Comparison of Net Portal Absorption with Predicted Flow of Digestible Amino Acids: Scope for Improving Current Models?

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ABSTRACT

This study was undertaken to determine the relationship between measured net portal absorptions (NPA) and flows of digestible essential amino acids (EAA) predicted with the National Research Council model (NRC, 2001) or the Cornell Net Carbohydrate and Protein System model (CNCPS, version 5.0.34). Net portal absorption data were obtained from 33 measurements of portal-arterial plasma EAA concentration differences among 8 treatments in lactating dairy cows, with plasma flow estimated from downstream dilution of *para* amino-hippurate. The predicted digestible flows from NRC (2001) related better than CNCPS to NPA observed in our studies, as shown by the lower standard errors on the slopes for all EAA and lower root mean prediction errors for all EAA except Met and Phe. However, the partitioning of the prediction error indicated a systematic underprediction (mean bias) for the NRC model (2001), with the exception of Ile. It is important to note that a relationship of unity was not expected, as discussed in the paper, because of losses of EAA through portal-drained viscera metabolism. A revised set of predictive equations for digestible EAA was obtained using a subset of data from NRC (2001) limited to trials conducted with dairy cows. This increased the predicted flows of digestible EAA by only 2%. Flows of digestible EAA were also estimated using a factorial approach, assuming an AA composition for each fraction of the duodenal flow estimated by NRC (undegradable, microbial, and endogenous proteins). This resulted in a slight improvement in the slope of the regression between predicted flows and measured NPA, but still yielded predicted digestive flows that were too low to support observed NPA. Finally, on the basis of literature values, increment of the digestibil-

ity of the undegradable fraction of forages and of microbial protein is suggested to improve the relationship between predicted digestible flows and NPA. Overall, this study indirectly confirms, across EAA, smaller losses through gut metabolism for His, Met, and Lys, intermediate losses for the branched-chain AA with the higher losses for Thr.

Key words: amino acid, net portal absorption, duodenal flow

INTRODUCTION

In recent years, major improvements have occurred in the formulation of dairy cow rations with regards to protein supply. It is now acknowledged that intake of CP is less than adequate to define the supply of protein to the animal and that at least minimally, supplies of both RDP and RUP fractions of feed protein plus energy intakes must be considered (e.g., NRC, 2001; Cornell Net Carbohydrate and Protein System (CNCPS), version 5.0.34, Cornell University, Ithaca, NY; Fox et al., 2004). This has led to the development of the MP systems. A further step forward will be to define the MP supply in terms of the requirement of the units used at the cell level for protein synthesis; that is, the individual AA. This type of approach has been successfully applied to nonruminant nutrition over the past decade and individual AA are routinely added to nonruminant rations to optimize animal performance.

Despite this improvement in the definition and quantification of supply in prediction models, the prediction of use of this supply still relies on fixed factors of conversion of absorbed essential AA (EAA), or of MP, for metabolic functions, including milk protein, irrespective of the supply until the requirements are met. These fixed factors are used even though it is recognized that the marginal recovery of absorbed AA into milk protein decreases with increasing supplies (Whitelaw et al., 1986; Hanigan et al., 1998a; Doepel et al., 2004). Raggio et al. (2004) observed that the decreased transfer of absorbed EAA to milk protein with increasing protein supply was associated with

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increased hepatic removal of some EAA (His, Met, Phe, and Thr) with probable increased oxidation of other EAA (Ile, Leu, Lys, Thr, and Val) by peripheral tissues including the mammary gland. There is a general consensus among several research groups that the best way to achieve accurate prediction of milk protein output would be the development of an integrative model of AA metabolism across major sites of utilization of AA in the dairy cow; that is, the portal-drained viscera (PDV), the liver, the muscle, and the mammary gland (e.g., Hanigan et al., 1998b, 2001; Cant et al., 2000; Hanigan, 2005). The first step in developing such a model would be to estimate net portal absorptions (NPA) of EAA from prediction of the flows of digestible EAA on the basis of analyses of feed ingredients and intakes, and taking into account the losses of EAA across the PDV due to oxidation and nonreabsorbed endogenous secretions (Figure 1).

Therefore, our first objective was to compare the NRC (2001) and CNCPS (version 5.0.34) models in their prediction of flows of digestible EAA with measurements of NPA in lactating dairy cows performed in our laboratory [Raggio et al., 2004; Berthiaume et al., 2006; H. Lapierre, unpublished data; R. Berthiaume, H. Lapierre, and M. C. Thivierge (Dept. Anim. Sci., Univ. Laval, Québec, QC, Canada), unpublished data]. Our second objective was to propose modifications, if needed, to the best model, in such a way that predicted flows of digestible EAA would fit with the

measurements of NPA, taking into account the potential losses of EAA from the site of digestion to NPA.

