Revised digestive parameter estimates for the Molly cow model

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ABSTRACT

The Molly cow model represents nutrient digestion and metabolism based on a mechanistic representation of the key biological elements. Digestive parameters were derived ad hoc from literature observations or were assumed. Preliminary work determined that several of these parameters did not represent the true relationships. The current work was undertaken to derive ruminal and postruminal digestive parameters and to use a meta-approach to assess the effects of interactions among nutrients and identify areas of model weakness. Model predictions were compared with a database of literature observations containing 233 treatment means. Mean square prediction errors were assessed to characterize model performance. Ruminal pH prediction equations had substantial mean bias, which caused problems in fiber digestion and microbial growth predictions. The pH prediction equation was reparameterized simultaneously with the several ruminal and postruminal digestion parameters, resulting in more realistic parameter estimates for ruminal fiber digestion, and moderate reductions in prediction errors for pH, neutral detergent fiber, acid detergent fiber, and microbial N outflow from the rumen; and postruminal digestion of neutral detergent fiber, acid detergent fiber, and protein. Prediction errors are still large for ruminal ammonia and outflow of starch from the rumen. The gain in microbial efficiency associated with fat feeding was found to be more than twice the original estimate, but in contrast to prior assumptions, fat feeding did not exert negative effects on fiber and protein degradation in the rumen. Microbial responses to ruminal ammonia concentrations were half saturated at 0.2 mM versus the original estimate of 1.2 mM. Residuals analyses indicated that additional progress could be made in predicting microbial N outflow, volatile fatty acid production and concentrations, and cycling of N between blood and the rumen. These additional corrections should lead to an even more robust representation of

the effects of dietary nutrients on ruminal metabolism and nutrient absorption, of animal performance, and the environmental impact of dairy production.

Key words: bovine, metabolism, digestion, model

INTRODUCTION

Accurate and precise models relating dietary inputs and animal performance are required to devise feeding strategies that minimize feed costs while maximizing animal performance and minimizing the environmental impact (Gregorini et al., 2010). A variety of nutrient requirement models have been developed over time to address this challenge (AFRC, 1993, 1998; NRC, 1978, 1984, 2001). These models can be adapted to simultaneously consider nutrient requirements and environmental loading (Hadrich et al., 2008), although this is limited to nutrients considered by the models. However, given the static nature of these models, it will not be easy to modify them to consider other management strategies that are time dependent, such as feeding frequency, supplementation timing, grazing methods, and reproductive management, among others (Beukes et al., 2010, 2011).

Mechanistic models such as Molly (Baldwin et al., 1987a,b,c; Danfær, 1990; Dijkstra et al., 1992) offer several potential benefits, including the ability to represent interactions between the animal and digestive processes, the ability to represent more processes that can be tested against empirical data and serves to integrate knowledge, and, in the case of the above dynamic models, the ability to represent time-dependent processes. Molly also offers the opportunity to predict directly most of the animal-related factors that affect the environment, including methane, CO₂, urinary urea, and fecal N. However, increased complexity increases the number of equations that must be parameterized. Unlike the above nutrient requirement models, Molly has not been subjected to extensive efforts to derive robust parameter estimates associated with the various digestive and metabolic processes considered in the model.

Digestion parameters in Molly were previously derived using data from the literature (Hanigan et al., 2009). Although these parameters were much better

Received September 21, 2012.

Accepted February 9, 2013.

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than the original parameters derived by Baldwin et al. (1987a,b,c), the prediction errors for ruminal pH, ammonia and VFA concentrations, and lipid and microbial N flow were biased (Hanigan et al., 2009 and subsequent unpublished work).

For these prior analyses, nutrient analyses of ingredients were used when available and missing values were filled with tabular NRC values to achieve a full nutrient profile. This approach can lead to potential bias when predicting dietary nutrients and may have contributed to the observed model prediction bias (Hanigan et al., 2009). Another limitation with the prior work was that ruminal pH was underpredicted by approximately 0.5 U, which depressed microbial activity. This could have contributed to the observed biases in VFA concentrations.

It was hypothesized that rederivation of ruminal pH, digestion, and microbial growth parameters in the Molly cow model, while attempting to accommodate potential nutrient input bias, would result in more accurate and precise predictions of ruminal digestion and nutrient outflow from the rumen and postruminal nutrient digestion than the current model. The objective of the current work was to use a data set constructed from the literature to solve for a set of parameters that resulted in unbiased predictions of ruminal pH, ruminal nutrient degradation, microbial growth, and postruminal digestion.

MATERIALS AND METHODS

The model used was that of Baldwin et al. (1987a,b,c), with modifications described by Baldwin (1995) and Hanigan et al. (2006, 2007, 2009). Simulations were

conducted with ACSL Math software (version 2.5.4; Aegis Technologies Group Inc., Huntsville, AL). The model was set to use observed DMI, to consume feed at a constant rate, to predict ruminal liquid volume from ruminal DM mass, to milk out twice daily, and to use the VFA stoichiometry set for a mixed forage and concentrate diet (Argyle and Baldwin, 1988) for all observations, which reflected the nature of the diets in the database used. The model was set to run 21 d before comparisons with observed data were made to ensure that it had reached steady-state conditions.

Data used for model reparameterization were assembled from the literature and represented 62 experiments with 233 treatment means (Hanigan et al., 2006). A list of the references is provided in Table 1, and a summary of the data are provided in Table 2. The data are a subset of those used by the NRC committee to formulate the 2001 nutrient requirement model (NRC, 2001). Of the 62 experiments, 3 were conducted with nonlactating heifers and the remainder with lactating cows. Four manuscripts did not report the breed of animal used, of which 3 were subsequently determined to have used Holsteins. One study used Swedish Red, 1 used Friesians, and the remainder used Holstein-Friesians.

Initial BW were set to match those reported in the literature. If no value was reported, the model was set to an initial BW of 600 kg. A BCS of 3.4 (scale 1 to 5) was assumed for all observations. Although it had no bearing on digestive predictions, starting DIM was set to a value of 21 d before the reported values so that DIM matched the observed days after the model was run 21 d. In no case did this result in a model starting point before calving. If DIM were not reported, it was set to 70.

Table 1. A listing of references for the experimental data used for model evaluations and parameter estimation

Aldrich et al. (1993)	Klusmeyer et al. (1991b)	Prange et al. (1984)
Armentano et al. (1986)	Kung et al. (1983)	Price et al. (1988)
Blauwiel et al. (1997)	Lu et al. (1988)	Putnam et al. (1997)
Calsamiglia et al. (1995)	Lykos and Varga (1997)	Robinson and Sniffen (1985)
Cameron et al. (1991)	Lykos et al. (1997)	Robinson et al. (1997)
Chan et al. (1997)	Lynch et al. (1991)	Santos et al. (1984)
Christensen et al. (1993)	Mabjeesh et al. (1996)	Sarwar et al. (1991)
Cunningham et al. (1994)	Mabjeesh et al. (1997)	Schwab et al. (1992a)
Cunningham et al. (1996)	Mansfield and Stern (1994)	Schwab et al. (1992b)
Doreau et al. (1991)	McCarthy et al. (1989)	Seymour et al. (1992)
Erasmus et al. (1994)	Merchen and Satter (1983)	Song and Kennelly (1989)
Espindola et al. (1997)	Murphy et al. (1987)	Stokes et al. (1991)
Feng et al. (1993)	Narasimhalu et al. (1989)	Tice et al. (1993)
Herrera-Saldana et al. (1990)	Ohajuruka et al. (1991)	Waltz et al. (1989)
Horner et al. (1988)	Oliveira et al. (1995)	Windschitl and Stern (1988)
Joy et al. (1997)	O'Mara et al. (1997)	Yang et al. (1997)
Kalscheur et al. (1997a)	Overton et al. (1995)	Yoon and Stern (1996)
Kalscheur et al. (1997b)	Pantoja et al. (1995)	Zerbini et al. (1988)
King et al. (1990)	Pena et al. (1986)	Zhu et al. (1997)
Klusmeyer et al. (1990)	Pires et al. (1997)	
Klusmeyer et al. (1991a)	Poore et al. (1993)	

