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Predictions of ruminal outflow of essential amino acids in dairy cattle

A. J. Fleming,[1] H. Lapierre,[2] R. R. White,[1,3] H. Tran,[3,4] P. J. Kononoff,[4] R. Martineau,[2] W. P. Weiss,[5] and M. D. Hanigan[1]*

[1]Department of Dairy Science, Virginia Tech, Blacksburg 24061
[2]Agricultural and Agri-Food Canada, Sherbrooke, QC, Canada J1M 0C8
[3]National Animal Nutrition Program, National Research Support Project, USDA, Washington, DC 20250
[4]Department of Animal Science, University of Nebraska, Lincoln 68583
[5]Department of Animal Sciences, The Ohio State University, Columbus 43210

ABSTRACT

The objective of this work was to update and evaluate predictions of essential AA (EAA) outflows from the rumen. The model was constructed based on previously derived equations for rumen-undegradable (RUP), microbial (MiCP), and endogenous (EndCP) protein outflows from the rumen, and revised estimates of ingredient composition and EAA composition of the protein fractions. Corrections were adopted to account for incomplete recovery of EAA during 24-h acid hydrolysis. The predicted ruminal protein and EAA outflows were evaluated against a data set of observed values from the literature. Initial evaluations indicated a minor mean bias for non-ammonia, non-microbial nitrogen flow ([RUP + EndCP]/6.25) of 16 g of N per day. Root mean squared errors (RMSE) of EAA predictions ranged from 26.8 to 40.6% of observed mean values. Concordance correlation coefficients (CCC) of EAA predictions ranged from 0.34 to 0.55. Except for Leu, all ruminal EAA outflows were overpredicted by 3.0 to 32 g/d. In addition, small but significant slope biases were present for Arg [2.2% mean squared error (MSE)] and Lys (3.2% MSE). The overpredictions may suggest that the mean recovery of AA from acid hydrolysis across laboratories was less than estimates encompassed in the recovery factors. To test this hypothesis, several regression approaches were undertaken to identify potential causes of the bias. These included regressions of (1) residual errors for predicted EAA flows on each of the 3 protein-driven EA flows, (2) observed EAA flows on each protein-driven EAA flow, including an intercept, (3) observed EAA flows on the protein-driven EAA flows, excluding an intercept term, and (4) observed EAA flows on RUP and MiCP. However, these equations were deemed unsatisfactory for bias adjustment, as they generated biologically unfeasible predictions for some entities. Future work should focus on identifying the cause of the observed prediction bias.

Key words: mechanistic model, amino acid, ruminal outflow, tissue

INTRODUCTION

Protein supplementation represents a substantial proportion of the total cost of dairy rations, and in many cases dairy cows are fed protein in excess of their requirements. Overfeeding protein results in inefficient N use, causing excess N to be excreted into the environment and unnecessarily increasing feeding costs (Pacheco et al., 2012). One reason protein may be overfed is that protein supply and requirements are generally stated in MP terms; however, the true requirements are for EAA within that protein. To ensure that variation in the EAA composition of the MP supply does not contribute to production losses, nutritionists feed protein above true EAA requirements either to overcome the variance in EAA composition or as a safety margin against uncertainty in composition and intake. To balance rations on an EAA basis (i.e., EAA supply matches requirements), reducing the excess N supply, requires accurate and precise predictions of both EAA supply and requirements.

Several ration formulation systems have been developed and evaluated over the last 3 decades, including the Nutrient Requirements of Dairy Cattle from the National Research Council (NRC, 2001), the Cornell Net Carbohydrate-Protein System (**CNCPS**; Fox et al., 2004), the feeding system guidelines of the Institut National de la Recherche Agronomique (INRA, 2018), the Dutch DVE/OEB system (Tamminga et al., 1994), the British Feed Into Milk model (Offer et al., 2002), and the Scandinavian NorFor evaluation system (Volden, 2011). These models all predict ruminal protein outflow

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*Corresponding author: mhanigan@vt.edu

as a function of DMI and ration composition. The NRC (2001) model predictions of microbial N (**MiN**) have been evaluated and reported to have a relatively small (27 g/d) but significant ($P = 0.02$) mean bias over the range of data evaluated (St-Pierre, 2003). Predictions of non-ammonia, non-microbial N (**NANMN**) flow from NRC (2001) had significant linear bias when compared with experimental data (Bateman et al., 2005). Pacheco et al. (2012) subsequently evaluated the duodenal EAA flow predictions from different feeding systems and reported that the predicted duodenal flows of Arg and His were underestimated compared with observed flows when using the NRC (2001), whereas duodenal flow of all EAA except for Leu and Thr were overestimated when using the Agricultural Modeling and Training Systems (AMTS) or the Cornell-Penn-Miner (CPM) system. The NRC (2001) model predicts ruminal EAA outflows from dietary protein based on predictions of the fraction of protein from each feed that escapes ruminal degradation and the EAA composition of the feed protein. The summation of the individual feed EAA contributions is subsequently adjusted empirically for each EAA, based on the proportion of RUP in the total duodenal protein flow. It is possible that these empirical adjustments were required due to biased predictions of protein outflows or biased estimates of the EAA composition of those proteins.

The objectives of this work were (1) to construct a revised model of rumen EAA outflows based on updated predictions of RUP and microbial and endogenous protein flows (**MiCP** and **EndCP**, respectively) and revised EAA compositions of each protein fraction; and (2) to evaluate predictions of ruminal EAA outflows by the revised model, using literature data reporting observed post-ruminal flows of EAA.

MATERIALS AND METHODS

Metadata

The data set used to evaluate prediction models for MiN and RUP included studies used by White et al. (2017b) plus studies listed in Table 1. The data set used to evaluate predicted EAA flows included a subset of those studies that reported ruminal EAA outflow (Table 2). Summary statistics for these data sets are listed in Table 3. A mixture of duodenal and omasal sampling techniques were used in the studies, with 18% of the studies using omasal sampling (n = 118 treatment means) and 82% using duodenal sampling (n = 549 treatment means) in the full protein data set. The sampling distribution for the EAA data set was similar, with 20% using omasal sampling (n = 52 treatment means) and 80% using duodenal sampling (n = 202 treatment means).