MATERIALS AND METHODS

Comparison of NRC and CNCPS Estimates of Digestible Flow Against Measured Portal Absorption

The first step in our approach was to decide which model to use to predict the flow of digestible EAA. Bateman et al. (2001) tested several models designed for North American conditions (CNCPS, version 3; CPM Dairy, version 1.0; Mepron Dairy Ration Evaluator, version 1.1; NRC, 1989; and University of Pennsylvania Release of the Net Carbohydrate and Protein System, version 2.12p) and reported that no single model could be identified as better in predicting flows of individual EAA to the duodenum. To complete the picture in terms of models currently available, we simulated the same publications included in Bateman et al. (2001) using NRC (2001) and concluded that the latest version of this model underestimates the mean flow of EAA to the duodenum (data not shown). This finding is in agreement with the conclusions presented by Bateman et al. (2001), who reported that, for most EAA (with the exception of Arg and Met), the aforementioned models resulted in underprediction errors associated with a systematic bias of 30 to 40%.

It has been acknowledged that the use of mean values from generic feed composition databases could be

Figure 1. Schematic representation of the fate of essential AA (EAA) flow in the portal-drained viscera (PDV). The difference between PDV arterial inflow and PDV venous outflow is designated as the net portal absorption (NPA). Given that $NPA = PDV \text{ arterial EAA inflow} - PDV \text{ venous EAA outflow}$, then $NPA = (\text{digestible endogenous EAA} + \text{digestible nonendogenous EAA}) - (\text{catabolism} + \text{digestible endogenous EAA} + \text{nondigestible endogenous EAA})$. Digestible endogenous EAA cancel each other in the previous equation resulting in: $NPA = \text{digestible nonendogenous EAA} - (\text{catabolism} + \text{nondigestible endogenous EAA})$.

Table 1. Dry matter intake, BW, and milk production of cows from the studies used in which measurements of net portal absorption were made

Item	Mean	SD	Range
DMI, kg/d	20.1	2.98	13.9–24.8
BW, kg	637	64.4	461–764
Milk yield, kg/d	32.9	5.27	23.7–43.4
Milk protein content, %	2.95	0.299	2.45–3.76
Milk protein yield, g/d	964	158.1	702–1,300
MP supply, ¹ g/d	1,910	311.2	1,262–2,554
ME, ¹ Mcal/kg	2.49	0.093	2.36–2.65
Portal plasma flow, L/h	1,302	171.5	1,084–1,972

¹Calculated with NRC (2001).

a contributor to this bias (Alderman et al., 2001). To use individual data generated for each cow, and assuming that accurate knowledge of the feed intake and composition would reduce systematic errors in the estimation of duodenal flows of EAA, we used studies conducted by our group in which NPA of individual EAA were measured (8 treatments comprising 33 individual observations: [Raggio et al., 2004; Berthiaume et al., 2006; H. Lapierre, unpublished data; R. Berthiaume, H. Lapierre, and M. C. Thivierge (Dept. Anim. Sci., Univ. Laval, Québec, QC, Canada), unpublished data]; see Table 1). Each measurement of NPA was the mean of 6 values (6 sampling times on 1 d of measurement) calculated by multiplying plasma flow (estimated from downstream dilution of *para*-amino hippurate) by veno–arterial concentration differences. Relevant information on the ingredients (including CNCPS fractions and AA analysis) and animals was entered into CNCPS (version 5.0.34) and NRC (2001) to compare the estimated flows of digestible EAA with measurements of NPA. Although neither model was designed to estimate NPA, we assumed that NPA of EAA is correlated with the corresponding digestible supply and that we would therefore be able to select a model that provides the best estimates of the flow of digestible AA for a given NPA. Before comparing flows of digestible EAA and NPA values, the estimated flows of digestible EAA from the NRC model (2001) were adjusted to remove the estimated contribution of the digested endogenous secretions. The endogenous proteins originate from various sources, including mucoproteins, saliva, sloughed epithelial cells, and enzyme secretions (Tamminga et al., 1995) and are secreted all along the lumen of the gut. Unlike the CNCPS model, the NRC model (2001) includes in its digestible flow the digested portion of the endogenous secretions flowing at the duodenum. As these secretions originate from arterial supply to the PDV (except for saliva), the predicted flows of digestible EAA were corrected by subtracting the con-

Table 2. Amino acid composition of microbial protein (MCP), abomasal isolate, and ileal endogenous protein used for the factorial approach

AA	MCP ¹	Abomasal isolate ²	Ileal endogenous protein ³
(% of AA)			
His	2.0	3.6	2.0
Ile	5.7	4.6	4.0
Leu	8.1	4.8	6.6
Lys	7.9	7.3	5.4
Met	2.6	1.5	2.0
Phe	5.1	4.6	7.1
Thr	5.8	6.5	7.9
Trp	1.6	1.9	1.7
Val	6.2	6.1	6.0

¹From Clark et al. (1992), except Trp from Storm and Ørskov (1983).

