} - F_{07}^{(s)} - F_{87} - F_{X7} \quad (7)$$

$$125 \quad \frac{dQ_8}{dt} = F_{87} - F_{08} - F_{78} \quad (8)$$

$$126 \quad \frac{dQ_9}{dt} = F_{91} + F_{92} + F_{96} - F_{09} \quad (9)$$

$$127 \quad \frac{dQ_X}{dt} = F_{X3} + F_{X4} + F_{X7} - F_{0X} \quad (10)$$

128 and for [^{13}C] labelled PHE and TYR:

$$129 \quad \frac{dq_1}{dt} = I_1 - e_1 (F_{61} + F_{91}) \quad (11)$$

$$130 \quad \frac{dq_2}{dt} = I_2 - e_2(F_{62} + F_{92}) \quad (12)$$

$$131 \quad \frac{dq_3}{dt} = I_3 - e_3(F_{73} + F_{X3}) \quad (13)$$

$$132 \quad \frac{dq_4}{dt} = I_4 - e_4(F_{74} + F_{X4}) \quad (14)$$

$$133 \quad \frac{dq_5}{dt} = e_6 F_{56} - e_5(F_{05} + F_{65}) \quad (15)$$

$$134 \quad \frac{dq_6}{dt} = e_1 F_{61} + e_2 F_{62} + e_5 F_{65} - e_6(F_{06} + F_{56} + F_{76} + F_{96}) \quad (16)$$

$$135 \quad \frac{dq_7}{dt} = e_3 F_{73} + e_4 F_{74} + e_6 F_{76} + e_8 F_{78} - e_7(F_{07}^{(o)} + F_{07}^{(s)} + F_{87} + F_{X7}) \quad (17)$$

$$136 \quad \frac{dq_8}{dt} = e_7 F_{87} - e_8(F_{08} + F_{78}) \quad (18)$$

$$137 \quad \frac{dq_9}{dt} = e_1 F_{91} + e_2 F_{92} + e_6 F_{96} - e_9 F_{09}$$

$$138 \quad (19)$$

$$139 \quad \frac{dq_X}{dt} = e_3 F_{X3} + e_4 F_{X4} + e_7 F_{X7} - e_X F_{0X} \quad (20)$$

140 and for [²H] labelled TYR:

$$141 \quad \frac{d\phi_3}{dt} = \Phi_3 - \varepsilon_3(F_{73} + F_{X3})$$

$$142 \quad (21)$$

$$143 \quad \frac{d\phi_4}{dt} = \Phi_4 - \varepsilon_4(F_{74} + F_{X4}) \quad (22)$$

$$144 \quad \frac{d\phi_7}{dt} = \varepsilon_3 F_{73} + \varepsilon_4 F_{74} + \varepsilon_8 F_{78} - \varepsilon_7(F_{07}^{(o)} + F_{07}^{(s)} + F_{87} + F_{X7}) \quad (23)$$

$$145 \quad \frac{d\phi_8}{dt} = \varepsilon_7 F_{87} - \varepsilon_8(F_{08} + F_{78}) \quad (24)$$

$$146 \quad \frac{d\phi_X}{dt} = \varepsilon_3 F_{X3} + \varepsilon_4 F_{X4} + \varepsilon_7 F_{X7} - \varepsilon_X F_{0X} \quad (25)$$

147 When the system is in steady state with respect to both total and labelled PHE and TYR,
148 the derivative terms in equations (1)-(25) are zero. For the scheme assumed, the enrichment
149 of intracellular export protein-bound pools equalizes with that of the respective free pool in
150 steady state (i.e. $e_5 = e_6$, $e_8 = e_7$, $\varepsilon_8 = \varepsilon_7$) otherwise equations (5) and (15), and (8), (18) and
151 (24) are inconsistent. It should be noted that the liver is a heterogeneous mixture of cells, and
152 enrichment may differ at different parts of the organ where synthesis of export protein or
153 synthesis of constitutive protein takes place. Within cells there are different sites of synthesis
154 and oxidation, and differences in the sites of synthesis of individual proteins, with labelling of
155 the tracer amino acid highest at points near the cell surface and lowest at points near the sites
156 of degradation (see Waterlow, 2006). After equating intracellular enrichments and
157 eliminating redundant equations etc., equations (11) to (25) yield the following useful
158 identities:

$$159 \quad I_1 - e_1(F_{61} + F_{91}) = 0 \quad (26)$$

$$160 \quad I_2 - e_2(F_{62} + F_{92}) = 0 \quad (27)$$

$$161 \quad e_1F_{61} + e_2F_{62} - e_6(F_{06} + F_{56} + F_{76} + F_{96} - F_{65}) = 0 \quad (28)$$

$$162 \quad e_3F_{73} + e_4F_{74} + e_6F_{76} - e_7(F_{07}^{(o)} + F_{07}^{(s)} + F_{87} + F_{X7} - F_{78}) = 0 \quad (29)$$

$$163 \quad e_1F_{91} + e_2F_{92} + e_6F_{96} - e_9F_{09} = 0 \quad (30)$$

$$164 \quad e_3F_{X3} + e_4F_{X4} + e_7F_{X7} - e_XF_{0X} = 0 \quad (31)$$

$$165 \quad \Phi_3 - \varepsilon_3(F_{73} + F_{X3}) = 0 \quad (32)$$

$$166 \quad \Phi_4 - \varepsilon_4(F_{74} + F_{X4}) = 0 \quad (33)$$

$$167 \quad \varepsilon_3F_{73} + \varepsilon_4F_{74} - \varepsilon_7(F_{07}^{(o)} + F_{07}^{(s)} + F_{87} + F_{X7} - F_{78}) = 0 \quad (34)$$

$$168 \quad \varepsilon_3F_{X3} + \varepsilon_4F_{X4} + \varepsilon_7F_{X7} - \varepsilon_XF_{0X} = 0 \quad (35)$$

169 To obtain steady state solutions to the model, it is assumed that PHE and TYR secreted
170 in export protein, CO₂ production and PHE and TYR removal from the hepatic vein pools
171 (i.e. F_{05} , F_{08} , $F_{07}^{(o)}$, F_{09} and F_{0X} , respectively) can all be measured experimentally. Further, it
172 is mathematically convenient to assume that percentage PHE extraction by the liver is the
173 same from the portal vein and hepatic artery supplies, giving:

$$174 \quad \frac{F_{91}}{F_{92}} \left(= \frac{F_{61}}{F_{62}} \right) = \frac{F_{10}}{F_{20}} \quad (36)$$