Table 2. A summary of the data contained in references listed in Table 1 and used for model evaluations and parameter estimation

Variable	n	Mean	SD	Minimum	Maximum
DMI (kg/d)	233	18.6	4.1	5.8	26.8
BW (kg)	187	590	51	480	731
DIM	131	103	57	16	213
Milk (kg/d)	188	26.6	7.6	0	43.2
Milk lactose (%)	30	4.80	0.12	4.54	4.98
Milk protein (%)	163	3.13	0.28	2.59	3.90
Milk fat (%)	163	3.37	0.43	2.19	4.63
Nutrient composition of the total diet (% of DM)					
DM	139	67.1	1.4	15.7	93.0
OM	204	92.5	1.7	85.4	96.9
CP	233	16.7	1.8	10.3	22.2
NDF	187	33.6	5.4	17.6	49.8
ADF	192	19.6	4.0	8.8	30.6
Starch	101	33.0	7.9	13.7	47.9
Fat	76	5.6	2.9	2.1	20.2
Ruminal pH and ammonia and VFA concentrations					
pH	181	6.1	0.3	5.5	6.7
Ammonia (mmol/L)	173	8.8	3.6	1.0	23.0
VFA (mmol/L)	180	106	21	11	148
Acetate (% of VFA)	177	61.6	4.3	52.1	74.9
Propionate (% of VFA)	177	23.0	3.9	15.3	33.0
Butyrate (% of VFA)	177	11.8	1.5	8.1	16.9
True ruminal digestion (% of intake)					
DM	32	47.3	8.2	27.0	59.0
OM	201	52.2	9.5	29.3	83.4
N	227	56.6	13.2	14.6	85.5
ADF	129	38.9	12.6	6.2	72.4
NDF	152	42.8	12.8	3.0	71.2
Starch	92	59.7	15.4	27.3	87.4
Fat	24	7.9	10.6	-17.0	25.0
Duodenal flow (kg/d)					
DM	57	11.9	3.7	3.5	21.3
OM	181	11.3	3.2	2.8	18.3
ADF	120	2.3	0.8	0.4	4.8
NDF	155	3.7	1.2	0.7	6.8
Starch	84	2.9	1.4	0.5	5.9
Fat	23	0.9	0.1	0.0	1.6
N (g/d)	214	501	139	154	930
Microbial N (g/d)	233	256	82	87	493
NANMN ¹ (g/d)	227	219	88	34	433
Total-tract digestibility (% of intake)					
DM	101	66.3	4.3	48.2	77.9
OM	183	68.2	4.4	49.4	81.4
N	193	68.6	12.4	40.3	155.0
ADF	115	42.7	9.7	18.0	72.1
NDF	137	49.2	10.7	25.1	82.9
Starch	77	92.7	5.7	77.4	99.3
Fat	41	69.8	9.3	40.2	89.9

¹Nonammonia, nonmicrobial N.

Primary nutrient inputs required by the model include CP, fat, starch, ADF, NDF, lignin, and ash, all expressed as a proportion of DM. Carbohydrate composition was further characterized with inputs of soluble starch, ruminally undegraded starch, ruminally undegraded ADF, and forage NDF, all expressed as a percentage of the parent material. Protein composition was characterized with inputs of soluble protein, RUP, NPN, and urea all expressed (in CP units) as a percentage of CP. Expressing carbohydrate and protein composition as a percentage of the parent material is a deviation from the original input description of Hani-

gan et al. (2006). Nonprotein nitrogen was assumed to represent NPN and non-urea sources such as nucleic acids. Contrary to some categorization schemes, free amino acids and peptides found in grasses and silages are represented as soluble protein rather than NPN. Urea was also specified separately due to its low carbon content, consistent with the original description of inputs (Baldwin, 1995).

If nutrient composition of individual ingredients was reported, which was generally the case with forages, those values were used in calculating dietary nutrients. Missing nutrients were generated from tabular values

Table 3. A summary of bias adjustments applied to predicted dietary nutrients

Item	Starch	CP	ADF	NDF	Fat
n ¹	96	225	181	177	75
Mean bias (% of DM)	-1.35	0.23	-0.28	2.03	0.00
Minimum bias (% of DM)	-12.52	-2.76	-9.37	-10.68	-0.02
Maximum bias (% of DM)	10.48	5.84	10.83	8.15	0.06
RMSPE ² (%)	19.5	10.5	18.4	14.9	49.4
Bias-adjusted RMSPE ³ (%)	15.1	5.7	11.7	8.7	34.2
Error reduction (%)	22.4	45.2	36.3	41.2	30.8

¹Represents the number of individual treatment means used for bias calculations.

²Root mean square prediction error (RMSPE) = error of prediction when using ingredient values to predict complete diet values.

³Error of prediction when using ingredient values to predict complete diet values after bias correction.

(NRC, 2001) in the following manner: starch content of individual ingredients was calculated as 80% of the ingredient NFC value, which is equivalent to total dietary ratios for common feeding programs (Batajoo and Shaver, 1994). Soluble starch (SolStarch) was assumed to be 20% of total starch, which reflects the high proportion of maize grain in most of the diets (Batajoo and Shaver, 1998). Ruminally undegraded starch (RuStarch) was calculated as follows:

$$\text{RuStarch} = (\text{Starch} - \text{SolStarch}) \times 0.631, \quad [1]$$

where 0.631 represented the mean proportion of insoluble starch appearing at the duodenum for the entire data set. The difference between SolStarch and RuStarch represents the insoluble but ruminally degraded fraction. Ruminally undegraded ADF (RuADF) was calculated as follows:

$$\text{RuADF} = \text{ADF} \times 0.405, \quad [2]$$

where 0.405 represented the mean observed proportion of intake ADF appearing at the duodenum.

Because diets were reconstructed from individual ingredients using a mix of tabular and observed values, potential for bias exists in the predicted dietary nutrient inputs to the model associated with the use of the tabular values. Such bias would translate into biased model parameter estimates if not corrected. For example, if dietary protein intakes were overestimated, then the ruminal protein degradation rate constant would have to assume a larger-than-appropriate value to bring protein outflow from the rumen in line with the, presumably, unbiased estimates of undegraded feed protein outflow. For this reason, it is critical to try to remove bias in feed inputs. For starch, protein, ADF, NDF, and fat, the predicted dietary concentrations as calculated from summation of the individual dietary ingredients were compared with the observed dietary concentrations when available and mean bias within

experiment was determined and used to correct the predicted values so that they aligned with the observed dietary values. This was achieved by determining the mean bias [$\Sigma(\text{observed} - \text{predicted})/N$] across treatments within an experiment and adding that value to the model-calculated nutrient values for each diet in that study. Bias factors were developed for starch, CP, ADF, NDF, and fat. A summary of the dietary bias adjustments are presented in Table 3. Unfortunately adjustments could not be developed for other nutrients, as dietary values were not reported in the literature.

Soluble carbohydrate (SolCHO; % of DM) was calculated on a dietary basis from dietary NFC and starch after bias adjustment:

$$\text{SolCHO} = \text{NFC} - \text{Starch}. \quad [3]$$

If SolCHO was less than 0, it was assumed to be 0, and the difference from 0 was subtracted from dietary starch. Because this adjustment was applied after calculation of SolStarch, it effectively assumes that any overestimate of NFC is due to an overestimate of insoluble starch.

Ruminally undegraded CP, starch, and ADF were then used to calculate ruminal degradation rate parameters for insoluble protein, starch, and cellulose, assuming a mean ruminal residence time (RRT) in the small particle pool of 8 h for starch and protein and 18 h for ADF (Hanigan et al., 2006). The rate constant for hemicellulose was calculated as a proportion of the cellulose rate constant as originally described (Baldwin, 1995).