²From Ørskov et al. (1986), except Trp from Stein et al. (1999).

³From de Lange et al. (1989), determined in pigs.

tribution of preduodenal endogenous secretions of the model (1.9 g of endogenous N per kg of DMI with a coefficient of digestibility of 0.80) and the AA composition of abomasal isolate (Table 2). Therefore, all subsequent references to flow of digestible AA will assume no contribution from endogenous sources, i.e., they will refer to the flow of nonendogenous digestible EAA.

Once the contribution of endogenous secretions was removed from the flows of digestible EAA, these flows were assessed as predictors of their corresponding NPA using the same assessment protocol as that used by Bateman et al. (2001), with the observed NPA (*y*) being the value modeled from the digestible supply data (*x*). Statistical analysis of the prediction error (root mean square prediction errors (**RMPSE**), mean bias, slope error, and random noise) was performed using SAS (SAS Institute, 2003) assuming that errors in *x* (the true digestible flow) would be equivalent between the NRC and CNCPS models.

Modifications to the NRC Model (2001)

Based on the results obtained from the steps described above, it was decided to reexamine the assumptions of the NRC model (2001) for the estimations of the flows of digestible EAA. This was undertaken in an effort to improve the predictions of flows of digestible EAA in relation to the measured NPA of EAA. First, the regression equations developed in NRC (2001) to predict duodenal flows of total EAA and the percentage of each in total EAA were reassessed, with published criticisms of the model taken into account. One criticism has been the use of combined data sets from lactating cows and growing cattle to generate the equations for predicting duodenal

Figure 2. Relationship between measured flow of essential amino acids (EAA) to duodenum and A) microbial CP, or B) EAA from RUP from database of studies used in NRC (2001) to derive equations for estimation of duodenal flows. Data from studies with dairy cows (□) and steers (■).

flows of protein and AA (Alderman et al., 2001). Indeed, 22% of the observations used to generate the equations that predict duodenal flows of total EAA and the proportion of each in total EAA are derived from data collected in experiments with growing cattle. Therefore, we removed the steer data from the

data set as they clustered at low levels of total EAA from RUP (Figure 2a) and microbial crude protein (MCP; Figure 2b). New equations were generated with the MIXED procedure from SAS (SAS Institute, 2003) using a random regression model as described by NRC (2001). Briefly, the regression equations in-

cluded a random slope effect for each publication in the database, with a simple variance-covariance structure matrix associated to random effects (publication) for partition of the residual variance. Additional modifications to the regression models included the use of weights for each observation depending on the error associated with the measurements in each study, as described by St-Pierre (2001).

A further step was to determine which parameter in the model had the greatest impact on the prediction of flow of digestible AA. Sensitivity analyses were performed, with each parameter increased or decreased by 10%. From these results of the sensitivity analyses, the digestibility of the RUP fraction of forages was first increased. On the basis of literature values that have estimated the true digestibility of AA from forage RUP in the range of 88 to 91% (Hvelplund et al., 1992), an average value of digestibility of 88% was used, whereas the values used by NRC (2001) are on average, 60, 65, and 70% for grass, legume, and corn silages respectively. The final step was to increase the digestibility of the MCP fraction from 80% (NRC, 2001) to 85%. This value was chosen because the digestibility of microbial AA was evaluated at 87% (Tas et al., 1981), although the average digestibility of crude MCP based on studies used for NRC (1985) and used subsequently by NRC (1989 and 2001) averaged 79%.

In parallel with these modifications to RUP and MCP digestibility, the factorial approach was also evaluated as an alternative to the random regression equations, in which the different protein fractions flowing to the intestine (RUP, MCP, endogenous proteins) as predicted by the NRC model (2001) were multiplied by the respective assigned AA composition (Table 2) and summed.

An Excel (Microsoft Office v. 2003 SP1; Microsoft Corp., Redmond, WA) version of the NRC model was built to allow changes in the running parameters of this model. Simulations from our Excel version were compared with values predicted from the original NRC software to ensure accuracy of the transcribed model. Simulations in the Excel model were automated by using the Visual Basic module from Excel and summary tables were generated for the different scenarios that were explored. Data were exported to SAS (SAS Institute, 2003) and the modified models were evaluated (sensitivity analysis, RMSPE, bias analysis) as described above.