175 Algebraic manipulation of equations (1)-(10) with the derivatives set to zero, together
176 with equations (26)-(36), gives the following unique solution:

$$177 \quad F_{10} = I_1 / e_1 \quad (37)$$

$$178 \quad F_{20} = I_2 / e_2 \quad (38)$$

$$179 \quad F_{30} = \Phi_3 / \varepsilon_3 \quad (39)$$

$$180 \quad F_{40} = \Phi_4 / \varepsilon_4 \quad (40)$$

$$181 \quad \overline{F_{56} - F_{65}} = \tilde{F}_{05} \quad (41)$$

$$182 \quad \overline{F_{87} - F_{78}} = \tilde{F}_{08} \quad (42)$$

$$183 \quad F_{X7} = \frac{(e_4 - e_3)(\varepsilon_X - \varepsilon_3) - (e_X - e_3)(\varepsilon_4 - \varepsilon_3)}{(e_4 - e_3)(\varepsilon_7 - \varepsilon_3) - (e_7 - e_3)(\varepsilon_4 - \varepsilon_3)} \tilde{F}_{0X} \quad (43)$$

$$184 \quad F_{X4} = \frac{(\varepsilon_X - \varepsilon_3) \tilde{F}_{0X} - (\varepsilon_7 - \varepsilon_3) F_{X7}}{(\varepsilon_4 - \varepsilon_3)} \quad (44)$$

$$185 \quad F_{X3} = \tilde{F}_{0X} - F_{X4} - F_{X7} \quad (45)$$

$$186 \quad F_{73} = F_{30} - F_{X3} \quad (46)$$

$$187 \quad F_{74} = F_{40} - F_{X4} \quad (47)$$

$$188 \quad F_{07}^{(s)} = \frac{\varepsilon_3}{\varepsilon_7} F_{73} + \frac{\varepsilon_4}{\varepsilon_7} F_{74} - \tilde{F}_{07}^{(o)} - \overline{F_{87} - F_{78}} - F_{X7} \quad (48)$$

$$F_{76} = \frac{e_7}{e_6} \left(\widetilde{F}_{07}^{(o)} + F_{07}^{(s)} + \overline{F_{87} - F_{78}} + F_{X7} \right) - \frac{e_3}{e_6} F_{73} - \frac{e_4}{e_6} F_{74} \quad (49)$$

$$F_{70} = \widetilde{F}_{07}^{(o)} + F_{07}^{(s)} + \overline{F_{87} - F_{78}} + F_{X7} - F_{73} - F_{74} - F_{76} \quad (50)$$

$$F_{91} = \frac{(e_9 - e_6) \widetilde{F}_{09}}{(e_1 - e_6) + (e_2 - e_6) \frac{F_{20}}{F_{10}}} \quad (51)$$

$$F_{92} = \frac{F_{20}}{F_{10}} F_{91} \quad (52)$$

$$F_{96} = \widetilde{F}_{09} - F_{91} - F_{92} \quad (53)$$

$$F_{61} = F_{10} - F_{91} \quad (54)$$

$$F_{62} = F_{20} - F_{92} \quad (55)$$

$$F_{06} = \frac{e_1}{e_6} F_{61} + \frac{e_2}{e_6} F_{62} - \overline{F_{56} - F_{65}} - F_{76} - F_{96} \quad (56)$$

$$F_{60} = F_{06} + \overline{F_{56} - F_{65}} + F_{76} + F_{96} - F_{61} - F_{62} \quad (57)$$

198 where for these equations the italics denote steady state values of flows and enrichments, the
 199 tilde identifies a measured flow, and the over-lining (not to be confused with statistical mean)
 200 indicates coupled flows (which cannot be separately estimated within the model). Equations
 201 (37)-(57) were obtained through analytical solution (solving by hand) using conventional
 202 linear algebra.

203

204 **3. Application**

205 Application of the model is illustrated using data from an experiment conducted in the
 206 UK at the University of Reading with multi-catheterised mid-lactation Holstein-Friesian dairy
 207 cows (average live-weight 667 kg). Animals were fed hourly by auto-feeders total mixed
 208 ration (TMR) diets consisting of a 50:50 mixture on a dry matter basis of forage and
 209 concentrate with the forage comprised of grass silage and chopped dried Lucerne in a 25:75

210 ratio on a dry matter basis. Concentrates were formulated to provide crude protein levels of
211 approximately 110 and 200 g per kg concentrate dry matter, such that average TMR crude
212 protein concentrations were 128 and 175 g/kg dry matter. Average daily dry matter intake
213 and milk yield for these animals were 22 kg/d and 30 L/d, respectively. The cows were given
214 constant abomasal infusions of water (18 L/d) for 4 d, followed by a buffered mixture of
215 essential amino acids for a further 6 d. The essential amino acids were administered at a
216 daily rate equivalent to the essential amino acids in 800 g milk protein. On the final day of
217 each abomasal infusion, animals received a primed, continuous infusion at a constant rate
218 into the jugular vein of [$1\text{-}^{13}\text{C}$]PHE (350 mg/h) and [$2,3,5,6\text{-}^2\text{H}$]TYR (100 mg/h) in sterile
219 saline for 8 h. Blood samples were taken simultaneously from catheters in the dorsal aorta
220 and the portal and hepatic veins at hourly intervals for the duration of the infusion for the
221 measurement of blood flow rate (by PAH dilution) and nutrient metabolism by the portal
222 drained viscera and liver. The final four blood samples taken 5-8 h after the infusion started
223 were averaged to provide steady state values.

224 The relevant experimental measurements are given in Table 2. They are reported for
225 three animals during the water infusion (2 low protein; 1 high protein diet) and one animal
226 during the amino acid infusion (high protein diet). Values are based on plasma rather than
227 whole blood. Phenylalanine and TYR measurements are based on free rather than total (i.e.
228 free plus bound) plasma PHE and TYR. The effective isotope infusion rates to the liver, I_1 ,
229 I_2 , and I_3 , Φ_3 , I_4 , Φ_4 were obtained from portal vein and arterial concentration and enrichment
230 of PHE and TYR and plasma flow rate in the portal vein and hepatic artery. The flows F_{09}
231 and F_{0X} were determined from hepatic vein PHE and TYR concentration and plasma flow
232 rate in the hepatic vein. The intracellular enrichments e_6 , e_7 and ε_7 and the flows F_{05} , F_{08}
233 were not measured in the trial and had to be prescribed. Unpublished observations from our
234 laboratories demonstrated an intracellular to extracellular enrichment ratio of 0.3 for PHE and

235 TYR. Therefore, the missing intracellular free PHE and TYR enrichments, e_6 , e_7 , and ε_7 were
 236 initially calculated as 0.3 times the corresponding arterial enrichments e_2 , e_4 , and ε_4
 237 respectively. The export protein flows F_{05} and F_{08} were assigned values of 33.8 $\mu\text{mol}/\text{min}$
 238 and 25.0 $\mu\text{mol}/\text{min}$ respectively based on Raggio et al. (2007) and the relative proportion of
 239 PHE and TYR in bovine serum albumin as a representative export protein (UniProt, 2017).
 240 The flow $F_{07}^{(e)}$ was obtained from labelled CO_2 elevation in plasma flow across the liver and
 241 hepatic vein PHE enrichment in sheep (Harris et al., 1992).

