A revised representation of ruminal pH and digestive reparameterization of the Molly cow model

Meng M. Li and Mark D. Hanigan*

Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg 24061

ABSTRACT

Ruminal pH is a critical factor to regulate nutrient degradation and fermentation. However, it has been poorly predicted in the Molly cow model, and recent improvements in the representation of nitrogen cycling across the rumen wall altered some of the modeled responses to feed nutrients, resulting in some model bias. The objectives of this study were to further improve the representation of pH and to refit parameters related to ruminal metabolism and nutrient digestion in the model to resolve this bias, and to use the improved model to estimate nitrogen and energy fluxes with varying rumen-degradable protein (RDP; 40 vs. 60%) and ruminally degraded starch (RDSt; 50 vs. 75%). A meta data set containing 284 peer reviewed studies with 1,223 treatment means was used to derive parameter estimates for ruminal metabolism and nutrient digestions. Refitting the parameters significantly improved the accuracy and precision of the model predictions for ruminal nutrient outflow [acid detergent fiber (ADF), neutral detergent fiber (NDF), total N, microbial N, nonammonia N, and nonammonia nonmicrobial N], ammonia and blood urea concentrations, and fecal nutrient outflow (protein, ADF, and NDF). The prediction error for body weight was decreased from 19.3 to 6.2% with decreased mean bias (from 76.0 to 11.5%) and slope bias (from 17.2 to 7.7%), primarily due to improved representations of ruminal dry matter and liquid pool size. Adding ammonia concentration as a driver to the pH equation increased the precision of predicted ruminal pH and, thereby, the precision of predicted volatile fatty acid (VFA) concentrations, due to improved representation of pH regulation of VFA production rates. Although minor mean and slope bias were observed for ruminal pH and VFA concentrations, the concordance correlation coefficients indicated that much of the observed variation in these variables remains unexplained.

Overall, the biological functions of nutrient degradation and digestion appear to be represented without bias. Simulated results indicated that decreasing RDP and RDSt proportions in an isonitrogenous and isocaloric diet can slightly improve N efficiency, and increasing RDSt proportions can increase energy efficiency.

Key words: mathematical model, simulation, nitrogen, energy

INTRODUCTION

For most dairy operations, feed is the single largest cost, representing approximately 70% of total operating costs (Short, 2004). As a result, improving the efficiency with which feed is converted into salable milk is a primary goal. Within a diet, protein and energy are the most expensive nutrients (St-Pierre and Glamočic, 2000), suggesting that careful ration balancing to optimize energy and protein supplies is a critical step toward optimizing feed efficiency.

Models such as the NRC (2001), Cornell Net Carbohydrate and Protein System (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992; O'Connor et al., 1993), NorFor (Volden, 2011), and the INRA Feeding System (Sauvant et al., 2017) are examples of nutrient requirement systems that are used for ration balancing. Least-cost formulation programs based on these models have been developed, and this approach has been extensively used to optimize the combination of feed ingredients to meet required nutrient needs with minimum cost (St-Pierre and Thraen, 1999). However, considering the level of aggregation of these requirement response models, the relationships between nutrient inputs and level of productivity are not always well matched (Huhtanen and Hristov, 2009; Vyas and Erdman, 2009; Hollmann et al., 2011; Leduc et al., 2017). Critical components of some metabolic reactions are not adequately captured, and thus they do not completely reflect the potential range of nutritional efficiency. It is also difficult to simulate time-dependent management strategies, given the static nature of these models. Alternatively, Molly is a dynamic and mechanistic model that integrates biological knowledge at the pathway level. Therefore, it

Received February 15, 2020.

Accepted August 2, 2020.

*Corresponding author: mhanigan@vt.edu

allows assessment of energy and nitrogen efficiency by comparing various biological measurements (Hanigan et al., 2006).

A series of efforts to improve representations of mechanistic functions and model parameter estimates of the Molly model have been conducted. These have focused on ruminal metabolism, nutrient digestion, and animal metabolism, and have yielded significant improvements in accuracy and precision (Hanigan et al., 2006; Hanigan et al., 2009; Hanigan et al., 2013; Gregorini et al., 2015). Most recently, the representation of nitrogen cycling between blood and the gut was improved, which addressed prior bias in predictions of ruminal ammonia concentration when evaluated with a small data set (Li et al., 2019b). Li et al. (2018) hypothesized, based on residual errors, that consideration of the effects of ammonia concentrations in the ruminal pH equation would improve the representation and thereby improve predictions of ruminal fiber digestion and VFA production. Ammonia was found to be an important driver of pH in the original equation by Briggs et al. (1957).