RESULTS AND DISCUSSION

Loss of AA Through Gut Metabolism

The slope of the regression between the flows of digestible EAA and the NPA was not observed to be

Table 3. Comparison of measured net portal absorptions of essential AA (g/d; mean $\pm$ SD) with predicted flows of digestible AA by the NRC (2001)¹ and Cornell Net Carbohydrate and Protein System (CNCPS v. 5.0.34) models

AA	Measured net portal absorption	Predicted by NRC	Predicted by CNCPS
His	43 $\pm$ 10.6	38 $\pm$ 7.4	51 $\pm$ 7.2
Ile	83 $\pm$ 13.9	81 $\pm$ 9.1	93 $\pm$ 9.6
Leu	147 $\pm$ 31.1	161 $\pm$ 22.1	156 $\pm$ 18.8
Lys	119 $\pm$ 20.9	111 $\pm$ 16.5	127 $\pm$ 15.4
Met	41 $\pm$ 8.7	32 $\pm$ 4.6	44 $\pm$ 4.6
Phe	106 $\pm$ 23.4	86 $\pm$ 12.2	93 $\pm$ 10.8
Thr	69 $\pm$ 15.3	81 $\pm$ 10.0	91 $\pm$ 10.4
Val	93 $\pm$ 18.9	99 $\pm$ 14.2	112 $\pm$ 13.5

¹Corrected to remove duodenal endogenous secretions.

equal to unity (Tables 3 and 4). This could be due to errors in the prediction of EAA duodenal flows and in the measurements of NPA, but also to foreseeable potential losses of EAA through PDV metabolism. Can we evaluate the magnitude of these losses? This would permit definition of the proportion of the digestible flow that would be recovered into portal circulation, after biological events have occurred into the gut.

A first consideration is to account for the endogenous proteins secreted in the gut and not reabsorbed at the ileum level, which would encompass undigested endogenous secretions flowing at the duodenum plus undigested endogenous proteins secreted into the intestine. These 2 fractions were estimated to be about equal (Lapierre et al., 2006). Total preduodenal endogenous secretions, including endogenous proteins and those incorporated into MCP were calculated as 4.4 g of N per kg of DMI (Ouellet et al., 2002). With a coefficient of digestibility of 80% (NRC, 2001), the undigested preduodenal endogenous proteins reaching the ileum level were calculated, and the endogenous secretions in the small intestine that were not reabsorbed were estimated to be equal to this amount. Using the AA composition of the abomasal isolate (Ørskov et al., 1986) for the preduodenal fraction and the AA composition of the ileal endogenous protein in pigs receiving a protein-free diet and infused intravenously with AA (de Lange et al., 1989) for the small intestine, the total ileal fraction of AA originating from undigested endogenous secretions was calculated (Table 5). As these AA originate from arterial supply to the PDV or from AA absorbed from the lumen, they will represent a net loss and therefore decrease the NPA by the amount of the loss.

The second factor to be taken into consideration is the oxidation of EAA by the gut. Oxidation of some EAA by the PDV has been observed in many species, including ruminants, but reported quantification of such events is scarce (Reynolds et al., 2000; Lapierre

Table 4. Analysis of the root mean square prediction errors (RMSPE) and decomposition of the prediction errors when net portal absorption is modeled as a function of digestible AA flow estimated with the NRC and Cornell Net Carbohydrate and Protein System (CNCPS) models

Model	AA	Slope ¹	RMSPE (% of mean)	Mean bias, %	Slope bias, %	Random noise, %
NRC	His	1.14 (0.03)	15.0	62	16	21
	Ile	1.03 (0.05)	12.7	4	0	96
	Leu	0.91 (0.05)	16.5	34	0	66
	Lys	1.07 (0.03)	9.6	47	4	49
	Met	1.28 (0.05)	23.9	81	11	8
	Phe	1.24 (0.05)	22.1	73	14	13
	Thr	0.87 (0.04)	19.5	69	5	26
	Val	0.94 (0.03)	11.1	39	7	54
CNCPS	His	0.83 (0.05)	26.2	60	0	40
	Ile	0.88 (0.06)	22.2	31	19	50
	Leu	0.94 (0.07)	21.0	7	10	83
	Lys	0.93 (0.04)	13.9	27	0	73
	Met	0.95 (0.06)	14.6	16	10	74
	Phe	1.14 (0.06)	18.9	40	11	49
	Thr	0.77 (0.05)	34.6	79	0	21
	Val	0.83 (0.05)	25.6	66	0	34

¹Slope indicates the marginal change in net portal absorption per unit of increase in digestible AA flow.

et al., 2002; Lobley et al., 2003). Calculations were performed for the AA for which such quantification has been done in ruminants: Leu, Lys, Met, and Phe. Leucine has certainly been the most studied EAA for PDV oxidation, and its oxidation averaged 25% of NPA in dairy cows (Lapierre et al., 2002), a value similar to that observed in sheep (Lobley et al., 2003). No oxidation of Phe was observed across the PDV in dairy cows (Reynolds et al., 2000). In sheep, the same observation was made for Phe and Lys, whereas Met oxidation was equivalent to 10% of NPA (Lobley et al.,