242 Calculated flows are presented in Table 3. Data provided were insufficient for
 243 comprehensive statistical analysis; therefore the results must be interpreted with a degree of
 244 caution. Initial solutions for each cow were non-physiological as some of the derived flows
 245 gave negative values. Therefore, error bands of $\pm 25\%$ were placed around the values given
 246 in Table 2 of the prescribed intracellular enrichments (e_6 , e_7 and ε_7) and the measured
 247 extracellular enrichments close to minimum detection levels (e_3 , e_4 and e_X), and the solution
 248 space mapped out by these bands was searched to find the best feasible solution for each cow.
 249 The best feasible solution was obtained by sum of squares minimisation, where the sum of
 250 squares to be minimised (SS) was defined as

$$251 \quad \text{SS} = \left(1 - \frac{F_{60}}{F_{06}}\right)^2 + \left(1 - \frac{F_{70}}{F_{07}^{(s)}}\right)^2 + \left(\frac{F_{07}^{(s)}}{F_{06}} - \frac{F_{70}}{F_{60}}\right)^2$$

252 The assumptions underlying SS are constitutive liver protein synthesis and degradation are
 253 moving towards equilibrium (as the cows were in mid-lactation), and the TYR to PHE ratio is
 254 the same (or similar) in synthesised and degraded liver tissue. If no feasible solution could be
 255 found, the error bands were expanded (up to $\pm 40\%$) and the search repeated. The final
 256 solutions are shown in Table 3.

257 An analysis of measurement errors in experimental enrichments and infusion rates on
 258 model solutions was conducted. Mean values across all datasets were assigned to e_1 , e_2 , e_3 ,

259 $e_4, e_6, e_7, e_9, e_x, \varepsilon_3, \varepsilon_4, \varepsilon_7, \varepsilon_x, I_1, I_2, \Phi_3, \Phi_4, F_{05}, F_{07}^{(o)}, F_{08}, F_{09}, F_{0X}$. Inputs were then perturbed
260 in turn by 0, $\pm 10\%$ and $\pm 20\%$. Each calculated flow (y , $\mu\text{mol}/\text{min}$) was then plotted against
261 the perturbation (x , %), and a five-point linear regression of y on x performed to determine
262 the slope of the line produced. The average slope was subsequently scaled by its
263 corresponding unperturbed average flow value, giving the scaled slopes dimensions of %
264 change in y per % change in x . Results of the error assessment are presented in Table 4. In
265 general, errors in infusion rates and measured flows had little impact on the sensitivity of
266 model solutions. However, errors in the measurement of isotopic enrichment and in assumed
267 intracellular enrichment values, cause marked changes in calculated flows, emphasising the
268 value of measuring intracellular enrichment directly.

269

270 **5. Discussion**

271 Improving nitrogen utilization in the ruminant, and consequently minimizing the
272 environmental impacts of ruminant production, is dependent on a clear understanding of post-
273 absorptive amino acid metabolism. The present model described the partitioning of the
274 indispensable amino acid, PHE (and TYR), in the bovine liver. The model gave estimates of
275 PHE flow across the liver, rates of PHE and TYR incorporation into constitutive and export
276 protein synthesis, and the rate of hydroxylation of PHE to TYR.

277 PHE and TYR enter the liver via the portal vein and hepatic artery. Amino acids
278 entering via the portal vein, the main blood supply to liver, represent recently absorbed or
279 released amino acids from the portal drained viscera and recirculating amino acids coming
280 from the mesenteric and hepatic arteries. Incoming amino acids via arterial blood reflect the
281 utilization of nutrients by peripheral tissues (Reynolds, 2006). The model predicted a greater
282 flow of PHE and TYR from the portal vein (90% of total inflow) than that from the hepatic

283 artery, which was expected as the portal vein accounted for the majority (84%) of liver blood
284 flow.

285 *In vivo* studies have observed a negative net flow of PHE across the bovine liver,
286 suggesting the liver is a major site of catabolism (Tagari et al., 2004, 2008; Reynolds, 2006;
287 Raggio et al., 2007; Berthiaume et al., 2006; Cantalapiedra-Hijar et al., 2014; Larsen et al.,
288 2015). In agreement, model solutions indicated a net negative flow of PHE and TYR across
289 the liver (efflow minus inflow, -396 and -298 $\mu\text{mol}/\text{min}$ PHE and TYR respectively). Other
290 efflows of PHE and TYR from the liver included oxidation and incorporation into secreted
291 hepatic proteins. Oxidation rates varied greatly in individual animals (range 146 to 525
292 $\mu\text{mol}/\text{min}$), the highest value observed in the animal receiving the essential amino acid
293 infusion. The range of oxidation rates in animals receiving the control water infusion were
294 similar to those previously reported in lactating dairy cows (Raggio et al., 2007) fed low and
295 high protein diets. Oxidation rates used in the present model represent the sum of PHE and
296 TYR oxidation and combined PHE and TYR oxidation accounted for 23% of total PHE and
297 TYR inflow to the liver. However, the current model enables the conversion of PHE to TYR
298 to be estimated (F_{76}) which enables the combined oxidation to be separated. Phenylalanine
299 may either be used for protein synthesis or be converted to TYR which is catalysed by the
300 enzyme phenylalanine hydroxylase and is the first and rate limiting step in the metabolic
301 disposal of PHE. Tyrosine is then transaminated to *p*-hydroxyphenylpyruvate and ultimately
302 yields fumarate and acetoacetate, so that PHE and TYR give rise to both glucogenic and
303 ketogenic fragments. Tyrosine is not considered essential, because it can be synthesised from
304 PHE in addition to that provided from the diet. On average the model estimated that 23% of
305 inflow of PHE from blood (sum of hepatic artery and portal vein inflows) to the liver was
306 converted to TYR. Of the 178 $\mu\text{mol}/\text{min}$ of PHE hydroxylated, 101 $\mu\text{mol}/\text{min}$ were directly
307 oxidised and 77 $\mu\text{mol}/\text{min}$ contributed 11% of the 717 $\mu\text{mol}/\text{min}$ total TYR inflow (sum of

308 TYR from hepatic artery and portal vein and from PHE hydroxylation). Individual amino
309 acid oxidations as a percentage of liver inflow were estimated to be 13% for PHE and 29%
310 for TYR. Measuring TYR oxidation directly represents a challenge experimentally. In
311 general, deuterium labelled PHE is inadequate for quantifying PHE kinetics and oxidation,
312 which requires the use of carbon labelled PHE and by default, deuterium labelled TYR
313 (reviewed by Matthews, 2007).