Observations were deemed outliers if they were more than 3 standard deviations (SD) from the mean and were excluded before model parameterization and as-

Table 1. Studies used in addition to that of White et al. (2017b) for model evaluation of protein outflows from the rumen

Citation		
Appuhamy et al. (2011)	Ardalan et al. (2010)	Armentano et al. (1997)
Arriola Apelo et al. (2014)	Benchaar et al. (1991)	Benchaar et al. (1994a)
Benefield et al. (2009)	Berthiaume et al. (2001)	Berthiaume et al. (2006)
Blum et al. (1999)	Boerman et al. (2017)	Broderick et al. (2009)
Broderick et al. (2008)	Chen et al. (2011)	Chilliard and Doreau (1997)
Chow et al. (1990)	Colmenero and Broderick (2006b)	Colmenero and Broderick (2006a)
Davidson et al. (2008)	de Souza et al. (2017)	Donkin et al. (1989)
Drackley and Elliott (1993)	Drackley et al. (1994)	Elliott et al. (1996)
Giallongo et al. (2016)	Girard et al. (2005)	Guinard and Rulquin (1995)
Jenkins and Jenny (1989)	Kowalski et al. (2003)	Kowalski et al. (1999)
Kröber et al. (2000)	Kudrna et al. (2009)	Lara et al. (2006)
Leonardi et al. (2003)	Loor et al. (2002)	Loor et al. (2004)
Madsen (1986)	Misciatteilli et al. (2003)	Moller (1985)
Ohajuruka et al. (1991)	Oliveira et al. (1995)	Ordway et al. (2009)
Overton et al. (1998)	Overton et al. (1996)	Palmquist (1991)
Pantoja et al. (1996)	Papas et al. (1984a)	Papas et al. (1984b)
Piantoni et al. (2013)	Piantoni et al. (2015)	Pisulewski et al. (2002)
Pisulewski et al. (1996)	Polan et al. (1991)	Preynat et al. (2009)
Pruekvimolphan and Grummer (2001)	Rico et al. (2014)	Robinson et al. (2000)
Rogers et al. (1987)	Rulquin and Delaby (1997)	Rulquin et al. (2006)
Samuelson et al. (2001)	Simas et al. (1997)	Simas et al. (1998)
Socha et al. (2005)	Soder and Holden (1999)	Stern et al. (1983)
Vanhatalo et al. (1999)	Varvikko et al. (1999)	Weisbjerg et al. (1992)
Weiss and Wyatt (2004)	Wonsil et al. (1994)	Yang et al. (1997)

Table 2. Studies used for model evaluations of EAA outflows from the rumen

Citation		
Ahvenjärvi et al. (2002)	Aldrich et al. (1993)	Armentano et al. (1986)
Benchaar et al. (1994a)	Benchaar et al. (1994b)	Bernard et al. (2004)
Blauwiekel et al. (1997)	Brito and Broderick (2006)	Brito et al. (2007a)
Brito et al. (2007b)	Brito et al. (2009)	Broderick and Reynal (2009)
Cameron et al. (1991)	Chibisa et al. (2012)	Christensen et al. (1993)
Christensen et al. (1996)	Cunningham et al. (1993)	Cunningham et al. (1994)
Erasmus et al. (1994)	Eramus et al. (1992)	Fanchone et al. (2013)
Halmemies-Beauchet-Filleau et al. (2014)	Ipharraguerre et al. (2002)	Ipharraguerre et al. (2005a)
Ipharraguerre et al. (2005b)	Jones-Endsley et al. (1997)	Joy et al. (1997)
King et al. (1990)	Klusmeyer et al. (1990)	Klusmeyer et al. (1991a)
Klusmeyer et al. (1991b)	Korhonen et al. (2002)	Lynch et al. (1991)
Mabjeesh et al. (1996)	Mabjeesh et al. (1997)	Mansfield and Stern (1994)
McCarthy Jr. et al. (1989)	Merchen and Satter (1983)	Murphy et al. (1987)
Narasimhalu et al. (1989)	O'Mara et al. (1997)	O'Mara et al. (1998)
Overton et al. (1995)	Palmquist et al. (1993)	Pena et al. (1986)
Prange et al. (1984)	Price et al. (1988)	Putnam et al. (1997)
Reynal and Broderick (2003)	Reynal et al. (2005)	Reynal et al. (2007)
Robinson et al. (1994)	Robinson (1997)	Santos et al. (1984)
Schwab et al. (1992a)	Schwab et al. (1992b)	Stern et al. (1983)
Stern et al. (1985)	Teller et al. (1992)	Vanhatalo et al. (2009)
Van Vuuren et al. (1992)	Volden (1999)	Waltz et al. (1989)
Windschitl et al. (1988)	Yang and Beauchemin (2004)	Zerbini et al. (1988)

sessment (4 NANMN treatment means were removed from the protein data set). Dietary inputs to the model were derived from a combination of observed and tabular values as described by Hanigan et al. (2013) and Li et al. (unpublished data). Briefly, the reported nutrient composition of dietary ingredients was used when available. No studies in the data set reported all values required to conduct the work, and thus a feed library,

Table 3. Statistical summary of literature data used to evaluate prediction models

Data set[1]	N[2]	Parameter[3]	Mean	SD	Minimum	Maximum
Protein	667	DMI, kg/d	19.0	4.4	5.8	31.8
		Microbial outflow, g of N/d	278	105	64	763
		NANMN outflow, g of N/d	204	85.4	31	576
		BW, kg	601	60.7	319	788
		DIM	106	58.9	0.0	323
		Milk yield, kg/d	28.1	8.3	0.0	47.0
		Milk CP, %	3.14	0.2	2.6	3.9
		CP of the ration, %	17.3	2.2	10.3	29.6
EAA	254	DMI, kg/d	19.6	3.8	9.1	30.4
		Microbial outflow, g of N/d	284	100	100	743
		NANMN outflow, g of N/d	224	170	32.6	2400
		BW, kg	600	45.7	490	717
		DIM	116	50.3	50	230
		Milk yield, kg/d	30.2	6.5	13.0	41.7
		Milk CP, %	2.9	0.2	2.2	3.4
		CP of the ration, %	16.8	2.1	11.0	29.6
		Arg outflow, g/d	117	40.0	4.9	222
		His outflow, g/d	56.4	21.2	2.0	122
		Ile outflow, g/d	119	43.5	5.4	261
		Leu outflow, g/d	220	86.6	8.4	452
		Lys outflow, g/d	154	62.1	7.5	488
		Met outflow, g/d	47.7	19.8	2.4	110
		Phe outflow, g/d	127	45.6	5.7	260
		Thr outflow, g/d	122	42.6	6.0	270
		Val outflow, g/d	136	50.3	5.4	279

[1]Protein = studies used for model evaluation of ruminal outflow of proteins (Table 1); EAA = studies used for model evaluation of ruminal outflow of EAA (Table 2).

[2]N = number of observations.

[3]NANMN = non-ammonia, non-microbial nitrogen.

constructed from a combination of the NRC (2001) feed library, a library derived from commercial laboratory data (collected by the National Animal Nutrition Program, www.animalnutrition.org; Tran et al., 2019), and the CNCPS feed library, was used to generate missing values for each study. Where duplicate nutrient information occurred, precedence was generally given to the commercial feed library, followed by the NRC (2001) library, followed by the CNCPS library. In general, the proximate nutrients and protein fractions came from the commercial library, and EAA composition came from the CNCPS library (Higgs et al., 2015). The calculated dietary nutrient intakes from each ingredient were subsequently summed by diet and compared with observed dietary CP, ADF, NDF, starch, FA, and ether extract intakes, and mean bias by study for each nutrient was calculated. It was assumed that this bias reflected nutrient specification bias due to the use of varying amounts of library data, although sampling and analytical error within each study cannot be ruled out. The mean bias for each study was used to adjust the nutrient content of each ingredient in the study, with weighting by the contribution of that ingredient to the overall study nutrient sum. The SD of the adjustment factors was determined for each nutrient across studies, and nutrient adjustments were truncated to ±1 SD before use in adjusting ingredient values (Bateman et al., 2008). This approach preserves the variance among diets but removes the mean bias for the study. All of the metadata are available on the National Animal Nutrition Program website (www.animalnutrition.org).