2003). El-Kadi et al. (2004) observed large incremental removals of the branched-chain AA and Lys (between 33 and 51% of the incremental supply) by the PDV in sheep that were fed a low protein diet and duodenally infused with incremental amounts of casein (0 to 105 g/d). These authors noted that the quantities removed were proportional to the increasing supply. Lesser amounts (7 to 19% of the incremental supply) of other EAA (His, Met, and Phe) were also removed by the gut. These values probably should not be directly transferred to dairy cows, as they are

Table 5. Theoretical digestible AA flow to duodenum required to account for the measurements of net portal absorption from the data set after correction for losses through oxidation across the intestine and undigested endogenous protein

AA	Net portal absorption, ¹ g/d	Oxidation		Undigested endogenous protein, ³ g/d	Theoretical digestible flow, ⁴ g/d	Theoretical slope ⁵ (portal/digested)
		% of portal absorption ²	g/d			
His	43			4.8	≥47.8	≤0.90
Ile	83			7.4	≥90.4	≤0.92
Leu	147	25	37	9.8	193.6	0.76
Lys	119	0	0	10.9	129.9	0.92
Met	41	10	4	3.0	48.1	0.85
Phe	106	0	0	10.1	116.1	0.91
Thr	69			12.4	≥81.4	≤0.85
Val	93			10.4	≥103.4	≤0.90

¹Average of the 33 individual measured means in the data set.

²Based on oxidation level measured in ruminants; see text for references; no value indicates that no observation is available for this AA in ruminants.

³From preduodenal and small intestine origin; see the text for calculation.

⁴Summation of net portal absorption and losses occurring through portal-drained viscera metabolism; when no data were available for oxidation, no values were added. Therefore, this estimation is the minimum estimated to account for the net portal absorption plus catabolism in the tissue and losses arising from nondigestible endogenous secretions.

⁵Slope indicates the marginal change in net portal absorption per unit of increase in digestible AA flow.

expressed for an incremental supply in sheep with a small metabolic demand receiving a large infusion of AA (up to 105 g/d). Although it is becoming more evident that PDV oxidation is related to total inflow of AA (Hanigan et al., 2004; Hanigan, 2005; Lapierre et al., 2005), the scarcity of data to quantify this relationship is such that we used available values of oxidation expressed as a proportion of the NPA. However, because AA use by the PDV is primarily from arterial source (MacRae et al., 1997), this step will need to be refined when additional data on PDV oxidation of AA in relation to total inflow becomes available. However, as arterial concentrations were found to be linearly related to increased absorption (Hanigan et al., 2004), this simplification should not introduce major bias. Therefore, assumed fractional oxidation values relative to NPA were 25% for Leu (Lapierre et al., 2002; Lobley et al., 2003), 10% for Met and 0% for Lys (Lobley et al., 2003), and 0% for Phe (Reynolds et al., 2000; Lobley et al., 2003). All of these measurements of oxidation across the gut were performed using labeled AA infused into the systemic circulation. In pigs, Lys oxidation has been demonstrated with an enteral infusion of the tracer but not with an intravenous infusion (van Goudoever et al., 2000). It is not clear whether this oxidation across the gut when the AA is infused enterally is due to the gut cells themselves or to microbial action. For the other EAA, we will have to keep in mind that the flow of digestible EAA will be equal to the NPA plus undigested endogenous secretions if there is no oxidation, or greater if any oxidation is further demonstrated.

With these assumptions in mind, the digestible flows to be expected as the sum of the measured NPA, the loss of endogenous proteins in the feces and PDV oxidation were estimated (Table 5). Then, a "theoretical" slope of the NPA in relation with the digestible flows was calculated, and the relationship between the predicted flow of digestible EAA and measured NPA was assessed relative to these slopes, rather than an attempt being made to reach unity.

Comparison of the NRC and CNCPS Models

A first look at the predicted flow of digestible EAA from the 2 models with measured NPA reveals that there is a reasonably good agreement between mean model predictions (excluding, when needed, predicted duodenal endogenous digestible flow) and mean NPA measurements (Table 3). Considering the different methodologies involved in estimating flows of digestible EAA using the models (mathematical models based on diet composition and intake) and the measurement of NPA of EAA (arteriovenous differences

and plasma flow estimation), this first observation was encouraging. To be used in modeling, however, the predicted values of digestible flow of EAA need to be better than a good approximation and should be quantitatively related to downstream values of NPA. The NRC model (2001) yielded lower estimates of the digestible flow for 5 of the EAA (His, Ile, Lys, Met, and Phe) than the measured NPA, whereas in the CNCPS model, this situation occurred only for Phe.