314 Another efflow of PHE and TYR considered in the model was their incorporation into
315 hepatic export protein. The liver makes several export proteins of various functions.
316 Albumin is important for the maintenance of vascular osmotic pressure. Due to this critical
317 role, albumin synthesis is maintained across various mild physiological challenges, although
318 in more severe situations of nutrient shortage, albumin synthesis may decrease. Raggio et al.
319 (2007) determined export plasma protein synthesis rate in lactating dairy cows and assumed
320 that export plasma protein contained 5% PHE. The value thus obtained by Raggio et al.
321 (2007) was used in the present model to represent the flow of PHE into export protein (F_{05}).
322 Since plasma proteins are also derived from sources other than the liver, the assumption that
323 total plasma export protein synthesis is all hepatic in origin may well have overestimated the
324 flow of PHE into export protein to some degree in the current model. The incorporation of
325 TYR (F_{08}) into albumin was unknown and assumed equivalent to that of PHE after correcting
326 for the relative proportions of PHE and TYR in albumin. This assumption is recognized as a
327 limitation of the current modelling exercise. Specifically, the incorporation of individual
328 amino acids depends on the type of export protein; some export proteins may require
329 proportionally higher amounts of PHE or TYR. This limitation was circumvented in our
330 bovine mammary model (Crompton et al., 2014) as the export protein was milk and isotope
331 enrichment in milk was easily measured.

332 A unique feature of the present model is the description of intracellular PHE and TYR
333 partitioning. Constitutive hepatic protein degradation was the largest contributor to the free
334 PHE and TYR pools, followed by portal vein and hepatic artery delivery (PHE 72%, 22%,
335 6%; TYR 65%, 22%, 5%). It should be noted that these estimates are influenced by
336 assumptions which had to be made with respect to intracellular enrichments, as discussed
337 later. Flow of PHE and TYR into constitutive protein synthesis comprised 98% of total
338 synthetic flow. The ratio of PHE to TYR in synthesised constitutive protein and degraded
339 constitutive protein was the same in each animal (range 1.06 to 1.66) and averaged 1.30. The
340 ratio of constitutive protein synthesis to degradation was 1.1 for both PHE and TYR.
341 However, in mid to late lactation dairy cows, the ratio of PHE and TYR synthesis to
342 degradation should be equal. Model estimates of intracellular PHE and TYR partitioning
343 must be interpreted with caution due to methodological limitations and imposed assumptions.
344 For example, samples were taken from whole blood and free PHE and TYR concentrations
345 were quantified and considered in the model. Therefore the extent to which peptide bound
346 PHE and TYR contribute to constitutive protein synthesis and degradation flows cannot be
347 determined. As hepatic tissues were not sampled, isotopic enrichment of the intracellular
348 pools was estimated based on the sampled precursor pools. The choice of precursor pool
349 enrichment is central in the present model as with any measurement of protein synthetic rate
350 in the whole body or tissues (see Waterlow, 2006). The assumption that intracellular
351 enrichments were 0.30 of plasma was based on unpublished observations from an unrelated
352 in vivo trial from our laboratory. Using the same isotopes as the present study, average liver
353 homogenate free enrichment for PHE and TYR was 0.30 (SD 0.07; range 0.19-0.38) of
354 plasma enrichment in lactating dairy cattle. Initially, the model solved to give some non-
355 physiological flows and had to be re-solved by adjusting the intracellular enrichments and the
356 extracellular ones close to minimum detection levels using a least-squares minimisation

357 routine. Besides, the actual precursor pool for various constitutive and export proteins may
358 differ. The lack of homogeneity in activity at various parts of the liver indicates that
359 intracellular enrichment is not uniform throughout the liver, and enrichment in export protein
360 and constitutive protein therefore may differ. In particular, the precursor enrichment for
361 constitutive protein synthesis may be lower than that for export protein synthesis (Connell et
362 al., 1997). Due to lack of specific measurements, this observation could not be included in
363 the present model.

364 Another means of estimating intracellular enrichment would be to measure the
365 incorporation of label into proteins having a fast turnover rate in plasma. For example,
366 studies in humans use plasma VLDL apolipoprotein-B (Apo-B) enrichment as a hepatic
367 intracellular marker protein (Rafi et al., 2008). However, to our knowledge this approach has
368 not been undertaken in lactating dairy cattle and would likely yield the enrichment of the
369 precursor pool used for export protein synthesis, which is not necessarily the same as the
370 precursor pool for constitutive protein synthesis as discussed above.

371

372 **5. Conclusions**

373 The present 10-pool model allows for quantitative interpretation of the metabolism of PHE
374 and TYR in the liver of dairy cattle by showing their partition among different fates. The
375 model builds upon the existing eight pool model to describe protein turnover in the mammary
376 gland of the lactating dairy cow (Crompton et al., 2014). The exchange of PHE and TYR
377 between extracellular and intracellular pools was described and the hydroxylation of PHE to
378 TYR was estimated. In addition, the extraction of amino acids from the liver via the portal
379 vein and hepatic artery pools was represented. Upon application to dairy cattle during
380 infusion of [1-¹³C] phenylalanine and [2,3,5,6-²H] tyrosine tracers, the model was shown to
381 be effective in providing information about the fates of PHE and TYR in the liver, and could

382 be used as part of a more complex system describing amino acid metabolism in the whole
383 animal.

384 Just as accurate measurements of precursor pool enrichment are critical for the use of
385 labelled amino acids to determine tissue protein synthesis generally, the measurement of
386 intracellular free PHE and TYR enrichment are crucial components of the present model, yet
387 had to be estimated along with liver export protein in order to apply the model using the data
388 available from the experiment. If the model is to be applied more rigorously, future *in vivo*
389 studies should measure simultaneously the intracellular free PHE and TYR pools and liver
390 export protein, along with the plasma enrichments and flow rates measured in the present
391 work. Furthermore, the model could not be solved by algebraic means alone, as initially it
392 solved to give non-physiological flows and had to be re-solved by adjusting selected
393 enrichments using a least-squares minimisation routine. Therefore, the mathematical exercise
394 of cleaving the combined model and solving the PHE and TYR sub-models independently
395 but sequentially merits future investigation.