Model Derivation

The model developed by White et al. (2017a) was used to predict RUP content ($RUP_{f,d}$, g/g) for each feed (f) within a diet (d), and RUP flow and the AA (i) associated with that flow ($RUPAA_{i,d}$, g/d) were calculated by summation for each diet:

$$RUP_{f,d} = \left[\alpha\left(CPa_{f,d}\right) + \beta\left(CPb_{f,d}\right) + CPc_{f,d}\right]$$

$$RUP_d = \sum_{f=1}^{n}\left[\frac{RUP_{f,d}}{100} \times \left(DMI_d \times \frac{DMIp_{f,d}}{100}\right)\right] \times 1{,}000 \quad [1]$$

$$RUPAA_{i,d} = \sum_{f=1}^{n}\left[\frac{FdAA_{i,f}}{RecAA_i \times 100} \times \frac{RUP_{f,d}}{100} \times \left(DMI_d \times \frac{DMIp_{f,d}}{100}\right)\right] \times 1{,}000,$$

where α and β were feed class–specific fractional passage coefficients; $CPa_{f,d}$ (% of DM) = CP that escapes from a ruminally incubated Dacron bag at time 0 for feed f within diet d; $CPb_{f,d}$ (% of DM) = CP that is insoluble and potentially degradable in the rumen for feed f within diet d; $CPc_{f,d}$ (% of DM) = CP that is undegradable in the rumen for feed f within diet d; and $FdAA_{i,f}$ represented the content of each AA in a feed (grams of AA per grams of CP), which were provided for use in the CNCPS library by Evonik (Hanau, Germany) as a complete data set reflecting their historical feed evaluations. In our model, DMI_d was dietary DMI in kilograms per day; and $DMIp_{f,d}$ represented the dietary inclusion rate of each feed (% of DM). $RecAA_i$ represented the recovery factor accounting for incomplete recovery of AA from a 24-h acid hydrolysis (grams per grams of true AA; Table 4), which was adapted from Lapierre et al. (2016b) by simple inversion of the factors. These are necessary, as recovery levels vary among AA based on their chemical characteristics. For example, the full release of the branched-chain AA (Ile, Leu, and Val) requires more than 24 h of acid hydrolysis due to the stable nature of peptide bonds formed with those AA (Blackburn, 1968), whereas the recovery of less-stable EAA (e.g., Thr) is incomplete due to partial degradation of the released AA during the 24 h of hydrolysis (Rees, 1946). Similarly, the recovery factors listed in Table 4 were used when calculating the microbial and endogenous protein flows to account for incomplete recovery of AA from a 24-h acid hydrolysis.

Equation [2], from Roman-Garcia et al. (2016), was used to predict MiN flows (grams per day):

$$MiN_d = -52.2 + 122 \times SMPLoc_d + 12.5 \times DMI_d + 1.23 \times RDStp_d + 2.23 \times \frac{RDSt_d}{RDNDF_d}, \quad [2]$$

where $SMPLoc_d$ was the sampling location (0 = duodenal and 1 = omasal); $RDStp_d$ was the apparent ruminal digestibility of starch (% of starch intake) for each diet; and $RDSt_d$ and $RDNDF_d$ represented the amount of starch and NDF degraded in the rumen (kilograms per day), respectively. The parameters $RDSt_d$ and $RDNDF_d$ were predicted using Equations [4] and [5] of White et al. (2016) with an intercept adjustment to address a significant mean bias ($P < 0.001$) observed in initial model evaluations:

$$RDStp_d = 58.5 - 1.45 \times DMI_d + 0.424 \times NDF_{Forage,d} + 1.39 \times St_d - 0.0219 \times St_d^2 - 0.154 \times Forage_{Wet,d}, \quad [3]$$

Table 4. Acid hydrolysis recovery factors and corrected EAA profiles of microbial true protein (MiTP) and endogenous crude protein (EndCP)

Item	Recovery[1]	MiTP profile[2]	EndCP profile[3]
Arginine	0.97	5.29	4.49
Histidine	0.98	2.08	2.75
Isoleucine	0.89	6.95	4.09
Leucine	0.93	9.22	7.70
Lysine	0.94	9.37	6.22
Methionine	0.95	2.61	1.26
Phenylalanine	0.91	6.43	4.09
Threonine	0.95	6.29	5.26
Valine	0.90	6.90	5.33

[1]Factors indicating the incomplete recovery of AA obtained after 24-h hydrolysis [1/correction factors proposed by Lapierre et al. (2016b)].

[2]Grams of AA per 100 g of true protein, corrected to account for incomplete recovery with a 24-h acid hydrolysis (Sok et al., 2017).

[3]Grams of AA per 100 g of CP; from Orskov et al. (1986) corrected for incomplete recovery with a 24-h acid hydrolysis (Lapierre et al., 2016b).

$$RDSt_d = \frac{RDStp_d}{100} \times StIn_d, \quad [4]$$

$$RDNDFp_d = -31.9 + 0.721 \times NDF_d - 0.247 \times St_d + 6.63 \times CP_d - 0.211 \times CP_d^2 - 0.387 \times \frac{ADF_d}{NDF_d} \times 100 - 0.121 \times Forage_{Wet,d} + 1.51 \times DMI_d, \quad [5]$$

$$RDNDF_d = \frac{RDNDFp_d}{100} \times (NDFIn_d), \quad [6]$$

where $StIn_d$ and $NDFIn_d$ represented dietary intakes of starch and NDF (kilograms per day), respectively. Forage NDF ($NDF_{Forage,d}$), dietary starch (St_d), wet forage ($Forage_{Wet,d}$), NDF_d, CP_d, and ADF_d were all expressed as a proportion of the dry matter from wet forage in the diet.

Microbial N flows from Equation [2] were converted to a true protein basis (***MiTP***, grams per day) assuming 16% N and 0.824 g of true protein per gram of CP (Sok et al., 2017):

$$MiTP_d = MiN_d \times 6.25 \times 0.824. \quad [7]$$

The microbial EAA ($MiEAA_{i,d}$, grams per day) flow to the duodenum was calculated as the product of $MiTP_d$ and the EAA profile of microbial protein ($MiProf_i$, grams of EAA per 100 g of MiTP; Table 4):

$$MiEAA_{i,d} = MiTP_d \times \frac{MiProf_i}{100}, \quad [8]$$

where i represented each individual EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val). The AA profile reflects a mix of fluid-associated bacteria, particle-associated bacteria, and protozoa (Sok et al., 2017), with corrections for incomplete recovery during hydrolysis.