However, the choice of the best model should relate not only to the absolute value of the mean prediction, but also to the ability of the model to minimize the error for individual observations. We acknowledge that neither model was designed to estimate NPA of EAA. However, because NPA of EAA is directly linked to the digestible supply of individual EAA, we would expect a high degree of association between the model estimates (particularly as all the ingredient data and feed intakes were known) and our measurements of NPA. Although the mean values from the CNCPS model appear to be good estimators of the mean NPA from the data set, estimates from the NRC model (2001) were better at simulating the direction of changes observed in NPA between experimental treatments: regression of NPA on predicted EAA duodenal supply from CNCPS returned slopes that were not significantly different from zero for Ile (slope 0.03) and Thr (slope 0.003). The predicted digestible flow from NRC related better to NPA observed in our studies, as demonstrated by the lowest standard errors on the slopes for all EAA and lower root mean prediction errors for all EAA except Met and Phe (Table 4). However, the partitioning of the prediction error indicated a systematic underprediction (mean bias) for the NRC model (2001), with the exception of Ile. The obvious question then became: "Does NRC underpredict the flows of digestible EAA or are our NPA measurements overpredictions?" There is no clear answer to this question. In our studies, however, the postliver supply of His, Met, and Phe was about equal to milk AA output; that is, large enough to support milk protein yield without invoking the use of body proteins, which was unlikely to occur at the stage of lactation of the cows used in these studies. This would suggest that our plasma measurements did not suffer from a systematic overprediction (Lapierre et al., 2005).

Changes to the NRC Model

Revision of the equations by deleting the data from steers and using weighted regression resulted in improved models as indicated by reductions of 2 to 14% on the values of Akaike's information criteria for non-weighted models. The modified set of equations is pre-

Table 6. Revised equations for predicting flows of total essential AA (EAA) to duodenum and percentage contribution of each to total EAA flow¹

Item	Equation	RMSPE ²
Total EAA	60.15 (34.68) + 0.9515 (0.0893) RUPEAAI + 0.4003 (0.0364) MCP	209.8
Arg	7.18 (0.45) + 0.265 (0.0386) AA% IN RUP	0.84
His	1.94 (0.29) + 0.482 (0.0459) AA% IN RUP	0.60
Ile	6.90 (0.74) + 0.525 (0.0571) AA% IN RUP – 0.0224 (0.0095) RUP % NAN	1.07
Leu	8.06 (0.68) + 0.448 (0.0311) AA% IN RUP + 0.0674 (0.0125) (RUP % NAN	1.26
Lys	11.96 (0.52) + 0.464 (0.0357) AA% IN RUP – 0.0688 (0.0090) RUP % NAN	1.07
Met	3.66 (0.35) + 0.289 (0.0714) AA% IN RUP – 0.01538 (0.0058) RUP % NAN	0.78
Phe	8.01 (0.71) + 0.229 (0.0551) AA% IN RUP + 0.01754 (0.0054) RUP % NAN	0.79
Thr	7.24 (0.88) + 0.493 (0.0830) AA% IN RUP – 0.0221 (0.0052) RUP % NAN	0.66
Val	8.15 (0.52) + 0.355 (0.0400) AA% IN RUP	1.14

¹Values in parentheses are the SE of the parameters; arginine is included as an essential AA in the NRC (2001) and Cornell Net Carbohydrate and Protein System (CNCPS) models.

²RMSPE = Root mean square prediction error, calculated from prediction from fixed effects only, without adjustment for random effects (publication).

sented in Table 6. The equation to determine duodenal flow of total EAA shows an improved relationship with biological events: the coefficient associated with the RUP fraction (0.95 in the new equation vs. 0.86 in NRC, 2001) suggests an almost complete recovery of EAA from the RUP fraction; the coefficient associated with MCP (0.40 in the revised equation) is in agreement with MCP containing 80% of true protein with an EAA content lower than 50% (Storm and Ørskov, 1983; Clark et al., 1992) and, finally, the intercept of 60 g/d of EAA would represent endogenous protein in the duodenal flow. This amount of EAA in endogenous CP is equivalent to 120 g/d of total AA (Table 2) and to about 240 g/d of endogenous CP, if it is assumed that the true protein content of endogenous protein is 50% (NRC, 2001). This amount of endogenous CP is equivalent to 38 g/d of N or, at an estimated intake of 20 kg of DMI, 1.9 g of N per kg of DMI, which is in agreement with the actual value used in the NRC

model (2001). However, these modifications to the NRC equations (2001) for AA increased the digestible flow of individual EAA by only 2% on average (Table 7), and the slopes relating NPA to the predicted digestible flows were still too high (Table 8). It was therefore concluded that the new estimates of digestible flows of EAA were still insufficient to account for the losses of AA across the PDV. The factorial approach yielded higher values than the regressions (except for Leu), even with the revised equations, but slopes greater than unity still exist for His, Lys, Met, and Phe; this continues to indicate insufficient provision of digestible EAA to account for the observed NPA of these EAA (Table 8).