396

397 **Acknowledgements**

398 This work was funded, in part, through DEFRA project LS3656, the European Union FP-7
399 REDNEX Project and the Canada Research Chairs Program. The experimental work was
400 funded by a consortium of DEFRA, BBSRC, the Milk Development Council, Purina Mills
401 LLC, and NUTRECO Inc.

402

403 **References**

- 404 Berthiaume, R., Thivierge, M.C., Patton, R.A., Dubreuil, P., Stevenson, M., McBride, B.W.,
405 Lapierre, H., 2006. Effect of ruminally protected methionine on splanchnic metabolism of
406 amino acids in lactating dairy cows. *J. Dairy Sci.* 89, 1621-1634.
- 407 Cantalapiedra-Hijar, G., Lemosquet, S., Rodriguez-Lopez, J.M., Messad, F., Ortigues-Marty,
408 I., 2014. Diets rich in starch increase the posthepatic availability of amino acids in dairy
409 cows fed diets at low and normal protein levels. *J. Dairy Sci.* 97, 5151-5166.
- 410 Connell, A., Calder, A.G., Anderson, S.E., Lobley, G.E., 1997. Hepatic protein synthesis in
411 the sheep: effect of intake as monitored by use of stable-isotope-labelled glycine, leucine
412 and phenylalanine. *Br. J. Nutr.* 77,255-271.
- 413 Crompton, L.A., France, J., Reynolds, C.K., Mills, J.A.N., Hanigan, M.D., Ellis, J.L.,
414 Bannink, A., Bequette, B.J., Dijkstra, J., 2014. An isotope dilution model for partitioning
415 phenylalanine and tyrosine uptake by the mammary gland of lactating dairy cows. *J.*
416 *Theor. Biol.* 359, 54-60.
- 417 Dijkstra, J., Oenema, O., Van Groenigen, J.W., Spek, J.W., van Vuuren, A.M., Bannink, A.,
418 2013. Diet effects on urine composition of cattle and N₂O emissions. *Animal* 7 (suppl. 2),
419 292-302.

420 Doelman, J., Curtis, R.V., Carson, M., Kim, J.J.M., Metcalf, J.A., Cant, J.P., 2015. Essential
421 amino acid infusions stimulate mammary expression of eukaryotic initiation factor 2B ϵ but
422 milk protein yield is not increased during an imbalance. *J. Dairy Sci.* 98:4499-4508.

423 Doepel, L., Hewage, I.I., Lapierre, H., 2016. Milk protein yield and mammary metabolism
424 are affected by phenylalanine deficiency but not by threonine or tryptophan deficiency. *J.*
425 *Dairy Sci.* 99, 3144-3156.

426 France, J., Hanigan, M.D., Reynolds C.K., Dijkstra J., Crompton, L.A., Maas J.A., Bequette,
427 B.J., Metcalf J.A., Lobley, G.E., MacRae, J.C., Beever, D.E., 1998. An isotope dilution
428 model for partitioning leucine uptake by the bovine liver. *J. Theor. Biol.* 198, 121-133.

429 Harris, P.A., Skene, P.A., Buchan, V., Milne, E., Calder, A.G., Anderson, S.E., Connell, A.,
430 Lobley, G.E., 1992. Effect of food intake on hind-limb and whole-body protein
431 metabolism in young growing sheep: chronic studies based on arterio-venous techniques.
432 *Br. J. Nutr.* 68, 389-407.

433 Hristov, A.N., Price, W.J., Shafii, B., 2004. A meta-analysis examining the relationship
434 among dietary factors, dry matter intake, and milk and milk protein yield in dairy cows. *J.*
435 *Dairy Sci.* 87, 2184-2196.

436 Katz, M.L., Bergman, E.N., 1969. Simultaneous measurements of hepatic and portal venous
437 blood flow in the sheep and dog. *Am. J. Physiol.* 216, 946-952.

438 Larsen, M., Galindo, C., Ouellet, D.R., Maxin, G., Kristensen, N.B., Lapierre, H., 2015.
439 Abomasal amino acid infusion in postpartum dairy cows: Effect on whole-body,
440 splanchnic, and mammary amino acid metabolism. *J. Dairy Sci.* 98, 7944-7961.

441 Matthews, D.E., 2007. An overview of phenylalanine and tyrosine kinetics in humans. *J.*
442 *Nutr.* 137, 1549S-1555S.

443 Rafei, M., McKenzie, J.M., Roberts, S.A., Steiner, G., Ball, R.O., Pencharz, P.B., 2008. *In*
444 *vivo* regulation of phenylalanine hydroxylation to tyrosine, studied using enrichment in

445 apoB-100 In vivo regulation of phenylalanine hydroxylation to tyrosine, studied using
446 enrichment in apoB-100. *Am J Physiol Endocrinol Metab*, 294(2), E475-E479.

447 Raggio, G., Lobley, G.E., Berthiaume, R., Pellerin, D., Allard, G., Dubreuil, P., Lapierre, H.,
448 2007. Effect of protein supply on hepatic synthesis of plasma and constitutive proteins in
449 lactating dairy cows. *J. Dairy Sci.* 90, 352-359.

450 Reynolds, C.K., 2006. Splanchnic metabolism of amino acids in ruminants, in: Sejrsen, K.,
451 Hvelplund, T., Nielsen, M.O., (Eds), *Ruminant Physiology: Digestion, metabolism and*
452 *impact of nutrition on gene expression, immunology and stress.* Wageningen Academic
453 Press, Wageningen, the Netherlands, pp. 225-248.

454 Rulquin, H., Pisulewski, P., 2000. Effects of duodenal infusions of graded amounts of Phe on
455 mammary uptake and metabolism in dairy cows. *J. Dairy Sci.* 83(Suppl. 1), 267-268.

456 Tagari, H., Webb Jr., K., Theurer, B., Huber, T., DeYoung, D., Cuneo, P., Santos, J.E.P.,
457 Simas, J., Sadik, M., Alio, A., Lozano, O., Delgado-Elorduy, A., Nussio, L., Nussio, C.,
458 Santos, F., 2004. Portal drained visceral flux, hepatic metabolism, and mammary uptake of
459 free and peptide-bound amino acids and milk amino acid output in dairy cows fed diets
460 containing corn grain steam flaked at 360 or steam rolled at 490 g/L. *J. Dairy Sci.* 87, 413-
461 430.