It was assumed that ruminally undegraded nutrient fractions, as determined from in sacco analyses, provided an accurate representation of the relative differences in rates of ruminal degradation among feeds, which has been demonstrated for protein (Madsen and Hvelplund, 1985). Use of the observed mean fractions of starch and ADF escaping the rumen in Equations 1 and 2 ensures that the relative differences among ingredients and

diets will be centered around the true observed mean escape. However, the absolute rate of degradation, as determined in sacco, may still be biased relative to the model structure where, among other things, nutrient passage from the rumen is represented. Thus, for data-fitting purposes, an adjustable variable (F_X ; d^{-1}) was introduced into all 3 rate parameter calculations (ADF, protein, and starch), yielding the following modified rate parameter calculation equation:

$$K_{DegX}(\text{d}^{-1}) = \frac{24}{\text{RRT}} \times -\ln \left[\frac{f_{\text{RumUndegX,Fd}}}{(f_{\text{TotalX,Fd}} - f_{\text{SolX,Fd}})} \right] + F_X, \quad [4]$$

where K_{DegX} , $f_{\text{RumUndegX,Fd}}$, $f_{\text{TotalX,Fd}}$, and $f_{\text{SolX,Fd}}$ represented the rate constant for ruminal degradation of nutrient X, the fractional proportion of ruminally undegraded nutrient X in feed, the fractional proportion of total nutrient X in feed, and the fractional proportion of soluble nutrient X in feed, respectively, where X represented starch, protein, and ADF (ADF was used to calculate the rate of degradation of cellulose directly and hemicellulose indirectly). The variable F_X was initially set to 0 and derived by fitting to the observed digestive nutrient flow data for nutrient X. The ratio $24/\text{RRT}$ represented the time conversion from the assumed 8-h residence time to a daily rate to be consistent with time units in the model. Again, using a residence time greater than 8 h would not appreciably change the relative differences across ingredients in calculated rates of degradation; however, it would result in a different solution for F_X .

Having defined all of the dietary nutrient and animal inputs required by the model, key ruminal and postruminal digestive parameters were estimated by fitting model predictions of ruminal pH, ammonia, and VFA concentration; ruminal outflow of DM, NDF, ADF, starch, lipid, total N, microbial N, and nonammonia, nonmicrobial N (NANMN); and fecal flows of NDF, ADF, starch, lipid, and N to observed values using the Nelder-Mead optimization algorithm (Press, 2007) within ACSL Math to maximize the log-likelihood function (LLF). Parameters to be fitted were identified through examination of the driving variables in the equations used to predict the outputs and by sensitivity analyses. In general, minimum and maximum bounds of 0.5 and 2 times the starting parameter estimate were used for fitting. These were adjusted each time an optimization run was initiated to maintain that general relationship with the current parameters estimates which ensured that none of the final parameter estimates were resting against or approaching a bound. The exception was

that for most parameters, a lower bound of 0 was maintained, as negative values were not biologically possible. Because of the size of the problem, the ruminal parameters were derived as a set first, and having fixed those parameters, the postruminal parameters were derived as a separate set. This prevented introduction of bias into, for example, postruminal predictions to minimize an opposite bias in ruminal predictions. Root mean square prediction errors (MSPE) and partitioning of MSPE into mean and slope bias was also conducted as described by Bibby and Toutenburg (1977) using the scripting language in ACSL Math.

Two independent efforts were undertaken to consider experiment effects during parameter estimation. In the first case, the LLF calculation was altered to calculate the mean deviation for each experiment and adjust the residuals for individual experiment deviations before calculation of the LLF. This approach ensured that only within-experiment responses influenced parameter estimation, consistent with the goals of a meta-analysis (St-Pierre, 2001). However, this approach proved nonviable, as the modified algorithms could not solve for a known set of digestion parameters and known experiment deviations when fitted to a set of data simulated using those digestion and experimental bias parameters.

The second approach involved coding a vector of bias factors ($\emptyset_j$, where j represented the experiment number) for each predicted output variable. These individual bias-adjustment factors were added to the model-predicted output variable ($\hat{Y}_{ij}$, where i represented individual treatment means) to create a set of unbiased model predictions ($\hat{\Psi}_{ij}$), which were compared with the observed data ($\text{residual}_{ij} = \text{observed}_{ij} - \hat{\Psi}_{ij}$). In other words, the model predictions were bias corrected before calculation of residuals:

$$\hat{\Psi}_{ij} = \hat{Y}_{ij} + \emptyset_j. \quad [5]$$

Thus, $\emptyset$ acts as a fixed set of intercept adjustments forcing the common slope to be derived from within-experiment observations (Figure 1); $\emptyset_j$ were initially set to 0 (no experiment bias), and included as adjustable parameters in the optimization run. The only difference between this approach and the prior one is that these parameters were estimated explicitly and, thus, were part of the parameter variance structure during the optimization run. This approach resulted in huge arrays of adjustable variables (a few model parameters plus an intercept for each experiment for each output variable) that required massive amounts of computing time for solutions and hampered the ability of the optimizers to reach a global solution. Repeated restarts

using parameter estimates from the prior run as the starting point for the next run resulted in an LLF value that reached a plateau after 4 or 5 starts. Although this approach likely resulted in better within-experiment parameter estimates, some of the derived digestion coefficients deviated substantially from estimates where experiment effects were not considered, which coincided with a large range in ϕ_j . By example, if the within-experiment slope is considerably less than the global slope (across experiments), the range in experiment intercepts would have to be large for the predicted values to remain within the range of observed data (Figure 1). Use of the derived within-experiment slope and an average experiment adjustment value ($\bar{\phi}$) results in overprediction of responses at very low inputs relative to observed values and underprediction at very high inputs when the within-experiment slope is less than the global slope. Unless ϕ_j can be predicted (i.e., the underlying cause of ϕ_j is represented), the derived model will do a very poor job of predicting throughout the data range.

It seemed likely that some of the apparent experiment effects could be caused by interactions among nutrients that were not adequately represented in the model. For example, if one experiment had dietary NDF much greater than the mean and another much less, and NDF was influencing digestive processes, residual errors from the model would present as random experiment effects if the model did not adequately represent those interactions. The residuals would also correlate with dietary NDF. Because of the potential for experiment effects to cloud the relationship between NDF, in this example, and residual errors, a post hoc analysis of residuals was

Figure 1. Example of fixed effects in digestion data. ϕ_i represents the effect of individual experiment and $\bar{\phi}$ represents the mean experimental effect. The global equation describes the response across experiments.

adopted that included experiment effects as a method to identify model elements that were missing or not properly represented. Analyses were conducted using backward elimination regression, with experiment included as a random class variable to identify potential factors affecting prediction accuracy. Residuals were regressed on observed DMI, dietary nutrient intakes, and DIM, and predicted BW, ruminal pH, and ruminal ammonia concentrations to determine relationships with key model inputs and driving model variables (pH and ammonia). When examining residual errors for predictions of ruminal pH or ammonia, they were not included in the regression model as independent variables. Variables were removed from the model if the associated *P*-value was greater than 0.10. Initial results from this work resulted in the addition of the parameters encoding fat and ammonia effects on microbial growth and fat effects on fiber and starch degradation to the optimization parameter set.