Endogenous crude protein flowing at the duodenum ($EndCP$, g/d) was predicted using the equation of Lapierre et al. (2016a):

$$EndCP_d = \left(15.4 + 1.21 \times DMI_d\right) \times 6.25, \quad [9]$$

where DMI was in kilograms per day. Total non-ammonia, non-microbial CP outflow ($NANMCP_d$, g/d) was the sum of RUP and endogenous protein flows:

$$NANMCP_d = RUP_d + EndCP_d. \quad [10]$$

The flow of each EAA associated with endogenous protein ($EndAA_{i,d}$, g/d) was estimated as the product of $EndCP_d$ multiplied by its EAA profile ($EndProf_i$, g of AA per 100 g of CP; Table 4):

$$EndAA_{i,d} = EndCP_d \times \frac{EndProf_i}{100}. \quad [11]$$

The AA profile was estimated as the mean of the ruminal and abomasal isolates from Orskov et al. (1986), except for Leu, where only the ruminal isolate was retained and therefore the AA profile was estimated only using the ruminal isolate. As for the other proteins, the observed profile was corrected for incomplete recovery during hydrolysis.

Total ruminal EAA outflows ($EAA_{outflow,i,d}$, grams per day) were the sum of AA flows from the 3 protein sources:

$$EAA_{outflow_{i,d}} = \left(RUPAA_{i,d} + MiEAA_{i,d} + EndAA_{i,d}\right). \quad [12]$$

Because all EAA flows represented our best estimates of the true flow, an additional set of flow predictions were required for direct comparison to observations from 24-h hydrolysis reported in the literature. This was achieved by multiplying the predicted true flows by the recovery factors listed in Table 4 ($RecAA_{i,d}$):

$$EAA_{outflow-24h_{i,d}} = \left(RUPAA_{i,d} + MiEAA_{i,d} + EndAA_{i,d}\right) \times RecAA_{i,d}. \quad [13]$$

Evaluating Prediction Errors

All work was conducted using R (Ver. 3.2.2; R Core Team, 2015) unless otherwise specified. Linear mixed effects regression was conducted using the lmer function of the lme4 package (Kuznetsova et al., 2017). When deriving EAA bias adjustments, EAA flow residuals were initially regressed on each of the individual protein flows, including a random effect of study in the model. Subsequently observed total EAA flows were regressed on the individual protein EAA flows both with and without random study effects. An additional bias adjustment was evaluated, where observed total EAA flows were regressed only on RUP and MiCP EAA flows with random study effects. Backward elimination was used to evaluate the bias adjustment approaches, with a significance level of 0.05 used for elimination of factors from the model. Model performance was evaluated using root mean squared error (**RMSE**) as described by Bibby and Toutenburg (1978), concordance correlation coefficient (**CCC**) as described by Lin (1989), and mean and slope bias with a significance level of 0.05. Variance associated with mean and slope biases was expressed as a percentage of mean squared error (**MSE**), and considered small, moderate, or large if <5%, 5–10%, or >10%, respectively.

RESULTS AND DISCUSSION

Initial evaluations of rumen protein outflows showed a small mean bias for NANMN (Table 5). Although significant ($P < 0.01$), it was biologically irrelevant at 16 g of nitrogen per day, representing 4% of MSE, and thus NANMN was deemed adequate for further work (Figure 1). We found no mean or slope bias for MiN predictions (Figure 2), and it was also deemed adequate for further work (Table 5), although a single study appeared to possibly be an outlier.

Evaluations of predicted flows, adjusted to reflect incomplete recovery from acid hydrolysis and observed ruminal EAA outflows, showed significant mean bias ($P < 0.05$) for all EAA except Leu and significant slope bias ($P < 0.05$) for Arg and Lys (Table 5). The residual plots for 4 EAA (His, Lys, Met, and Val) are shown in Figure 3. Mean bias ranged from 0.00 to 25.9% of MSE, all being overpredicted, with CCC ranging from 0.34 to 0.55. Because MiN and NANMN predictions were unbiased, the expectation was that predictions of post-ruminal flows of EAA should be similarly unbiased. The prediction errors could be driven by (1) missing dietary nutrient interactions, (2) incorrectly specified feed composition, (3) systematic bias in the AA composition of one or more protein fractions, or (4) poorer acid hydrolysis recovery in practice than reflected in the adjustment factors used. Except for the last, residuals could be expected to be correlated with the driving components. To test this hypothesis, several regression approaches were evaluated, including (1) residuals from each EAA prediction regressed on the corresponding predicted microbial, RUP, and endogenous EAA flows including an intercept term, (2) observed EAA prediction flows regressed on the corresponding predicted microbial, RUP, and endogenous EAA flows including an intercept term, (3) observed EAA prediction flows regressed on the predicted protein EAA flows without the inclusion of an intercept term, and (4) observed EAA prediction flows regressed on predicted RUP and MiCP EAA flows including an intercept.

Using the first regression approach, all 3 predicted protein flows were found to be correlated ($P < 0.05$) with the residuals for Arg, His, Ile, Lys, Thr, and Val. For Leu, only EndCP was found to be correlated with the residuals (Table 6). We found no significant correlations for Met or Phe. Because the majority of EAA had strong correlations between the protein flows and residuals, this suggests that the prediction errors may be caused by errors in the specification of the AA composition of those protein fractions. It is perhaps not surprising that RUP appeared to contribute to the prediction errors, as the passage of RUP is a function of multiple ingredients, which may not all be accurately predicted, and thus this system increases the chance of prediction problems (White et al., 2017a). Indeed, one or more ingredients may have poor predictions of RUP outflow, the latter being based on tabular values of rumen degradability. In addition, the AA composition of

Table 5. Summary of evaluations of predictions of ruminal protein and EAA outflows, adjusted to reflect incomplete recovery from acid hydrolysis; observed values are as reported