The sensitivity analyses run for individual AA to determine which parameter in the model had the greatest impact on the prediction of flow of digestible EAA are averaged in Table 9. Besides the intercept of the equations for individual EAA that could not be

Table 7. Amino acid flows (g/d) from measured net portal absorptions, or estimated digestible flows with suggested changes to NRC¹ (Mean ± SD)

Essential AA	Net portal absorption	Flows of digestible AA					
		Revised equations only	Revised equations and increment of RUP digestibility	Revised equations and increment of RUP and microbial CP digestibility	Factorial	Factorial and increment of RUP digestibility	Factorial and increment of RUP and microbial CP digestibility
His	43 ± 10.6	38 ± 7.9	41 ± 9.1	43 ± 9.2	41 ± 8.6	44 ± 9.8	46 ± 10.0
Ile	83 ± 13.9	82 ± 9.1	87 ± 10.4	91 ± 10.8	82 ± 9.9	87 ± 11.2	91 ± 11.7
Leu	147 ± 31.1	164 ± 23.6	175 ± 27.0	181 ± 27.6	150 ± 22.2	161 ± 25.6	166 ± 26.1
Lys	119 ± 20.9	113 ± 18.7	119 ± 21.1	125 ± 21.7	113 ± 18.9	120 ± 21.4	125 ± 22.0
Met	41 ± 8.7	33 ± 4.9	35 ± 5.6	37 ± 5.8	37 ± 5.8	39 ± 6.5	41 ± 6.7
Phe	106 ± 23.4	88 ± 13.0	94 ± 14.9	97 ± 15.3	85 ± 12.5	92 ± 14.4	95 ± 14.8
Thr	69 ± 15.3	82 ± 10.7	87 ± 12.2	91 ± 12.6	85 ± 11.3	90 ± 13.0	94 ± 13.4
Val	93 ± 18.9	101 ± 15.3	108 ± 18.0	113 ± 18.4	104 ± 16.4	112 ± 19.3	116 ± 19.7

¹The digestible AA flows are corrected to remove the contribution from preduodenal endogenous secretions, estimated according to the NRC model (2001).

Table 8. Revised slopes¹ (SE in parentheses) of the portal absorptions being modeled from the flows of digestible AA estimated with the revised NRC equations²

Essential AA	Revised equations only	Revised equations and increment of RUP digestibility	Revised equations and increment of RUP and microbial CP digestibility	Factorial	Factorial and increment of RUP digestibility	Factorial and increment of RUP and microbial CP digestibility
His	1.12 (0.03)	1.03 (0.03)	1.00 (0.03)	1.04 (0.03)	0.97 (0.02)	0.94 (0.02)
Ile	1.00 (0.05)	0.95 (0.05)	0.91 (0.04)	1.00 (0.05)	0.94 (0.05)	0.90 (0.04)
Leu	0.89 (0.04)	0.84 (0.04)	0.81 (0.04)	0.98 (0.05)	0.91 (0.05)	0.89 (0.04)
Lys	1.05 (0.03)	0.99 (0.03)	0.95 (0.02)	1.04 (0.03)	0.99 (0.03)	0.94 (0.02)
Met	1.24 (0.04)	1.18 (0.04)	1.13 (0.04)	1.11 (0.04)	1.05 (0.04)	1.01 (0.04)
Phe	1.21 (0.04)	1.13 (0.04)	1.09 (0.04)	1.24 (0.05)	1.16 (0.04)	1.12 (0.04)
Thr	0.85 (0.04)	0.80 (0.03)	0.76 (0.03)	0.82 (0.04)	0.78 (0.03)	0.74 (0.03)
Val	0.92 (0.03)	0.85 (0.03)	0.82 (0.03)	0.89 (0.03)	0.83 (0.03)	0.79 (0.03)

¹Slope indicates the marginal change in net portal absorption per unit of increase in digestible AA flow.

²The digestible AA flows are corrected to remove preduodenal endogenous secretions, estimated according to the NRC model (2001).

changed, the parameters with the greatest impact on the estimation of the digestible flow were the digestibility of both the RUP and the MCP fractions, and the AA composition of the microbial fraction for the factorial approach. With the proposed increment of the digestibility of grass hay, grass silage, and corn silage to 88%, the predicted digestible flow of EAA increased by an average of 9% for both the regression and the factorial approaches (Table 7), but as indicated by the slopes, the digestible flow still appeared to be too low. The final step was to increase the digestibility of the MCP fraction from 80 to 85% with a resulting increment of the predicted flow of digestible EAA (Table 7). It has also been proposed (Hanigan et al., 2004) that the coefficient of intestinal digestibility

should be increased to allow sufficient absorption of lysine to account for endogenous secretions.