Global sensitivity of ruminal pH, ruminal VFA concentrations, ruminal ammonia concentrations, and outflow of microbial nitrogen and undegraded feed nutrients from the rumen to ruminal model parameters was undertaken using the Fourier amplitude sensitivity test provided in acslX software (version 3.1; AEgis Technologies Group Inc.) as described by Saltelli et al. (1999). The parameters were sampled using boundaries of 70 and 130% of the final parameter estimates, except for the constant for the effect of fat on fiber digestion (K_{FatHb} ; g of endogenous fat/g of added fat) and the constant for the effect of fat on protein digestion (K_{FatPi} ; g of endogenous fat/g of added fat), which were set to the much wider bounds of 0 and 0.01 to reflect the uncertainty regarding their inclusion in the model. Resampling was set to 4 and the interference factor was set to 4. These settings resulted in a sampling population of 204,792 runs from which the global sensitivity coefficients were derived. All statistical summaries of the observed data and regression analyses were conducted using PROC MEANS, GLM, and MIXED of SAS (version 9.1; SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Feed Inputs

It was apparent from the residuals analyses of dietary nutrient predictions that the use of tabular nutrient values for individual ingredients to supplement the incomplete observed ingredient data resulted in biased predictions if no adjustments were applied (Table 3). Without bias adjustment, mean bias (% of dietary DM) for starch, CP, NDF, ADF, and fat was -1.35 , 0.23 , 2.03 , -0.28 , and 0 , respectively, where negative values

denote overpredictions and positive values underpredictions. Of course, the range in bias was much greater than the mean, indicating that dietary inputs for some studies deviated even more from observed values in the absence of a correction. Applying within-study bias corrections where dietary nutrient concentrations were reported reduced prediction errors for dietary starch, CP, ADF, NDF, and fat from 22 to 45%, depending on the nutrient. Assuming the observed dietary nutrients were accurate representations of the true diet, using correction factors to adjust dietary inputs so they matched observed inputs resulted in reduced input variation, which should lead to more accurate estimates of digestion parameters. Unfortunately no studies reported the full range of dietary nutrients required by the model and many studies did not report even this limited set of nutrients. In those cases, no corrections could be made, and based on the observations from studies that reported data, it seems likely that some bias may still remain in nutrient intake predictions.

Nutrient Digestion and Ruminal Metabolism

An evaluation of prediction errors for the initial model (Hanigan et al., 2009) was conducted to assess model performance with bias-adjusted nutrient inputs (Table 4). Some minor differences existed compared with the prior evaluation using this data set (Hanigan et al., 2006) that were associated with these adjustments; however, the results were generally consistent. Predictions exhibiting prediction errors greater than 20% included ruminal ammonia and VFA concentrations, the proportion of VFA as propionate, passage of all nutrients and microbial protein to the small intestine, and lower-tract digestion of ADF and NDF. Ammonia, molar proportions of all 3 VFA, lipid passage, and intestinal ADF digestibility also exhibited substantial proportions of the prediction error as slope bias. Total N and microbial N passage exhibited substantial proportions of error as mean bias.

Although ruminal pH values had relatively low prediction errors, essentially all of the error was due to mean bias associated with an underprediction by approximately 0.5 pH units. This is problematic, as microbial activity predictions are dependent upon ruminal pH (Argyle and Baldwin, 1988). Underpredicting pH by 0.5 results in significant depression in predictions of microbial growth and metabolism, which affect essentially all of the ruminal output predictions, as evidenced by the sensitivity of those outputs to pH prediction equation parameters (Table 5).

The model predicts pH from total VFA concentrations using an adaptation of the equation of Briggs et

al. (1957) as described by Argyle and Baldwin (1988). Using just the observed data, the validity of that prediction equation was checked by regressing observed pH on observed VFA concentrations. The resulting equation had an intercept of 6.50 ± 0.10 and a VFA coefficient of $-3.62 \pm 0.88 \text{ mol}^{-1}$ ($P < 0.0001$, $R^2 = 0.10$) as compared with 7.2 and -10.0 mol^{-1} , respectively in the equation of Briggs et al. (1957). Addition of an ammonia term to the regression model as in the original work did not result in a significant improvement ($P > 0.05$) and, thus, does not explain the difference in the relationships. As the original work was conducted with data from sheep, it seems likely that the substantial differences in the relationships are due to species differences and suggests that this equation has been problematic since its adoption some 24 yr ago.

Having identified the model predictions that were biased, an iterative series of parameter estimations was undertaken, which was followed by residual analyses (with consideration of experiment effects) in an attempt to correct the observed problems. The final parameter estimates resulting from this series are presented in Table 6. In general, the parameters were well defined by the data, as reflected by relatively small standard deviations for the parameter estimates. Although the model is highly integrative, the correlations among ruminal parameters were relatively low, with most having correlations of less than 0.2 (Table 7). Exceptions were the slope for the VFA effect on pH (K_{pHVFA} ; mol^{-1}) and the rate constant for ammonia absorption (K_{AmAbs} ; -0.252), the intercept for pH prediction (Int_{pH}) and the scalar for the cellulose degradation rate constant calculation (F_{KRuADF} ; -0.227), F_{KRuADF} and the scalar for the protein degradation rate constant calculation (F_{KRUP} ; -0.221), the constant to calculate the effect of fat on microbial growth (K_{FatFG} ; g of DM/g of dietary fat) and the rate constant for propionate absorption (K_{AbsPr} ; -0.215), and K_{pHVFA} and the rate constant for acetate absorption (K_{AbsAc} ; -0.204). Given the independence of postruminal nutrient flows, it was surprising that intestinal starch, fiber, and fat digestibilities were relatively highly correlated, with coefficients of 0.57 for the intestinal digestion coefficient for starch ($K_{Starch,LGut}$; g/g) and the intestinal digestion coefficient for lipid ($K_{Lipid,LGut}$; g/g), 0.685 for $K_{Lipid,LGut}$, and the intestinal digestion coefficient for fiber ($K_{Fiber,LGut}$), and 0.613 for $K_{Starch,LGut}$ and $K_{Fiber,LGut}$. Correlation coefficients for the intestinal digestion coefficient for protein ($K_{Protein,LGut}$) with $K_{Starch,LGut}$, $K_{Fiber,LGut}$, and $K_{Lipid,LGut}$ were 0.145, 0.054, and 0.005, respectively (data not shown). The greater correlations may have been an artifact of fitting to fecal NDF and ADF, which are correlated due to the common subnutrients within those fiber fractions.

Table 4. Residual error analyses for predictions by Molly with parameter estimates of Hanigan et al. (2009; initial) and the parameters listed in Table 6 (final)¹

Variable	n	Observed mean	Predicted mean		RMSPE ² (% of observed)		Mean bias (% of MSPE)		Slope bias (% of MSPE)	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
Ruminal metabolism										
pH	166	6.12	5.74	6.11	7	4	69	0.1	1	0.4
Ammonia (mmol/L)	159	8.53	7.60	6.02	59	67	5	20	47	43
VFA (mmol/L)	164	106	97	106	22	21	15	0.0	1	7
Acetate (% of VFA)	161	61.8	62.9	63.7	9	8	6	14	26	13
Propionate (% of VFA)	161	22.9	25.0	24.3	20	19	18	10	14	4
Butyrate (% of VFA)	161	11.8	12.1	12.0	15	14	2	1	31	21
Ruminal outflow (kg/d)										
DM	47	12.8	11.76	12.4	16	15	6	5	22	9
NDF	133	3.82	4.08	3.77	28	26	11	0.2	3	5
ADF	104	2.37	2.35	2.41	25	23	0.01	0.5	2	1
Starch	78	2.84	2.90	3.13	40	41	0.01	6	7	8
Lipid	23	0.98	0.97	1.0	23	23	0.2	1	24	25
Nitrogen (g/d)	199	499	433	477	23	19	38	9	0.2	2
Microbial N (g/d)	211	258	218	266	30	27	26	1	0.001	2
NANMN ³ (g/d)	211	218	212	209	33	35	1	2	6	8
Postruminal digestion (g/g of flow)										
NDF	131	0.483	0.428	0.479	26	22	22	1	13	4
ADF	108	0.424	0.446	0.436	28	22	4	2	38	6
Starch	75	0.924	0.934	0.926	6	6	2	0.01	6	2
Lipid	41	0.721	0.694	0.698	13	13	0.3	0.1	1	2
Protein	183	0.686	0.694	0.668	19	9	0.5	0.004	5	13

¹Dietary nutrients were adjusted to remove mean bias across diets within an experiment using the bias-adjustment factors summarized in Table 3.