Variable[1]	Adjustment[2]	N[3]	Observed mean	Predicted mean	RMSE,[4] % of observed mean	Mean bias, % of MSE[5]	Slope bias, % of MSE	CCC[6]
NANMN, g/d		236	212	197	37.2	3.97*	0.02	0.35
MiN, g/d		236	294	290	24.0	0.20	0.11	0.68
dStRum, kg/d		55	3.68	3.64	34.0	0.09	11.9*	0.49
dNDFRum, kg/d		125	4.02	4.10	21.2	0.86	6.81*	0.47
Arg, g/d	None	229	120	138	31.8	23.9*	2.2*	0.44
	1			119	25.4	0.0	0.3	0.55
	2			113	26.2	4.1*	0.9	0.51
	3			112	27.8	5.4*	0.3	0.48
	4			117	27.1	0.4	0.8	0.50
His, g/d	None	234	57.9	60.9	31.0	2.8*	0.6	0.44
	1			57.5	28.8	0.1	0.0	0.50
	2			55.6	29.1	1.9*	0.1	0.49
	3			56.9	29.9	0.3	1.5	0.41
	4			58.0	31.0	0.0	1.0	0.43
Ile, g/d	None	234	122	138	31.4	16.1*	1.3	0.45
	1			122	27.5	0.0	0.5	0.49
	2			103	32.3	25.2*	1.7*	0.36
	3			111	29.9	10.4*	2.8*	0.38
	4			105	32.0	19.5*	0.0	0.39
Leu, g/d	None	234	227	228	28.2	0.0	1.4	0.54
	1			226	27.3	0.0	0.1	0.61
	2			200	29.3	15.6*	3.5*	0.54
	3			212	29.3	4.6*	1.6	0.51
	4			215	29.0	3.1*	1.4	0.52
Lys, g/d	None	232	158	190	40.6	25.9*	3.2*	0.34
	1			157	31.8	0.0	1.2	0.43
	2			144	33.1	7.1*	1.8*	0.40
	3			151	33.4	1.6	4.8*	0.32
	4			154	34.8	0.5	1.4	0.36
Met, g/d	None	233	49.1	55.5	32.1	16.2*	1.4	0.53
	1			48.6	32.0	0.1	1.9*	0.45
	2			46.0	29.7	4.6*	0.4	0.59
	3			46.4	29.6	3.5*	0.4	0.59
	4			46.0	29.7	4.6*	0.4	0.59
Phe, g/d	None	234	131	143	28.0	10.4*	0.2	0.51
	1			130	26.1	0.1	0.9	0.51
	2			112	29.8	23.0*	1.2	0.43
	3			120	27.8	10.0*	0.2	0.48
	4			120	27.7	9.4*	0.1	0.48
Thr, g/d	None	234	125	138	26.8	15.1*	0.0	0.55
	1			123	23.6	0.2	1.6	0.61
	2			114	26.0	10.8*	3.3*	0.52
	3			119	25.4	3.7*	0.2	0.55
	4			118	25.5	4.1*	0.3	0.55
Val, g/d	None	234	140	152	30.6	7.6*	0.6	0.44
	1			139	28.2	0.0	0.2	0.48
	2			119	31.9	22.1*	1.9*	0.39
	3			128	30.7	7.4*	4.6*	0.33
	4			121	32.4	16.9*	0.0	0.37

[1]NANMN = non-ammonia, non-microbial N flow (g/d); MiN = microbial N flow (g/d); dStRum = digestible starch in the rumen (kg/d); dNDFRum = digestible NDF in the rumen (kg/d).

[2]None = ruminal EAA outflows (g/d) as predicted by the model; 1 = model predictions (g/d) adjusted using regressions of residual EAA flows on protein EAA flows including an intercept; 2 = model predictions (g/d) adjusted using regressions of observed EAA flows on protein EAA flows including an intercept; 3 = model predictions (g/d) adjusted using regressions of observed EAA flows on protein EAA flows excluding an intercept; 4 = model predictions (g/d) adjusted using regressions of observed EAA flows on RUP and microbial CP flows including an intercept.

[3]N = number of observations.

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

[5]MSE = mean squared error.

[6]CCC = concordance correlation coefficient.

*$P \leq 0.05$.

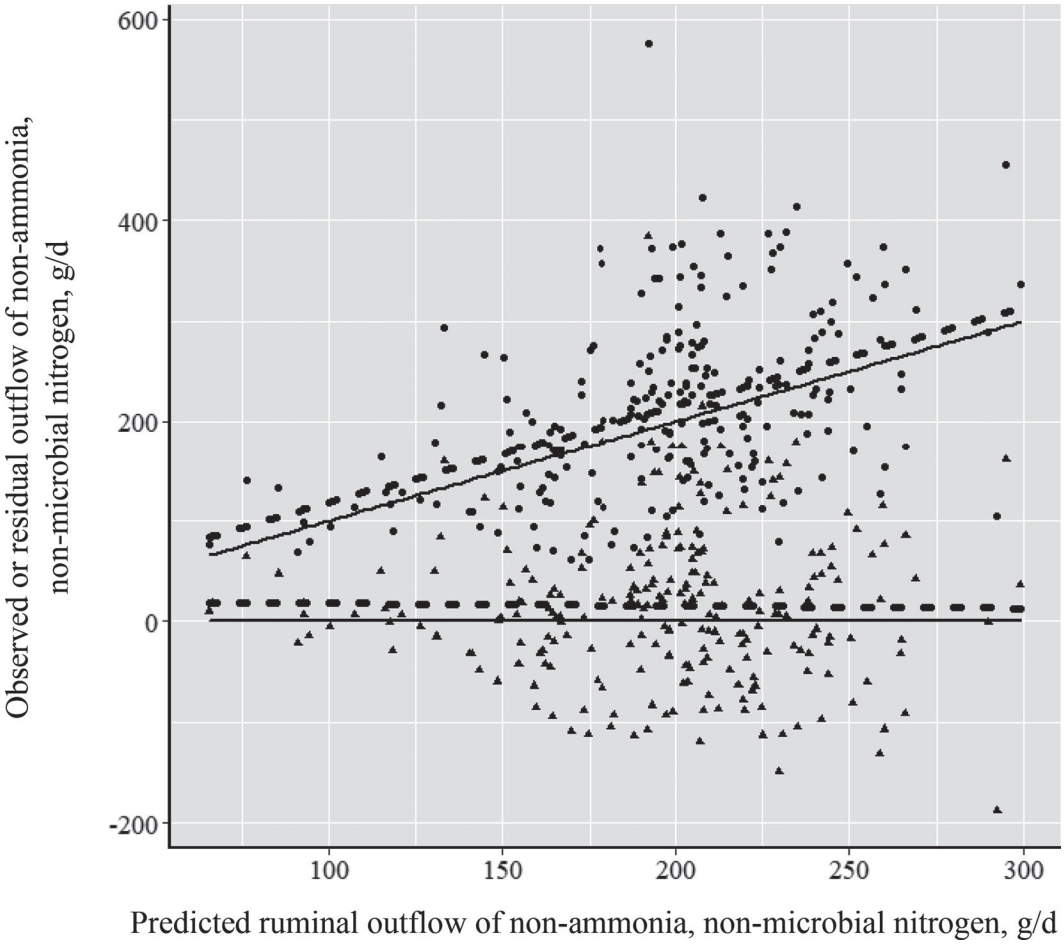

Figure 1. Predictions of ruminal outflow of non-ammonia, non-microbial nitrogen using Equation [10]. The solid lines represent unity, and the dashed line is the best-fit linear equation relating observed or residuals to predicted. Circles represent observed values, and triangles represent predicted values.

RUP was assumed to be similar to the AA composition of the feed ingredient, which is known to be false in some cases (Maxin et al., 2013), but not enough data are available to address this assumption. However, if these were the root causes of the problem, it seems more likely they would manifest as random variations, given the large range in diets used, and thus would not be correlated with RUP flow.

Endogenous protein composition may also contribute to prediction errors because of the very few observations of its AA composition (Lapierre et al., 2006). It is more surprising that MiCP flows were significantly correlated with the residuals, because the composition of MiCP has been well characterized and is thought to be fairly constant (Clark et al., 1992).