462 Tagari, H., Webb Jr., K., Theurer, B., Huber, T., DeYoung, D., Cuneo, P., Santos, J.E.P.,
463 Simas, J., Sadik, M., Alio, A., Lozano, O., Delgado-Elorduy, A., Nussio, L., Bittar,
464 C.M.M., Santos, F., 2008. Mammary uptake, portal-drained visceral flux, and hepatic
465 metabolism of free and peptide-bound amino acids in cows fed steam-flaked or dry-rolled
466 sorghum grain diets. *J. Dairy Sci.* 91, 679-697.

467 UniProt Consortium, 2017. UniProt: the universal protein knowledgebase. *Nucleic Acids*
468 *Res.* 45, D158-D169.

469 Waterlow, J.C. 2006. *Protein turnover.* CAB International, Wallingford, UK.

470

471 **Table 1.** Principle symbols used for the kinetic model.

F_{ij}	Flow of PHE ^a or TYR ^a to pool i from j ; F_{i0} denotes an external flow into pool i and F_{0j} denotes a flow from pool j out of the system	μmol/min
I_i	Effective rate of constant infusion of ¹³ C labelled PHE or TYR into primary pool i	μmol/min
Φ_i	Effective rate of constant infusion of ² H labelled TYR into primary pool i	μmol/min
Q_i	Quantity of PHE ^a or TYR ^a in pool i	μmol
q_i	Quantity of ¹³ C labelled PHE or TYR in pool i	μmol
ϕ_i	Quantity of ² H labelled TYR in pool i	μmol
e_i	Enrichment of ¹³ C PHE or TYR in pool i : ($= q_i/Q_i$)	atoms % excess/μmol
ε_i	Enrichment of ² H TYR in pool i : ($= \phi_i/Q_i$)	atoms % excess/μmol
t	Time	min

472

473 ^aTotal material (*i.e.* tracee + tracer).

474

475 **Table 2.** Experimental and other inputs.
 476

Cow		323/14	341/29	6031/42	6132/43 ^a	
Dietary CP (g/kg)		175	128	128	175	
Milk yield (kg/d)		25.8	24.1	24.2	25.3	
Plateau	e_1	2.19	3.37	2.79	2.10	
enrichment (APE)	e_2	3.58	5.51	4.31	3.32	
	e_3^b	0.15	0.18	0.18	0.32	
	e_4^b	0.28	0.30	0.30	0.43	
	e_6^b	1.07	1.65	1.29	1.00	
	e_7^b	0.08	0.09	0.09	0.13	
	e_9	2.14	3.54	2.70	2.00	
	e_X^b	0.25	0.32	0.28	0.39	
	ε_3	0.76	1.16	0.85	0.79	
	ε_4	1.12	1.97	1.21	1.15	
	ε_7^b	0.34	0.59	0.36	0.35	
	ε_X	0.73	1.23	0.83	0.74	
	Flow ($\mu\text{mol}/\text{min}$)	I_1	52.6	44.5	53.7	56.9
		I_2	24.2	36.6	16.5	9.55
		Φ_3	19.0	12.2	16.5	16.3
Φ_4		8.49	10.4	4.99	2.72	
F_{05}		33.8	33.8	33.8	33.8	
$F_{07}^{(o)}$		211	146	351	525	
F_{08}		25.0	25.0	25.0	25.0	
F_{09}		2595	1777	1938	2473	
F_{0X}		2858	1399	2034	2012	

477
 478 ^aEssential amino acid infusion

479 ^bInitial value

480

481

482 **Table 3.** Phenylalanine and tyrosine uptake and partition by the liver for four lactating dairy
 483 cows obtained using the ten-pool model (symbols are defined in the text and Table 1).
 484

Cow		323/14	341/29	6031/42	6132/43
Flow ($\mu\text{mol}/\text{min}$)	F_{10}	2403	1319	1921	2711
	F_{20}	676	665	382	288
	F_{30}	2486	1048	1951	2072
	F_{40}	756	530	414	237
	$\overline{F_{56} - F_{65}}$	33.8	33.8	33.8	33.8
	$\overline{F_{87} - F_{78}}$	25.0	25.0	25.0	25.0
	F_{X7}	547	308	215	300
	F_{X4}	534	412	287	187
	F_{X3}	1778	679	1532	1525
	F_{73}	708	369	419	547
	F_{74}	223	118	127	50
	$F_{07}^{(s)}$	2323	1225	1713	1476
	F_{76}	138	70	172	332
	F_{70}	2037	1147	1586	1397
	F_{91}	1597	967	1366	1935
	F_{92}	449	487	272	205
	F_{96}	548	323	300	333
	F_{61}	806	353	555	776
	F_{62}	227	178	110	82
	F_{06}	2455	1568	2071	2444
F_{60}	2143	1465	1911	2284	
Adjusted plateau enrichment (APE)	e_3^a	0.19	0.19	0.16	0.25
	e_4^a	0.32	0.38	0.35	0.54
	e_6^a	0.81	1.09	0.79	0.61
	e_7^a	0.10	0.11	0.11	0.16
	e_X^a	0.20	0.23	0.18	0.26
	ε_7^a	0.25	0.39	0.22	0.21