Because ammonia and pH are regulators of microbial growth, which in turn regulates nutrient degradation and fermentation, any changes in those representations necessitates reparameterization work to recenter predictions of ruminal metabolism and nutrient digestion in the observed data. Therefore, the objectives of this study were (1) to alter the representation of pH to include ammonia; (2) to refit parameters related to ruminal metabolism and nutrient digestion; and (3) to use the improved model to predict nitrogen and energy fluxes with varying RDP and ruminally degraded starch. We hypothesized that the model would better reflect the underlying biological functions of ruminal metabolism and nutrient digestion after the pH changes and reparameterizations.

MATERIALS AND METHODS

Data Collection

The data set used to derive parameters and evaluate the model predictions was assembled from studies used by Li et al. (2018) and Fleming et al. (2019). A list of the studies is provided in Supplemental Table S1 (<https://doi.org/10.3168/jds.2020-18372>), and the whole data set can be download at the National Animal Nutrition Program website (<https://animalnutrition.org/modeling-database>). The selection criteria for inclusion of studies in the database were as follows: the work was conducted in beef or dairy cattle; DMI and the ingredient composition of the diets were reported; and either ruminal fermentation variables (pH, ammonia, and

VFA concentrations) or nutrient digestion variables (ADF, NDF, starch, protein, or fat digestibility, ruminal outflow, or fecal outflow) were reported or could be calculated. In total, 284 studies with 1,223 treatment means were included in the data set. A descriptive summary of the data set is presented in Table 1.

Model Description

The model used was that of Baldwin et al. (1987a,b,c) with modifications described by Baldwin (1995), Hanigan et al. (2006, 2007, 2009, 2013), Gregorini et al. (2015), and Li et al. (2019b). It is important to note that the work of Li et al. (2019b) included significant changes to the representation of urea entry into the rumen, ammonia absorption from the rumen, and ammonia effects on urea entry and microbial growth. Thus, the behavior of the model after this update would be expected to deviate from the behavior before those changes were implemented. Required model input variables included DMI, BW, chemical composition of the diet (CP, starch, NDF, ADF, and fat), ruminal solubility and degradability of protein and starch, ruminal degradability of ADF, forage NDF, and diet particle size distribution. These variables were prepared following the same procedure described by Li et al. (2019b).

The original pH equation was developed by Briggs et al. (1957) and adapted by Argyle and Baldwin (1988) without considering ammonia concentrations. Hanigan et al. (2013) added an intercept scalar ($K_{pH,Rum}$) into the equation to adjust mean bias of predicted pH. Based on the residual analyses conducted by Li et al. (2018), ammonia concentrations were added to the existing ruminal pH equation to improve representations of acid-base balance in the rumen:

$$pH = K_{VFApH,Rum} \times C_{VFA,Rum} + 1.5 \times C_{La,Rum} - K_{AmpH,Rum} \times C_{Am,Rum} + K_{pH,Rum} \quad [1]$$

where $C_{VFA,Rum}$, $C_{La,Rum}$, and $C_{Am,Rum}$ represented total VFA, lactate, and ammonia concentrations; $K_{VFApH,Rum}$ and $K_{AmpH,Rum}$ were VFA and ammonia coefficients for ruminal pH; and $K_{pH,Rum}$ was an intercept scalar to adjust ruminal pH.

In addition to changes in the ruminal pH equation, initial model evaluations indicated that BW was substantially overestimated. Body weight is calculated by adding total gut contents to empty BW (Baldwin, 1995). We identified that the overestimated BW is largely due to overestimated ruminal DM and liquid pool size when rumen volume was defined as a function of observed DMI. Thus, to ensure a more stable estimate, the initial rumen volume equation ($RumVol$)

Table 1. Descriptive summary of observed nutrient intake, apparent ruminal digestibility, ruminal outflow and fermentation, and apparent total-tract digestibility in the fitting database