Even though the analyses indicated that all 3 protein flows were contributing to the prediction errors, the contribution of EndCP was predicted to be much greater than that of RUP or MiCP. This suggests that endogenous EAA composition or protein flow may be poorly specified. The bias-adjusted predictions reduced mean bias to less than 1% of MSE and yielded CCC ranging from 0.43 to 0.61. We discovered small but significant slope biases for Met ($P < 0.05$; 1.9% MSE) and Thr ($P < 0.10$; 1.6% MSE). However, the results obtained from this regression approach were not biologically feasible. Most of the duodenal AA flow is comprised of RUP and MiCP, with EndCP representing typically less than 20% of the total duodenal flow (Lapierre et al., 2006), yet EndCP-based adjustments suggested by the residual analysis were larger than the actual duodenal flows, with partially offsetting negative adjustments associated with MiCP and RUP. This obviously is not reflective of any potential bias in the individual protein-based flows.

Using the second regression approach, where the 3 protein-driven EAA flows were regressed on observed EAA flows with an intercept term, resulted in signifi-

cance for RUP and endogenous EAA flow for all AA flows excluding Met, which had significant terms for RUP and microbial flows. The resulting predictions resulted in mean bias ($P < 0.05$; > 2% MSE) for all AA and slope bias for all AA except Arg, His, Met, and Phe ($P < 0.05$), yielding CCC ranging from 0.36 to 0.59. As above, this approach resulted in biologically unfeasible solutions, with the majority of bias associated with EndCP flow and minimal contribution from the remaining proteins.

Using the third regression approach did not address all of the mean and slope biases for the majority of the AA ($P < 0.005$) and resulted in CCC ranging from 0.32 to 0.59. Using this approach, His, Ile, Lys, and Val flow predictions were correlated with EndCP flows that were still several fold greater than the predicted flows for each protein, and thus this regression approach was not biologically feasible (Table 6).

A possible reason for EndCP being such a large driver in the regression approaches was its single input of DMI, which perhaps introduces less variation into the regression model than the more complicated MiTP and RUP equations. The final regression approach evaluated was performed using only RUP and MiCP terms against the observed AA flows and excluded the EndCP term. This approach yielded more biologically sensible parameters but still large adjustments, ranging from 0.5 to 0.9 g per day for RUP and 0.9 to 1.2 g per day for MiCP. Using this approach still resulted in significant mean bias for all EAA excluding Arg, His, and Lys ($P < 0.05$), with CCC ranging from 0.36 to 0.59.

Evaluating the 4 methods used to adjust protein flows based purely on statistical performance, bias adjustment based on protein flow using EAA residuals as the regressor provided the best accuracy and precision (Figure 4). However, the adjustments are outside of

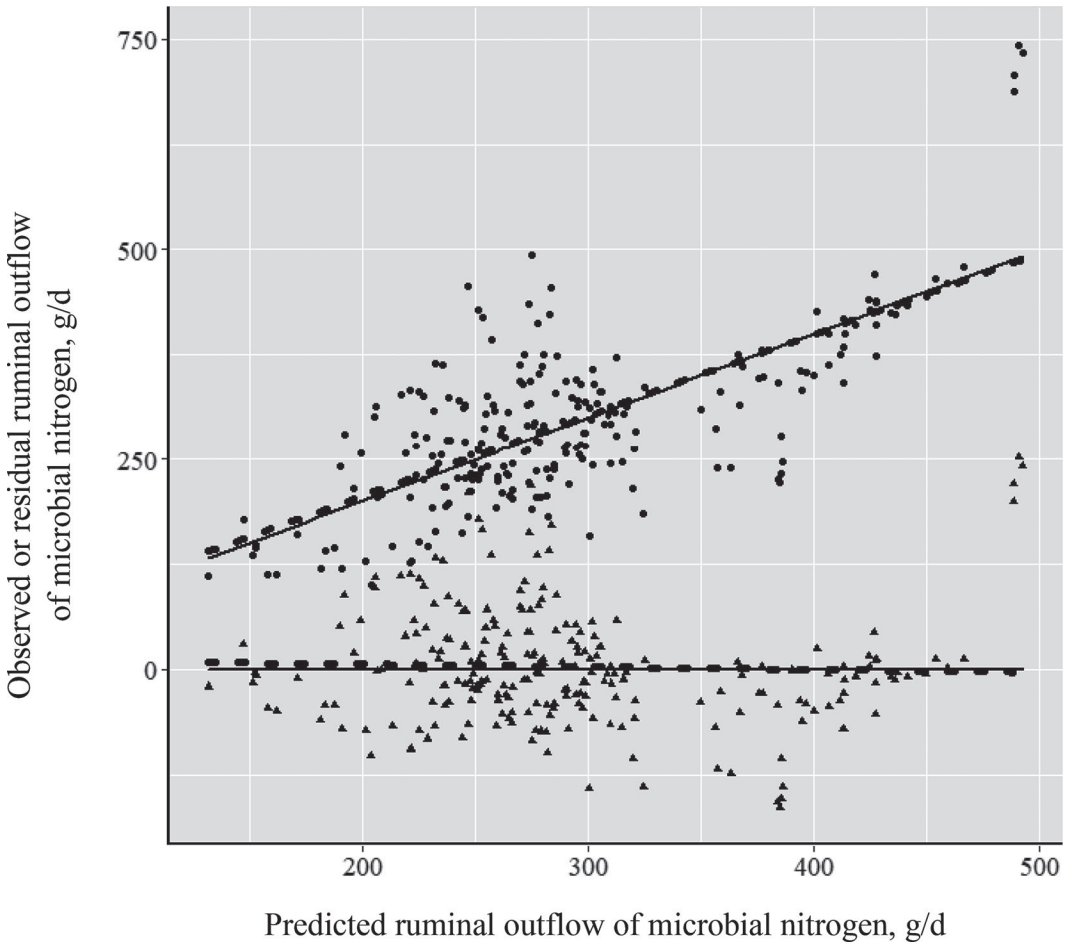

Figure 2. Predictions of ruminal outflow of microbial nitrogen using Equation [2]. The solid lines represent unity, and the dashed line is the best-fit linear equation relating observed or residuals to predicted. Circles represent observed values, and triangles represent predicted values.