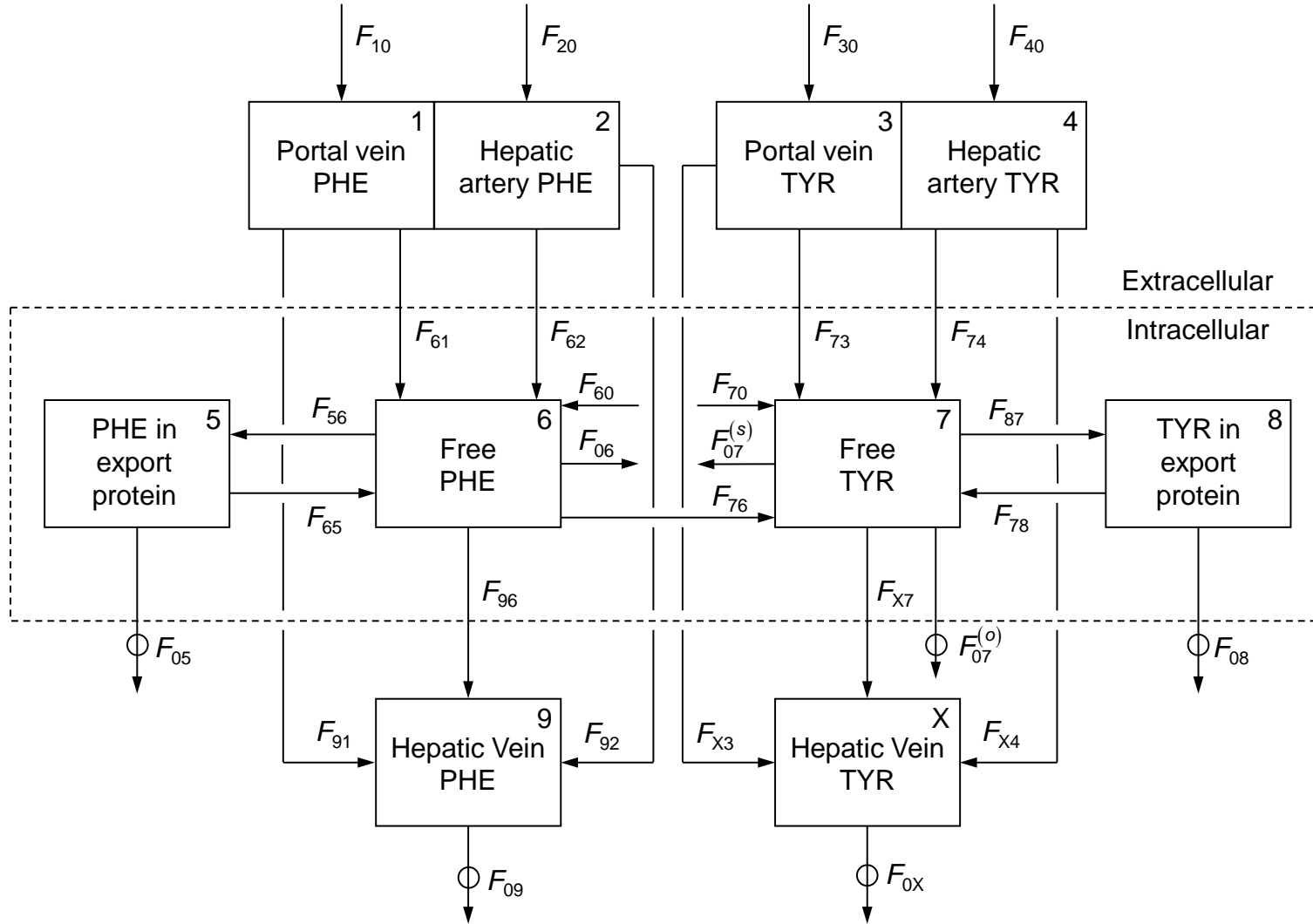
485
 486 ^aFinal value
 487

Table 4. Average slope (%) for each of the flows calculated by the model obtained by perturbing each input in turn^a.

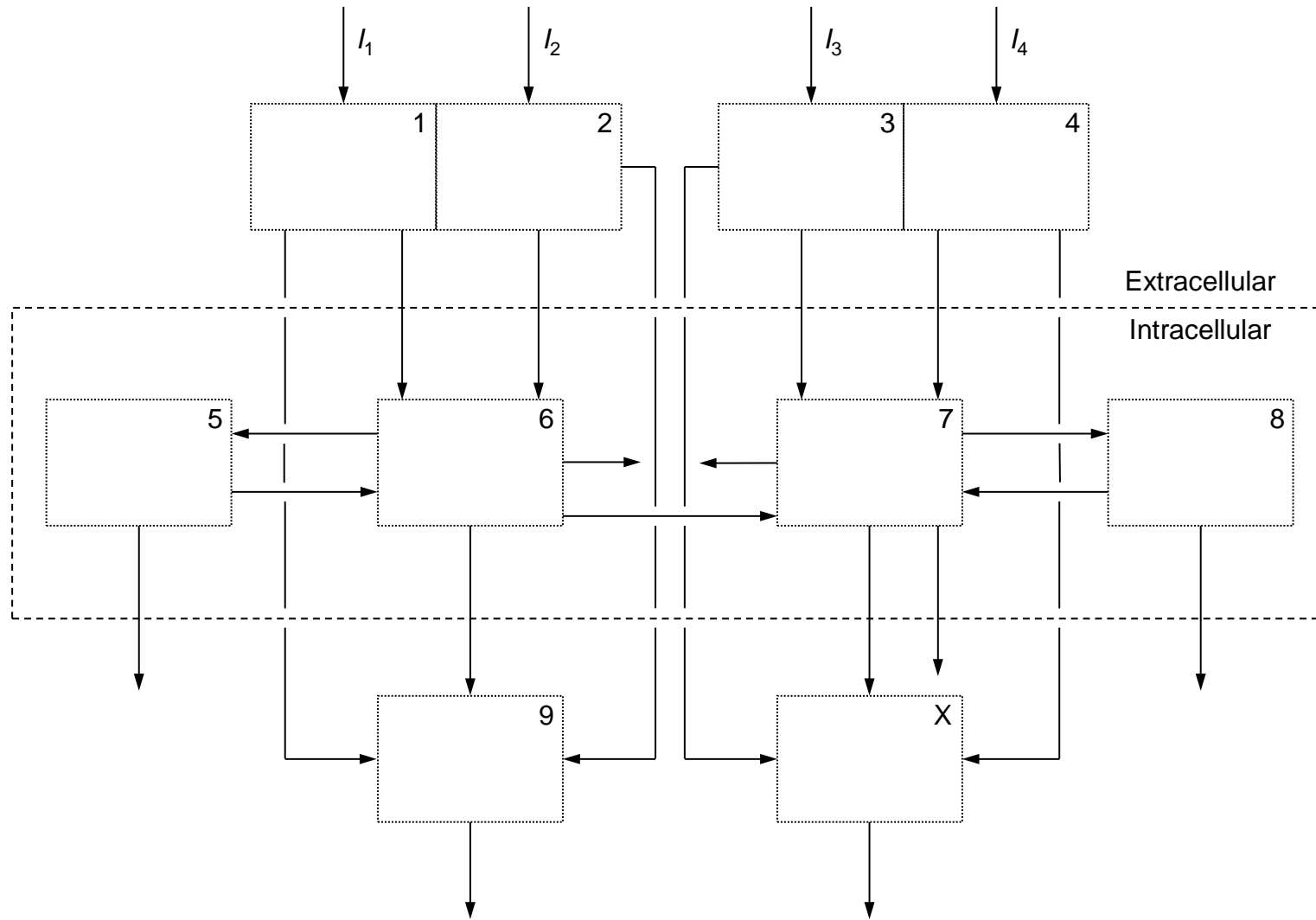
Flow	Unperturbed ($\mu\text{mol}/\text{min}$) ^b	Input perturbed ^c																						
		e_1	e_2	e_3	e_4	e_6	e_7	e_9	e_X	ε_3	ε_4	ε_7	ε_X	I_1	I_2	Φ_3	Φ_4	F_{05}	$F_{07}^{(o)}$	F_{08}	F_{09}	F_{0X}		
F_{10}	2086													1.0										
F_{20}	547														1.0									
F_{30}	1862															1.0								
F_{40}	519																1.0							
$\overline{F_{56} - F_{65}}$	34																	1.0						
$\overline{F_{87} - F_{78}}$	25																				1.0			
F_{X7}	260			-7.6	-3.4		-0.64		9.2	16	3.8	0.62	-16											1.0
F_{X4}	309			-8.5	-3.8		-0.71		10	5.2	1.2	0.20	-5.2											1.0
F_{X3}	1522			3.0	1.4		0.25		-3.7	-3.8	-0.91	-0.15	3.8											1.0
F_{73}	341			-13	-6.1		-1.1		16	17	4.1	0.65	-17			5.5								-4.5
F_{74}	210			12	5.6		1.0		-15	-7.7	-1.8	-0.29	7.6				2.5							-1.5
$F_{07}^{(s)}$	1626							0.18		3.8	1.6	-1.2	-4.2			3.8	1.6		-0.18	-0.02				-4.2
F_{76}	136			-3.3	-1.9	-1.0	2.1		4.1	6.6	2.8	-2.1	-7.4			3.3	1.0							-3.3
F_{70}	1512			0.30	0.17	0.09	-0.19		-0.37	3.5	1.5	-1.1	-3.9			2.6	1.3							-2.9
F_{91}	1488	-1.0	-0.41				-0.08	1.5						0.33	-0.33									1.0
F_{92}	391	-1.0	-0.41				-0.08	1.5						-0.68	0.67									1.0
F_{96}	366	5.2	2.1				0.39	-7.5						-0.64	0.63									1.0
F_{61}	598	2.5	1.0				0.19	-3.6						2.7	0.82									-2.5
F_{62}	157	2.5	1.0				0.19	-3.6						1.7	1.8									-2.5
F_{06}	2163	3.1	1.3	0.21	0.12	-1.1	-0.13	-3.3	-0.26	-0.42	-0.18	0.14	0.46	3.1	1.3	-0.21	-0.06	-0.02					-3.3	0.21
F_{60}	1944	3.4	1.4				-1.2	-3.6						2.3	1.2									-2.5