²Root mean square prediction error (MSPE).

³Nonammonia, nonmicrobial N.

Table 5. Global analyses of the sensitivity of model digestive predictions to digestive parameters¹

Model parameter ²	Predicted model output ³																			
	Concentration			Molar proportion			Ruminal outflow								Fecal output					
	pH	Am	VFA	Ac	Pr	Bu	DMP	NDFP	ADFP	HaP	LipidP	NitP	MiNP	NANMNP	Starch	NDF	ADF	Protein	Lipid	
Ruminal (proportion of total variation)																				
K_{AmAbs}	0.001	0.431	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.002	0.002						
Int_{pH}	0.449	0.074	0.397	0.120	0.094	0.068	0.357	0.368	0.413	0.256	0.461	0.314	0.384	0.272						
K_{pHVFA}	0.255	0.040	0.221	0.071	0.042	0.030	0.193	0.219	0.206	0.139	0.225	0.165	0.237	0.147						
K_{AbsAc}	0.030	0.002	0.057	0.197	0.126	0.108	0.035	0.031	0.031	0.024	0.032	0.012	0.026	0.030						
K_{AbsPr}	0.009	0.001	0.014	0.217	0.398	0.027	0.009	0.007	0.010	0.007	0.010	0.002	0.008	0.008						
K_{AbsBu}	0.001	0.001	0.004	0.056	0.007	0.387	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.003						
F_{KRuADF}	0.033	0.002	0.069	0.001	0.001	0.0004	0.067	0.057	0.063	0.015	0.030	0.014	0.024	0.022						
F_{KRUP}	0.003	0.113	0.005	0.002	0.003	0.001	0.004	0.001	0.001	0.003	0.002	0.152	0.001	0.140						
F_{KRuSt}	0.003	0.0005	0.004	0.002	0.001	0.001	0.006	0.002	0.001	0.199	0.002	0.001	0.002	0.002						
K_{HcCsI}	0.011	0.001	0.020	0.001	0.001	0.001	0.025	0.019	0.003	0.004	0.008	0.004	0.008	0.006						
K_{FGAm}	0.0004	0.001	0.0004	0.0005	0.001	0.001	0.001	0.0005	0.0005	0.001	0.001	0.002	0.002	0.001						
K_{FatFG}	0.0003	0.003	0.0003	0.001	0.001	0.001	0.0004	0.001	0.001	0.002	0.003	0.005	0.003	0.002						
K_{FatHb}	0.0004	0.001	0.0004	0.001	0.0005	0.0005	0.0004	0.001	0.0002	0.001	0.0003	0.001	0.001	0.001						
K_{FatPi}	0.0002	0.001	0.0003	0.001	0.0004	0.0004	0.001	0.001	0.0004	0.001	0.0004	0.001	0.001	0.001						
Postruminal																				
$K_{Starch,LGut}$															0.754	0.041	0.021	0.017	0.025	
$K_{Fiber,LGut}$															0.003	0.286	0.350	0.006	0.008	
$K_{Lipid,LGut}$															0.003	0.018	0.016	0.004	0.414	
$K_{Protein,LGut}$															0.001	0.016	0.019	0.630	0.007	

¹Parameter bounds were set to 70 and 130% of the final parameter estimates (Table 6), except for K_{FatHb} and K_{FatPi} , which were set to 0 and 0.01. Data are expressed as a fraction of the total variance.

²Definitions for each of the model parameters are given in Table 6.

³Am = ammonia; Ac = proportion of VFA as acetate; Pr = proportion of VFA as propionate; Bu = proportion of VFA as butyrate; DMP = DM passage; NDFP = NDF passage; ADFP = ADF passage; HaP = start passage; LipidP = lipid passage; NitP = nitrogen passage; MiNP = microbial nitrogen passage; NANMNP = nonammonia, nonmicrobial nitrogen passage.

The global sensitivity analyses did not demonstrate the same level of interdependence of these model parameters (Table 5).

Derived parameters for the ruminal pH equation within the model also deviated considerably from the original parameters, but corresponded closely with the above direct regression results, with an intercept of 6.53 and a VFA coefficient of -3.94 mol^{-1} . Based on this equation, a 10-mM increase in VFA concentrations could be expected to reduce the ruminal pH by 0.04 pH units. These changes reduced the root MSPE from 7 to 4% and removed all of the mean bias. The lack of slope bias suggests that the equation form and derived parameters are consistent with observations, and that the minimal bias in VFA predictions did not significantly influence the outcome.

Altered pH predictions contributed to changes in several additional parameters, as the flow of all nutrients, including microbial protein from the rumen, and concentrations of VFA were sensitive to alterations in the pH prediction parameters (Table 5). The very large reduction in the parameter value for F_{KRuADF} was due to alleviation of the predicted pH suppression of fiber degradation (Argyle and Baldwin, 1988). Restoration of pH predictions to more normal ranges removed the predicted inhibition, allowing adoption of a more reasonable value for F_{KRuADF} while maintaining appropriate predictions of fiber passage from the rumen. Using the derived value for F_{KRuADF} and the mean observed nutrient inputs to calculate $K_{Deg, Cellulose}$ (Equation 4) yielded a value of 6.63, which is only 10% greater than the original estimate of 6.0 by Baldwin (1995). The scalar relating hemicellulose to cellulose degradation increased approximately 2 fold to a value of 0.96, which indicates that the rate of degradation of cellulose and hemicelluloses are almost equal, again more consistent with the original settings by Baldwin (1995). Small changes in rate parameters for protein and starch degradation reflect the lesser sensitivity of those flows (Table 5) to the indirect effects of pH on microbial-driven degradation of those nutrients in the model (Baldwin, 1995).

Overall error in microbial N predictions was reduced modestly from 30 to 27%, and the mean bias was removed, with essentially no slope bias. The reduction in overall error resulted primarily from improved predictions of pH, with a minor contribution from improved predictions of fiber fermentability (Table 5). Although refined estimates of the effects of ammonia and fat contributed to improved model performance, sensitivity to those parameters was exceedingly small compared with the large pH effects. Improved microbial flow predictions contributed to a reduction in errors for total N

flow from 23 to 19%, due largely to a reduction in mean bias.

Nonammonia, nonmicrobial N prediction error increased very slightly from 33 to 35% with essentially no mean bias and possibly a small amount of slope bias. Regression of residuals for NANMN on $K_{Deg, RUP}$ (Equation 4) indicates that the variation in that parameter, which is driven by specified feed inputs, is not the cause of the variation in NANMN flow predictions (Figure 2). A slight trend exists for an underprediction of NANMN flow when calculated rate constants are high (low-RUP diets) and the reverse with low settings, suggesting that minor gains may be possible if Equation 4 were reformulated to create greater change in the resulting rate constant as dietary RUP estimates change. The relatively large additive term $F_{K_{Deg, RUP}}$ effectively dampens the proportional response, resulting in a mean rate constant of 3.57 and a range of 85 to 123% of that value, whereas the negative logarithm of RUP divided by insoluble protein ranged from 65 to 133% of the mean value.

Very little reduction in prediction errors was observed for ruminal VFA concentrations, with an overall prediction error of 21%. The reduction was associated with removal of mean bias, with prior predictions averaging 97 mM versus the observed 106 mM. Some of this variance is likely associated with heterogeneity in the observed data caused by variation in ruminal sampling technique. However, predictions of VFA production, and the simple mass action representation for absorption of the VFA likely contribute to the problem. The much greater sensitivity of concentrations to factors affecting production (i.e., pH, as compared with those regulating absorption; Table 5) suggest that the problem is primarily associated with predictions of production. Ghimire et al. (2012) observed large errors associated with predictions of VFA production rates. In addition to reported pH effects on transport (Dijkstra et al., 1993), Storm et al. (2011, 2012) found that ruminal blood flow also regulates VFA uptake. Neither of these effects is captured in the current absorption prediction equations. Although the observed prediction errors are worrisome from the point of predicting animal production responses to dietary nutrients, VFA exert no direct effect on any ruminal elements other than pH; thus, the sensitivity of nutrient outflow from the rumen to VFA absorption parameters simply reflects the effects of VFA on pH (Table 5).