At this last step, neither the regression nor the factorial approach is better for all the EAA. After the adjustments in RUP and MCP digestibility, the regression and factorial approaches were not different for Ile, Leu, Lys, Phe, Thr, and Val ($P > 0.15$), whereas the factorial approach gave a lower slope for His ($P = 0.03$) and Met ($P = 0.02$). The reason why the regression approach could not yield a slope lower than 1.13 for Met, even with all the corrected factors, might be the preparation of the digesta samples for the AA analyses in studies used to build the equations. Prior to hydrolysis, pretreatment of the sample is needed, otherwise Met may be lost during the hydrolysis: the

Table 9. Sensitivity analyses on the prediction of digestible AA flow (% of variation from the mean obtained with the current factors)

Variable	Change, %	Method	
		Regression	Factorial
Fraction A of protein intake	-10	0.7	0.8
	+10	-0.9	-1.0
Fraction B of protein intake	-10	-0.8	-0.9
	+10	1.0	1.2
Fraction C of protein intake	±10	±0.4	±0.4
Degradation rate	±10	±0.6	±0.7
Digestibility of RUP	±10	±3.9	±4.0
Digestibility of microbial CP	±10	±4.5	±4.3
Endogenous secretions at 1.9 g/kg of DMI	±10	±0.8	±0.2
Endogenous secretions at 4.4 g/kg of DMI ¹	±10	-2.5	-9.8
Intercept of the total essential AA (TEAA) equation	±10	±0.5	
Slope of the RUP in TEAA equation	±10	±3.9	
Slope of the microbial CP in TEAA equation	±10	±3.9	
Intercept of the individual AA equations	±10	±6.1	
Slopes of RUP ² for individual AA	±10	±3.7	
Slopes of RUP ³ for individual AA	±10	±0.2	
AA composition of the microbial CP	±10	±0.9	±6.2

¹From Ouellet et al., 2002.

²Slopes for individual AA as a percentage of RUP (all essential AA).

³Slopes for RUP as a percentage of duodenal protein (His, Ile, Leu, Lys, Met, Phe, and Thr).

preferred treatment is oxidation with performic acid, which transforms Met into Met sulfone (AOAC, 1996). Of the 199 treatment means used to build the equations, only 54 were obtained after protection of Met during the hydrolysis using either performic acid oxidation ($n = 34$) or phenol plus dithiopropionic acid ($n = 20$). The proportion of Met relative to total EAA averaged 4.5% when performic acid was used and 4.2% when no pretreatment was reported, indicating some potential loss of Met without adequate pretreatment of samples. With the factorial approach, the slope equals unity, leaving no room for oxidation and loss of the nonreabsorbed intestinal endogenous secretions. The reason for this might also relate to the lack of protection of Met for the hydrolysis. For example, of the 17 studies used by Clark et al. (1992) on AA composition of MCP, only 3 reported a pretreatment with performic acid. Therefore the concentration of Met in rumen bacteria might be slightly underestimated. Interestingly, in review papers reporting the AA composition of bacteria, Met always has the highest coefficient of variation (Storm and Ørskov, 1983; Clark et al., 1992; Rulquin et al., 1998). The reason why Phe has a slope higher than unity remains unsolved.

Other than Met and Phe, the ranking amongst EAA in terms of potential losses indicated by the magnitude of the slope relating the NPA to the predicted flow (Table 8) follows the biological events that were discussed earlier: some loss of all EAA through the nonreabsorption of intestinal endogenous secretions, minimal oxidation of His, limited oxidation of Lys, considerable oxidation of the branched-chain AA, and a loss of Thr larger than those of other EAA due to the relatively high contribution of Thr to endogenous proteins (Table 2). Except for His for which a major use across PDV was reported, this ranking also agrees with the model developed by Hanigan et al. (2004).

Another issue arising from this study concerns the absorption of EAA into blood circulation as peptides. It has been proposed that, also in dairy cows, AA may be absorbed in peptide form (Tagari et al., 2004) and therefore not detected by the standard methods to quantify free AA absorption in the portal vein. In contrast, uptake of peptides from the lumen would be included in the predicted digestible flow of EAA based on duodenal flows. Because currently predicted flows of digestible AA cannot account for measured NPA, especially when taking into account potential losses of EAA through the gut, it appears unlikely that significant quantities of EAA were absorbed into the portal vein as intact peptides in these various studies.

CONCLUSIONS

This study was undertaken to reconcile 2 “realities” evolving in parallel: models predicting flows of digest-

ible EAA from measured gastrointestinal events (rate of rumen degradability, rate of passage, intestinal digestibility) and diet composition with direct measurements of NPA of EAA. Making the link between these 2 worlds increases our ability to propose refinements to the existing models to achieve better prediction. From this data set, it is suggested that the true intestinal digestibility of both the RUP and the MCP fractions in the NRC model (2001) should be increased to obtain a better fit between observed NPA of EAA and predicted flow of digestible EAA. In many studies, the contribution of Met to total AA may be underestimated owing to a lack of sample processing before hydrolysis. This study also confirms the ranking of potential losses amongst the different EAA through gut metabolism: lower losses for His, Met, and Lys, followed by the branched-chain AA, and the higher losses for Thr.

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