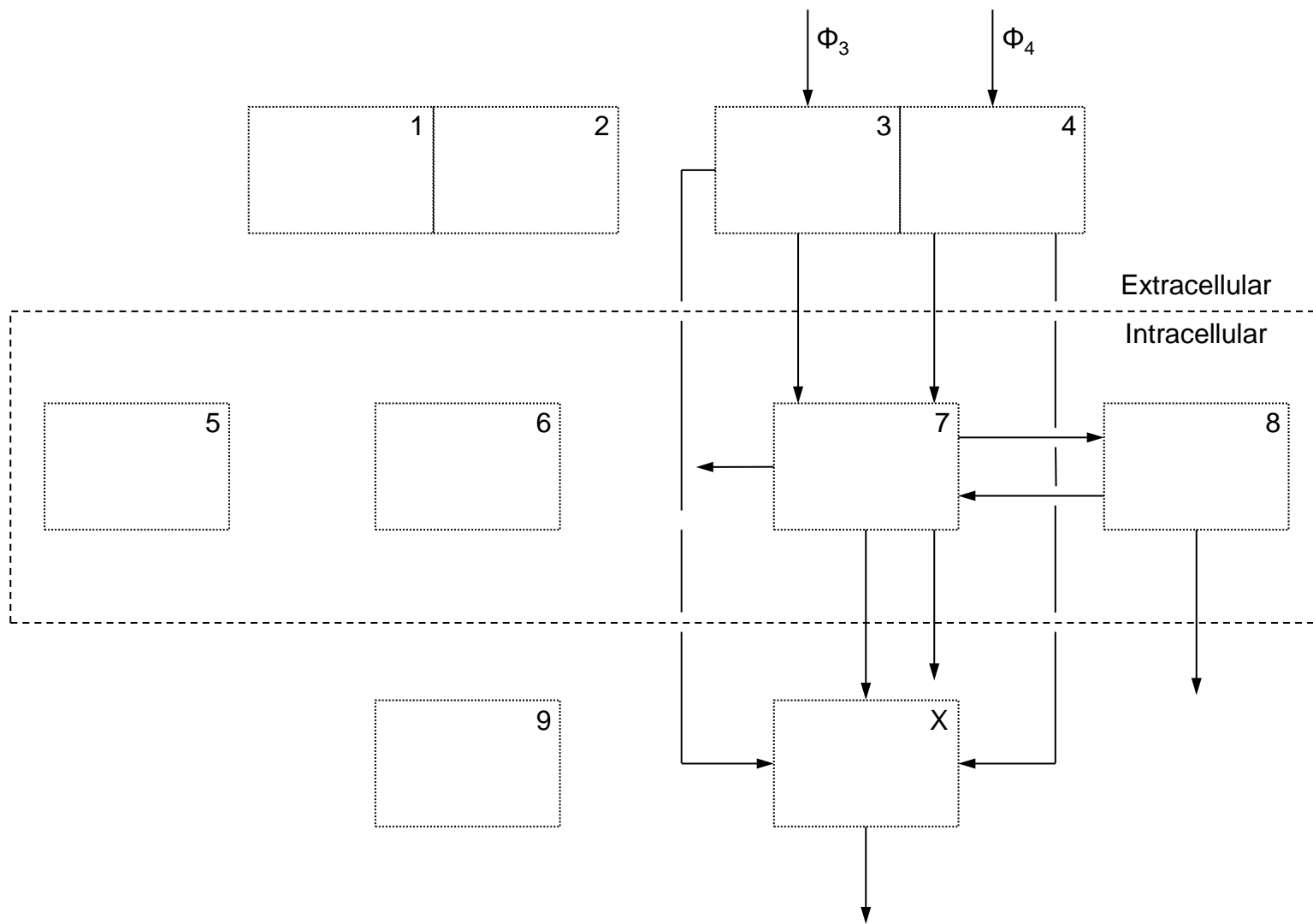
^a The slope for each flow is expressed relative to the value of the flow obtained when no perturbation is made. Only slopes which differ from zero are shown.^b Values calculated from the mean of inputs reported in Table 2.^c Model solved by perturbing each input in turn by 0%, $\pm 10\%$ and $\pm 20\%$.

494 **Figure 1.** Scheme for the uptake and utilisation of PHE and TYR by the liver of lactating dairy cows: (a) total PHE and TYR, (b) [¹³C] labelled
 495 PHE and TYR and (c) [²H] labelled TYR. Nomenclature is defined in Table 1. Represented pools are numbered 1 to X and labelled in Figure
 496 1a. The small circles in Figure 1a indicate flows out of the system which need to be measured experimentally.



497 (a)





500 (c)