Prediction errors for ammonia concentrations actually increased substantially due primarily to an increase in mean bias. Predicted ammonia concentrations are most sensitive to the settings for the rate constant for absorption, ruminal pH, and the rate of protein degra-

Table 6. Model parameters derived when the model was fitted to the data summarized in Table 2

Model parameter	Description ¹	Previous value ²	Final estimate	SE
Ruminal ³				
K_{AmAbs}	Rate constant for ammonia absorption	23.07	22.02	0.08
Int_{pH}	Intercept for pH prediction	7.2 ⁴	6.53	0.002
K_{pHVFA}	Slope for VFA effect on pH (mol^{-1})	10.0 ⁴	3.94	0.01
K_{AbsAc}	Rate constant for acetate absorption	6.26	5.91	0.02
K_{AbsPr}	Rate constant for propionate absorption	11.68	8.79	0.03
K_{AbsBu}	Rate constant for butyrate absorption	10.39	7.96	0.03
F_{KRuADF}	Scalar for cellulose degradation rate constant calculation	59.78	5.44	0.02
F_{KRUP}	Scalar for protein degradation rate constant calculation	3.66	2.84	0.01
F_{KRuSt}	Scalar for starch degradation rate constant calculation	3.01	3.18	0.01
K_{HcCs1}	Scalar to calculate the hemicellulose degradation rate constant from the cellulose rate constant	0.415	0.959	0.003
K_{FGAm}	Constant to calculate the effect of ammonia on microbial growth (mol^{-1})	0.0012 ⁴	2.01×10^{-4}	6.2×10^{-7}
K_{FatFG}	Constant to calculate the effect of fat on microbial growth (g of DM/g of dietary fat)	0.03 ⁴	0.0683	0.0002
K_{FatHb}	Constant for the effect of fat on fiber digestion (g of endogenous fat/g of added fat)	0.03 ⁴	1.94×10^{-7}	6.3×10^{-10}
K_{FatPi}	Constant for the effect of fat on protein digestion (g of endogenous fat/g of added fat)	0.00077	4.46×10^{-7}	1.5×10^{-9}
Postruminal				
$K_{Starch,LGut}$	Intestinal digestion coefficient for starch (g/g)	0.819	0.808	0.01
$K_{Fiber,LGut}$	Intestinal digestion coefficient for fiber (g/g)	0.132	0.118	0.003
$K_{Lipid,LGut}$	Intestinal digestion coefficient for lipid (g/g)	0.698	0.711	0.01
$K_{Protein,LGut}$	Intestinal digestion coefficient for protein (g/g)	0.663	0.68	0.007
	Log-likelihood function	161	1,043	

¹Units are d^{-1} unless specified otherwise.²From Hanigan et al. (2009).³Ruminal parameters were derived as a set and fixed for subsequent derivation of postruminal parameters.⁴From the original model description by Baldwin (1995).

Table 7. Correlations among ruminal parameters¹

Item	K_{AmAbs}	Int_{pH}	F_{KRuADF}	F_{KRUP}	F_{KRuSt}	K_{HeCsI}	K_{FatFG}	K_{pHVFA}	K_{FatHb}	K_{FatPi}	K_{AbsAc}	K_{AbsPr}	K_{AbsBu}	K_{FGAm}
K_{AmAbs}	1.000													
Int_{pH}	-0.145	1.000												
F_{KRuADF}	-0.037	-0.227	1.000											
F_{KRUP}	0.046	-0.126	-0.221	1.000										
F_{KRuSt}	-0.072	-0.116	-0.144	-0.070	1.000									
K_{HeCsI}	-0.155	-0.057	-0.147	-0.092	-0.190	1.000								
K_{FatFG}	-0.140	-0.119	-0.093	0.078	-0.193	0.085	1.000							
K_{pHVFA}	-0.252	0.147	-0.111	-0.182	-0.073	-0.081	-0.177	1.000						
K_{FatHb}	-0.062	0.024	-0.075	-0.143	-0.078	-0.177	-0.156	-0.062	1.000					
K_{FatPi}	-0.060	0.023	-0.051	-0.146	-0.082	-0.178	-0.155	-0.060	-0.059	1.000				
K_{AbsAc}	-0.129	-0.165	-0.121	-0.068	-0.002	-0.102	-0.079	-0.204	-0.101	-0.127	1.000			
K_{AbsPr}	-0.076	-0.070	-0.015	-0.168	-0.023	-0.189	-0.215	0.046	-0.124	-0.121	-0.143	1.000		
K_{AbsBu}	-0.102	-0.096	-0.021	-0.178	-0.136	-0.049	-0.135	-0.075	-0.052	-0.051	-0.014	-0.101	1.000	
K_{FGAm}	-0.084	-0.099	-0.041	-0.119	-0.105	-0.061	-0.106	-0.152	-0.132	-0.132	-0.041	-0.094	-0.178	1.000

¹Definitions of the ruminal parameters are given in Table 6.

dation in the rumen (Table 5). Given that all of those parameters were optimized, the results indicate that the prediction problems are not related to substrate degradation or ruminal pH effects on microbial growth. This problem is discussed further in the following section.

Some of the imprecision in predicting ruminal concentrations of hydrogen ions, VFA, and ammonia could be due to potential problems with predictions of ruminal volume and liquid passage. The former was considered as a constant proportion of ruminal DM contents and the latter as a constant fractional proportion of the ruminal pool (Baldwin, 1995). Deviations from these assumptions would introduce bias in concentration predictions, which could contribute to the observed prediction errors. Argyle and Baldwin (1988) provided predictions of ruminal volume and liquid passage based on a mechanistic representation; however, those equations have not been thoroughly tested. Additionally, because liquid volume is predicted from osmotic po-

tential in that submodel, soluble ash is a critical model input that is completely missing from the current data and, thus, those equations were not used to predict ruminal volume for this work. In addition to soluble ash inputs, it is critical to predict nutrient degradation and solubilization to predict osmotic potential and ruminal fluid balance. Thus, the problem is circular, requiring that both fluid balance and nutrient degradation and fermentation parameter sets be derived simultaneously to achieve the ideal solution. We are currently working to assess the fluid volume and passage rate equations and will revisit parameterization of absorption coefficients in conjunction with that work.

The new parameter set resulted in substantial reductions in prediction errors for postruminal protein digestion and smaller reductions in prediction errors for postruminal fiber digestion. The proportion of MSPE associated with slope bias increased slightly for protein digestion but decreased substantially for fiber digestion. Although the proportion of error associated with slope bias for postruminal protein digestion more than doubled, the sums of squares associated with slope bias were essentially constant, indicating that reparameterization did not alter the problem; it simply reduced the overall error, resulting in slope bias assuming a greater percentage of the error.

Figure 2. Residual errors for predictions of nonammonia, nonmicrobial nitrogen (NANMN) versus the calculated rate constant for degradation of insoluble protein in the rumen (Equation 4).