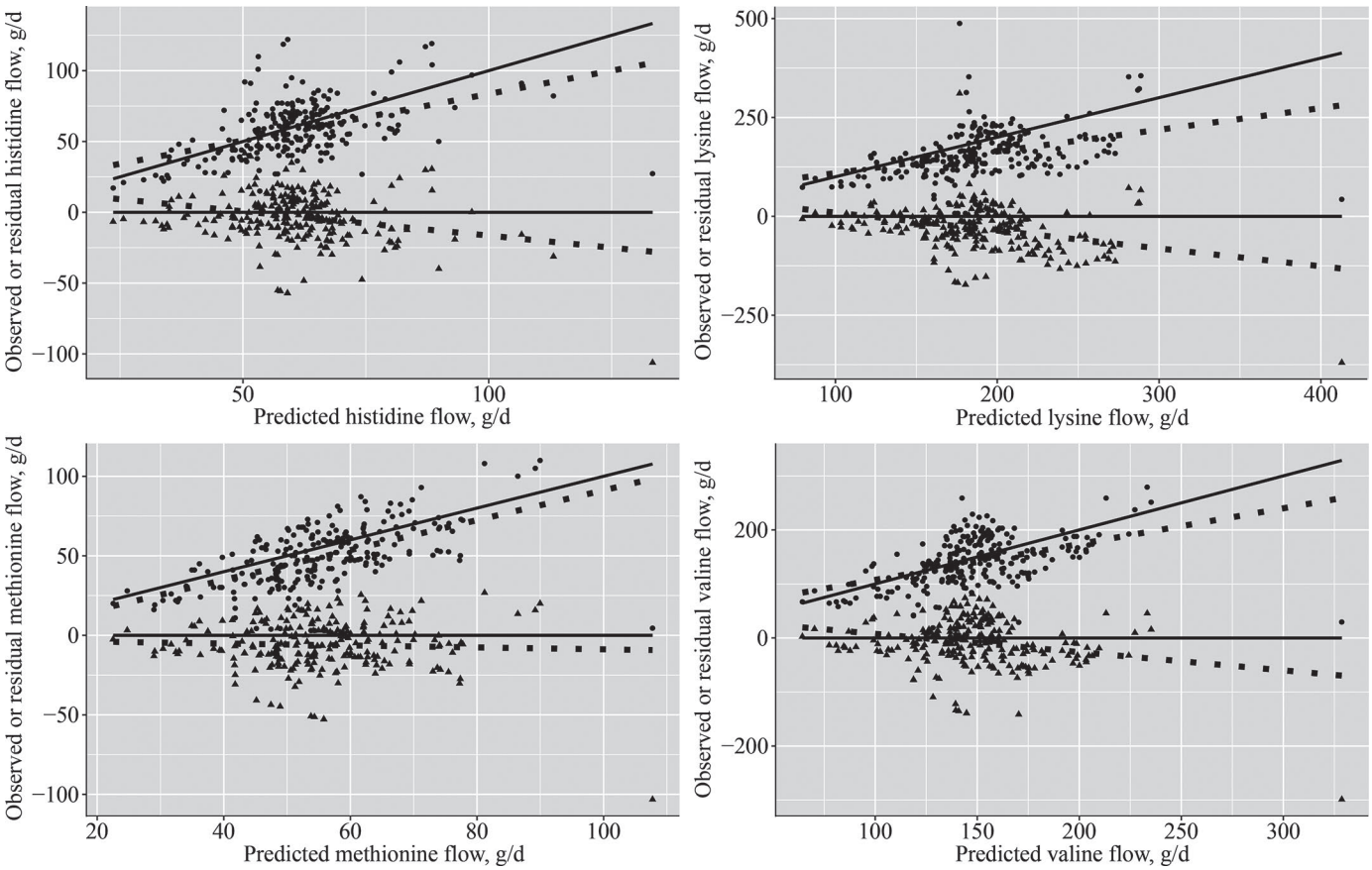

Figure 3. Duodenal EAA flows (g/d) as predicted by the model. The solid lines represent unity, and the dashed lines are the best-fit linear equations relating observed or residuals to predicted. Predicted values (triangles) were adjusted to reflect incomplete recovery from acid hydrolysis, to match the observed data (circles).

the likely range, suggesting that the problem is rooted in some aspect of the measurement. The generally low observed values, compared with predicted, suggest that the problem may be rooted in inadequate techniques when conducting the acid hydrolysis, resulting in generally lower recoveries than the values reported by Lapierre et al. (2016b) in the majority of laboratories. Such a conclusion is consistent with the observed residual errors. It also may explain the correlations with the protein flows. If recovery were, for example, 90% of the true values, the magnitude of the bias would be greater as true flows increased, which would present as a correlation with the predicted protein flows, assuming they are predicted without bias. Although we cannot test this hypothesis with the current data, it is the explanation that best aligns with the observed errors.

Given that Leu is predicted without bias and the mean biases for Val and Ile are relatively less than for other EAA, it seems more likely that the problem, if it is due to acid hydrolysis recovery, may be too much time or temperature. The opposite would be expected to result in incomplete recovery of the branched-chain AA. However, the relative error for Thr might be expected to be greater than for other EAA if that were the case, and such is not clearly evident. Recently, we have adopted a newer technique using much higher temperatures and a shorter duration (Walsh et al., 2014) and found that the recovery of EAA is improved. Although one cannot rule out other factors as the potential source of the bias, it is more difficult to conceive of other sources of variation that would cause the observed pattern in errors. More work is needed to determine the cause of the bias.

CONCLUSIONS

Based on the work herein, EAA flows can be predicted with moderate accuracy and precision from RUP, microbial, and endogenous CP flows if the observed data are corrected for losses during hydrolysis. The

observed negative mean and slope bias may be due to prediction problems or under-reporting of true flows in the literature due to recovery problems.

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Table 6. Results of backward elimination regression approaches of observed or residual EAA flows on predicted EAA flows (g/d) arising from RUP, microbial, and endogenous protein flows from the rumen[1]