Nutrient Interactions

Several nutrient interactions on digestion are encoded in the model (i.e., inhibition of ruminal fiber and protein digestion by fat). If these interactions are inappropriately represented or parameterized or if additional interactions should be represented but have not been included in the model, the model would exhibit bias where those nutrients were substantially altered from the mean. Inappropriate representations or miss-

ing representations would be apparent in the residual errors when they are regressed on the interacting variables using a meta-approach to restrict inference space to within an experiment. For example, if fat does not significantly inhibit ruminal protein degradation (which appears to be the case) when the model represents such an interaction, one would observe a correlation between dietary fat content and residual errors for protein passage from the rumen. The same would be the case if some other nutrient affected protein degradation but that interaction was not encoded in the model. Finally, fat may suppress degradation or digestion of another nutrient, but if the effect is improperly represented, residual errors will be correlated with dietary fat, suggesting that additional model development is needed. When regressing residuals on inputs or other model variables, positive correlations indicate that the model underpredicted responses to the independent variable and negative correlations indicate overprediction of response as compared with observed responses.

Initial model evaluations indicated that model predictions of microbial growth responses to ammonia and dietary fat, and ruminal protein and fiber degradation responses to dietary fat were inappropriate. These interactions are controlled by the variables K_{FGAm} (the constant to calculate the effect of ammonia on microbial growth; mol^{-1}), K_{FatFG} , K_{FatHb} and K_{FatPi} , respectively, and thus they were added to the parameters being estimated, which resulted in a reduction in the correlations (Table 8) and a reduction in model prediction errors.

The model represents microbial growth as a function of fermentable substrate availability and efficiency of conversion of ATP derived from the fermentable substrate to microbial mass (Y_{ATP}). Ruminal ammonia affects Y_{ATP} in a saturable manner and long-chain FA in a linear manner. Baldwin (1995) used a Michaelis-Menten equation with a maximal effect (V_{max}) of 1 and an ammonia affinity constant (K_{FGAm}) of 1.2 mM to encode the ammonia response. After fitting to the data herein, K_{FGAm} solved to a value of 0.2 mM as compared with the average ruminal ammonia concentrations of 8.8 mM (Table 2). This results in microbial growth being essentially saturated with ammonia at average concentrations. Reducing ruminal ammonia from 8.8 to 1 mM, the lowest value in the observed range, would result in only a 15% predicted reduction in growth rate and increasing it to 23 mM, the highest value in the observed range, would only stimulate growth by 2%, given a K_{FGAm} value of 0.2 mM. Satter and Slyter (1974) observed increases in microbial yields that were maximized at ruminal ammonia concentrations of 3.5 mM, which would be predicted to yield 95% of maximal growth at a K_{FGAm} of 0.2 mM. However, Kang-Meznarchik and Broderick (1980) and Reynal and Broderick

(2005) reported that microbial protein synthesis was not maximized until ruminal $\text{NH}_3\text{-N}$ concentrations exceeded 6.0 and 8.4 mM, respectively, which correspond to 96.8 and 97.7% of maximal growth with K_{FGAm} set to 0.2 mM. Although microbial N flow predictions were improved using the new estimate, residual errors were still positively correlated with ruminal ammonia predictions, indicating that the model was underresponsive. Therefore, the true value for the half-maximal response may be somewhat greater than the current estimate, but almost certainly less than the value of 1.2 mM. However, the large slope bias in predicted ruminal ammonia concentrations limits the confidence one can have in determining the actual minimal ruminal ammonia concentrations and underscores the need to improve those predictions (Arriola Apelo et al., 2011).

The effect of long-chain FA derived from fats and oils on microbial growth is thought to be due to energy sparing resulting from alleviating the need to synthesize FA de novo in support of growth (Doreau and Ferlay, 1995) or inhibition of bacterial predation (Palmquist and Jenkins, 1980; Pantoja et al., 1995). The energy-sparing effect is consistent with observations that 8% of dietary lipid intake is converted to microbial lipid (Jenkins, 1993). The model assumes that this increase in microbial growth efficiency is restricted to fats and oils added to the diet; however, the observed correlations of microbial N passage errors with endogenous dietary lipid intake supports the use of other types of plant lipids to offset microbial FA synthesis. The linear effect of long-chain FA derived from oilseeds, grease, tallow, and calcium salts of FA, among other sources, on microbial growth efficiency is scaled with the parameter K_{FatFG} , where a value of 0 results in no stimulatory effect. No gains in efficiency were assumed from endogenous plant lipids present in the diet (Baldwin, 1995). After fitting to the data, K_{FatFG} solved for a value of 0.068, which is slightly more than double the value of 0.03 used by Baldwin (1995). Assuming that endogenous plant lipids average approximately 3% of dietary DM yields a 1% increase in microbial efficiency for every 1 percentage unit increase in dietary fat on a DM basis when K_{FatFG} is set to 0.03, and a 2.27% increase in efficiency when K_{FatFG} is set to 0.068.

Baldwin (1995) also included factors to inhibit fiber and protein digestion when oils and fats were added to the diet. These effects were captured as linear functions of the ratio of added fat over endogenous plant lipid, again multiplied by scalars (K_{FatHb} and K_{FatPi} , respectively) to alter sensitivity. Although reports exist of the inhibitory effects of fat on fiber degradation in the rumen (Palmquist and Jenkins, 1980; Pantoja et al., 1995), it appears these effects are restricted to high levels of unsaturated fat feeding (Doreau et al., 1991;

Table 8. Regression analyses of residual errors from final model predictions¹

Regression variable ²	Predicted variable												
	Ruminal metabolism						Ruminal outflow (kg/d)						
	pH	Amm (mol/L)	VFA (mol/L)	Acet (% of VFA)	Prop (% of VFA)	Buty (% of VFA)	Starch	NDF	ADF	Lipid	N	MiN	NANMN
DMI			0.003	−0.94	0.65		−0.15	−0.16	−0.19		0.020		
Nutrient intake													
CP	0.21	−0.006		7.28	−3.19			0.01	0.44		−0.051	−0.055	0.063
Soluble CP				4.76					−0.66				
Urea		−0.022						2.18	−0.51			−0.262	0.209
RUP	−0.24	0.006		−3.08	2.76						−0.091	0.036	−0.092
Starch	−0.04	0.002				0.56							
Soluble starch		−0.006				−2.76	1.24						
NDF	−0.06								0.30	0.57	−0.038		
ADF				−1.99					−0.71	−0.79		−0.014	
Lignin		0.007	−0.024					−1.57					−0.065
Added fat											−0.032		
Endogenous lipid ³									7.29			0.264	
Ash	−0.19	0.005		−4.18	3.83		2.74			−0.24			
BW					−0.03						0.001		0.001
Ruminal pH			0.352		33.4						0.817	0.489	−0.309
Ruminal ammonia	−14.0			−539	258	16.14		−94.8				11.5	−10.50

¹Residuals for ruminal pH, ammonia (Amm), VFA, acetate (Acet), propionate (Prop), and butyrate (Buty), and ruminal outflow of starch, NDF, ADF, lipid, nitrogen, microbial nitrogen (MiN), and nonammonia, nonmicrobial nitrogen (NANMN) were regressed on DMI, nutrient intakes, BW, predicted ruminal pH, and predicted ruminal ammonia concentrations. Experiment was included as a random effect in the regression model. All variables remaining in the model were significant ($P < 0.10$). Positive values denote underpredictions and negative values denote overpredictions.

²Independent variables have units of kilograms per day except for BW (kg), ruminal pH, and ammonia (mol/L).

³Lipids naturally present in forage and grain.

Klusmeyer et al., 1991a; Ohajuruka et al., 1991; Chan et al., 1997). Within the current data set with reported total dietary fat ranging up to 20% of DM, there was essentially no support for fat inhibition of protein digestion (Table 6). Baldwin (1995) noted that fat effects on fiber and protein degradation were poorly supported and, thus, tentative additions. Because K_{FatPi} and K_{FatHb} both solved for values that were essentially 0, any effects are very small. However, the lack of an effect of fat on fiber digestion may also reflect the fact that few of the studies examined the effects of unsaturated and unprotected fat sources. Because the model is not configured to represent differential effects of saturated and unsaturated fat, it is not able to capture any potential effects of unsaturated FA and, thus, may fail when challenged with data derived from diets supplemented with oils.

Although the above changes improved predictions of microbial yield, residual errors were still correlated with N, ADF, and lipid intakes as well as predictions of ruminal pH and ammonia (Table 8), indicating that the model does not adequately represent those interactions, or, in the case of pH and ammonia, bias in model predictions of those variables may be contributing to error in microbial yield predictions. Residual errors for ruminal pH were correlated positively with CP intake and negatively correlated with RUP, starch, NDF, ash intake, and ruminal ammonia concentration (Table 8). Correlations with the nutrient intakes may reflect inappropriate predictions of the relationships between available substrate supply and rates and patterns of ruminal fermentation, given the moderate errors in nutrient-degradation predictions. Although observed ammonia concentrations were not found to correlate with observed ruminal pH, ruminal ammonia concentrations were found to be correlated with residual errors for pH predictions suggesting that addition of ammonia to the pH prediction equation may have some merit. But although such an addition may improve model performance, it does not appear to be supported by observational data and may simply reflect model problems in predicting ammonia.

Ash, starch, protein, and NDF all contribute to osmotic balance in the rumen, which plays a role in determining the concentration of VFA and H in the rumen (Argyle and Baldwin, 1988). As noted above, because additional assumptions would have been required to define ash inputs to the water balance submodel, simulations were conducted with that submodel turned off and water volume predicted as a constant proportion of ruminal DM. Therefore, it cannot be determined whether lack of consideration of osmotic pressure and water balance across the rumen wall is contributing to pH prediction bias.

Ruminal ammonia residual errors were negatively correlated with intakes of CP, urea, and soluble starch intakes, and positively correlated with RUP, starch, lignin, and ash intakes. The negative correlations with CP, urea, and soluble starch intakes and positive correlation with RUP intake suggest that ammonia absorption and urea entry into the rumen may not be properly represented or parameterized. The model considers urea entry into the rumen by saliva, urea transport across the rumen wall, and inhibition of urea entry by ruminal ammonia, all of which are supported by experimental observations (Kennedy and Milligan, 1980; Huntington, 1989). Collectively, the residual slopes suggest that cycling of N from blood urea into the gut and back to urea via ammonia is too slow, allowing ammonia concentrations to respond too much to changes in N supply and stimulation of ammonia use by ruminal microbes in response to increased fermentable substrate (Kennedy and Milligan, 1980; Clark et al., 1992; Lobley et al., 2000). Increased rates of cycling would allow ruminal ammonia concentrations to be buffered from the blood urea pool, thereby dampening responses. Because residual analyses included experiment effects, it is less likely the observed correlations are due to diversity in sampling location or time after feeding (Storm et al., 2011) across experiments and, thus, must represent a fundamental deficiency in the model. Correcting this problem would require observations of urea recycling to the rumen and ammonia absorption.

Residual errors for VFA were positively correlated with DMI and ruminal pH and negatively correlated with lignin intake (Table 8). Dry matter intake was also negatively correlated with residuals for starch, NDF, and ADF flow from the rumen. The latter suggests that predicted passage rates of these nutrients increases too much as DMI increases, which is consistent with underpredictions of VFA concentrations (i.e., substrate is predicted to pass out of the rumen instead of being fermented to VFA). The positive correlation of VFA concentrations with ruminal pH suggests that VFA absorption should be stimulated by low ruminal pH, which has been observed experimentally (Stevens and Stettler, 1966), but is not encoded in the model (Baldwin, 1995). The negative correlation with lignin may reflect the lack of consideration of the inhibitory effect of lignin on cellulose and hemicellulose digestion. If lignin renders some portion of the normally digestible fiber indigestible (Van Soest, 1982), failure to consider that effect would result in too much fiber degradation and VFA production at high lignin intakes.

The correlations between the molar proportion of acetate and intake of DM and several nutrients (CP, RUP, and ash) and ruminal concentrations of ammonia, and correlations of the opposite sign for the same

independent variable effects on molar proportion of propionate strongly suggest that the submodel to determine VFA stoichiometric coefficients is inadequate, which is supported by a direct assessment of the VFA model using observed *de novo* synthesis data (Ghimire et al., 2012). Individual VFA are predicted as a function of total VFA production using a set of fixed molar proportions unique to each major nutrient class (soluble carbohydrate, protein, and fiber; Murphy et al., 1982). The opposing signs on the correlations are clear indicators that these stoichiometric coefficients or the entire approach to deriving fractional production of each VFA is inappropriate (Bannink et al., 1997). Given the importance of acetate as an energy source, propionate as a glucose source and a stimulator of insulin secretion, and the effect of the ratio of the two on methane production (Czerkawski, 1969; Whitelaw et al., 1984), correcting this deficiency in the model is extremely important. However, such a correction cannot be achieved with certainty using only VFA concentration data, as VFA absorption is not simply a function of ruminal VFA concentrations and pH (Storm et al., 2011). Thus, concentrations are not always proportional to production as has previously been assumed (Murphy et al., 1982; Bannink et al., 1997). Addressing the VFA production problem is supported by the work herein, as predictions of fermentable nutrient supply are required as inputs to the VFA submodel.

Ruminal concentrations of butyrate are generally less variable due possibly to reduced variation in butyrate production and the importance of butyrate in regulating ruminal blood flow and VFA absorption (Storm et al., 2011). Because butyrate concentrations drive the rate of uptake of butyrate from the rumen, concentration changes will be dampened with respect to production rates, thereby minimizing variation that could correlate with independent variables. However, the regulation of blood flow by butyrate would also have profound effects on acetate and propionate absorption, which may explain a portion of the prediction errors for acetate and propionate concentrations.

Residual errors for starch, NDF, and ADF passage to the small intestine were all negatively correlated with DMI, indicating that the model overpredicted outflow of these nutrients at high intake. Because the degradation rates were fitted to the observed intakes and flows, these correlations may suggest that particle passage rate is being predicted inappropriately. The version of the model used defines small particle passage rate as a mass action function of the small particle pool size. Earlier versions of the model (Baldwin, 1995) predicted small particle passage from BW and DMI using a regression equation derived from literature data (Evans, 1981; Robinson et al., 1985). Because residuals

for these 3 nutrients were correlated with DMI in the current work, reinstating the prior passage equation may improve residual bias. The lack of correlation of errors with BW may simply reflect an inadequate range in BW within this data set.

CONCLUSIONS

Although a meta-analysis should provide superior response parameter estimates, it is unclear how those parameters can be used in a global model such as Molly in the absence of knowledge of the cause of differences among experiments. A meta-approach was used, however, to guide model enhancements to capture some of the underlying reasons for experiment effects, thus explicitly representing that knowledge rather than treating it as a random event. Using such an approach in refitting digestive parameters to a large set of lactating cow observations resulted in significant improvements for predictions of some variables and more modest improvements for others. Key improvements were removal of mean bias in predictions of ruminal pH, NDF and microbial N outflow from the rumen, and postruminal NDF digestion, and reductions in slope bias for predictions of the proportions of all 3 VFA and postruminal digestion of fiber. Key findings supporting the improved performance were that pH responses to ruminal VFA concentrations are one-third of the previously estimated slope, the half-maximal microbial growth response to ruminal ammonia was one-sixth of the previous estimate, the efficiency of microbial growth in response to added dietary fat appears to be twice the previous estimate, and added fat had no observable negative effect on protein and fiber degradation. Results indicated that additional improvements in model performance could be achieved through better representations of particle passage from the rumen, VFA production, the effects of ruminal pH on microbial growth efficiency, cycling of urea and ammonia across the rumen wall, and postruminal fiber digestion.

ACKNOWLEDGMENTS

Funding support was provided by the Virginia State Dairymen's Association (Bridgewater, VA) and the College of Agriculture and Life Sciences of Virginia Tech. (Blacksburg). Salary support for M. D. Hanigan was provided by the New Zealand Dairy Farmers through DairyNZ Ltd. (Hamilton, New Zealand).

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