EAA	Regression approach[2]	N	INT ± SE	RUP ± SE	MiCP ± SE	EndCP ± SE	AICc	BIC
Arg	1	229	−55.2 ± 21.3	−0.5 ± 0.1	−0.7 ± 0.3	10.4 ± 3.6	1,987	2,007
	2		−64.6 ± 19.2	0.4 ± 0.1		14.5 ± 1.8	1,986	2,003
	3			0.5 ± 0.1	1.2 ± 0.1		1,992	2,006
	4		NS	0.5 ± 0.1	1.2 ± 0.1		1,992	2,006
His	1	234	−30.8 ± 11.8	−0.2 ± 0.1	−1.1 ± 0.4	9.9 ± 3.1	1,763	1,783
	2		−28.7 ± 10.7	0.8 ± 0.1		9.8 ± 1.6	1,761	1,778
	3			0.8 ± 0.1		5.7 ± 0.4	1,766	1,779
	4		NS	0.9 ± 0.1	1.2 ± 0.1		1,771	1,784
Ile	1	234	−48.1 ± 20.4	−0.3 ± 0.1	−0.7 ± 0.2	11.5 ± 3.9	2,010	2,030
	2		−56.3 ± 18.4	0.5 ± 0.1		15.6 ± 1.9	2,008	2,025
	3			0.6 ± 0.1	0.5 ± 0.2	5.1 ± 2.4	2,013	2,030
	4		NS	0.6 ± 0.1	0.9 ± 0.1		2,015	2,029
Leu	1	234	−85.6 ± 35.8			4.4 ± 1.9	2,319	2,333
	2		−135 ± 35.5	0.9 ± 0.1		15.0 ± 1.9	2,318	2,335
	3			0.9 ± 0.1	1.1 ± 0.1		2,325	2,339
	4		NS	0.9 ± 0.1	1.1 ± 0.1		2,325	2,339
Lys	1	232	−76.3 ± 31.6	−0.2 ± 0.1	−1.1 ± 0.3	13.4 ± 3.9	2,196	2,217
	2		−69.9 ± 28.6	0.7 ± 0.1		12.7 ± 1.9	2,194	2,211
	3			0.7 ± 0.1		8.3 ± 0.5	2,198	2,212
	4		NS	0.8 ± 0.1	0.9 ± 0.1		2,205	2,219
Met	1	233					1,583	1,593
	2		−10.5 ± 5.2	0.8 ± 0.1	1.2 ± 0.1		1,583	1,600
	3			0.7 ± 0.1	1.0 ± 0.1		1,585	1,598
	4		−10.5 ± 5.2	0.8 ± 0.1	1.2 ± 0.1		1,583	1,600
Phe	1	234					2,051	2,061
	2		−68.5 ± 20.1	0.7 ± 0.1		16.3 ± 2.0	2,051	2,068
	3			0.7 ± 0.1	1.0 ± 0.1		2,054	2,068
	4		NS	0.7 ± 0.1	1.0 ± 0.1		2,054	2,068
Thr	1	234	−50.9 ± 18.8	−0.3 ± 0.1	−0.5 ± 0.2	7.2 ± 2.7	1,974	1,994
	2		−65.2 ± 17.1	0.7 ± 0.1		12.6 ± 1.4	1,975	1,992
	3			0.8 ± 0.1	1.0 ± 0.1		1,978	1,992
	4		NS	0.8 ± 0.1	1.0 ± 0.1		1,978	1,992
Val	1	234	−56.1 ± 23.3		−0.7 ± 0.3	9.0 ± 3.4	2,071	2,088
	2		−62.7 ± 17.1	0.1 ± 0.8		1.4 ± 12.4	2,068	2,086
	3			0.8 ± 0.1		7.8 ± 0.5	2,075	2,089
	4		NS	0.8 ± 0.1	0.9 ± 0.1		2,076	2,090

[1]Terms were retained in the model at $P < 0.05$. N = number of observations; INT = intercept; MiCP = microbial CP; EndCP = endogenous CP; AICc = second-order Akaike information criterion; BIC = Bayesian information criterion.

[2]Regression approaches: 1 = model predictions (g/d) adjusted using regressions of residual EAA flows on protein EAA flows including an intercept; 2 = model predictions (g/d) adjusted using regressions of observed EAA flows on protein EAA flows including an intercept; 3 = model predictions (g/d) adjusted using regressions of observed EAA flows on protein EAA flows excluding an intercept; 4 = model predictions (g/d) adjusted using regressions of observed EAA flows on RUP and MiCP flows including an intercept.

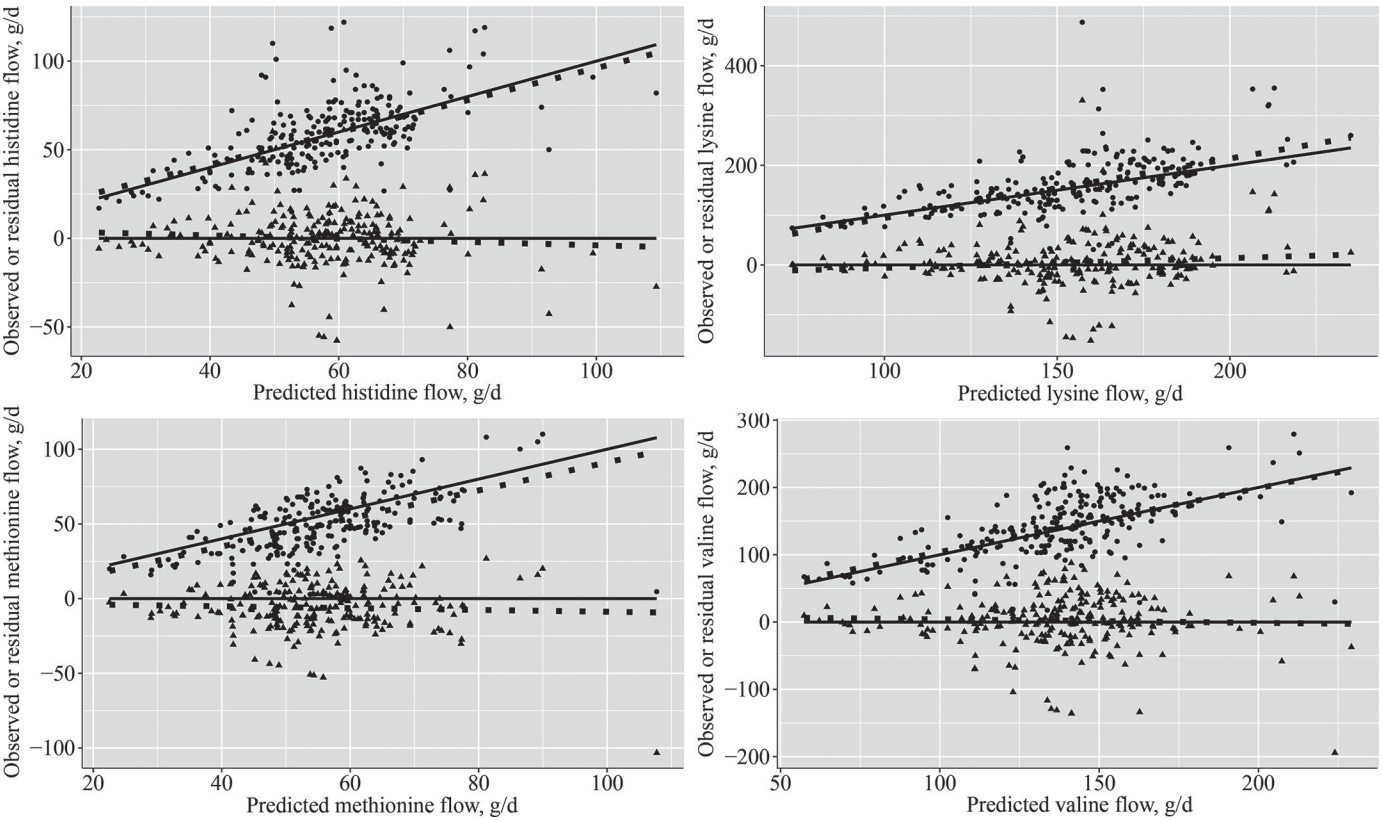

Figure 4. Predicted duodenal EAA flows (g/d) using bias adjustments based on the component proteins. The solid lines represent unity, and the dashed lines are the best-fit linear equations relating observed (circles) or residuals to predicted (triangles). All data are unadjusted for hydrolysis recovery.

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ORCIDS

A. J. Fleming https://orcid.org/0000-0002-6122-5962
H. Lapierre https://orcid.org/0000-0002-1162-7226
P. J. Kononoff https://orcid.org/0000-0001-6069-2174
W. P. Weiss https://orcid.org/0000-0003-3506-4672
M. D. Hanigan https://orcid.org/0000-0002-5639-9677