Item	n	Mean	SD	Minimum	Maximum
BW, kg	1,154	582.76	95.14	248.00	807.00
Rumen pool size, kg					
DM	94	12.0	5.87	5.90	42.8
Liquid	66	57.4	17.6	30.1	92.4
Intake, kg/d					
DM	1,223	17.6	5.83	3.99	31.8
ADF	396	3.72	1.28	0.30	6.90
NDF	549	6.10	2.07	1.01	11.3
Fat	199	0.91	0.46	0.16	3.2
Starch	347	5.23	1.99	0.07	11.0
Protein	1,014	3.01	1.18	0.62	6.01
Apparent ruminal degradability, %					
Protein	380	59.18	14.5	5.7	103
ADF	305	39.95	12.2	6.20	77.1
NDF	454	44.08	12.3	11.40	83.0
Starch	297	66.54	18.8	9.70	96.7
Ruminal outflow, kg/d					
DM	176	14.12	4.27	3.50	25.7
ADF	229	2.31	0.81	0.23	4.76
NDF	337	3.64	1.32	0.74	7.35
Starch	206	2.34	1.64	0.09	7.12
Fat	39	0.96	0.37	0.40	2.00
Total N	508	0.50	0.16	0.11	1.00
Microbial N	629	0.27	0.10	0.05	0.60
Nonammonia N	598	0.49	0.15	0.07	0.93
Nonammonia nonmicrobial N	599	0.21	0.09	0.003	0.46
Ruminal fermentation					
pH	593	6.16	0.28	5.44	6.92
Ammonia, mM	536	7.23	2.90	0.71	16.76
Total VFA, mM	575	112	22.5	11.00	183
Acetate, mM	562	68.2	21.6	6.51	102
Propionate, mM	562	25.7	11.1	2.43	78.0
Butyrate, mM	562	13.3	4.87	1.51	33.7
Blood urea N, mg/dL	117	12.8	4.05	2.6	23.4
Apparent total-tract digestibility, %					
DM	402	0.69	0.06	0.50	0.86
Protein	587	0.68	0.06	0.44	0.87
ADF	333	0.45	0.11	0.12	0.76
NDF	529	0.51	0.11	0.20	0.84
Starch	303	0.94	0.05	0.76	1.00
Fat	161	0.72	0.11	0.37	0.94

was altered to calculate the pool size as a proportion of initial body weight (iBW):

$$RumVol = iBW \times 0.15 \quad [2]$$

Previously we assumed that ruminal urea entry across the rumen epithelium and postruminal urea entry were proportional to DM disappearance in the rumen and intestines (Li et al., 2019b), the latter being calculated by difference from instantaneous rates. However, because ruminal DM outflow is a mass function of ruminant DM pool size, the ruminal DM outflow could be greater than DMI when the model is initiated or when reducing DMI during a simulation. This can result in negative, instantaneous values for apparent ruminal or intestinal DM disappearance and, subsequently, negative urea entry into the rumen or intestines, leading to model

instability. Because blood urea recycled to the intestine on a net basis should be a function of microbial activity in the hindgut, total urea entering the rumen and intestine was assumed to be proportional to fermentable DM in each compartment ($F_{FermDM,Rum}$ and $F_{FermDM,Int}$, mol/d):

$$F_{FermDM,Rum} = F_{Ac,RumBld} + F_{Ac,RumInt} + F_{Pr,RumBld} + F_{Pr,RumInt} + F_{Bu,RumBld} + F_{Bu,RumInt} \quad [3]$$

$$F_{FermDM,Int} = F_{HcAc,Int} + F_{HcPr,Int} + F_{HcBu,Int} + F_{CeAc,Int} + F_{CePr,Int} + F_{CeBu,Int} + F_{MiPr,Int} + F_{MiBu,Int} \quad [4]$$

$$f_{FermDM} = \frac{F_{FermDM,Rum}}{F_{FermDM,Int}}, \quad [5]$$

where $F_{Ac,RumBld}$, $F_{Pr,RumBld}$, and $F_{Bu,RumBld}$ represent ruminal acetate, propionate, and butyrate absorption rate (mol/d); $F_{Ac,RumInt}$, $F_{Pr,RumInt}$, and $F_{Bu,RumInt}$ are ruminal outflow of acetate, propionate, and butyrate (mol/d); $F_{HcAc,Int}$ and $F_{CeAc,Int}$ represent conversions of hemicellulose and cellulose to acetate in the hindgut (mol/d); $F_{HcPr,Int}$, $F_{CePr,Int}$, and $F_{MiPr,Int}$ are conversions of hemicellulose, cellulose, and microbes to propionate (mol/d) in the hindgut; $F_{HcBu,Int}$, $F_{CeBu,Int}$, and $F_{MiBu,Int}$ are conversions of hemicellulose, cellulose, and microbes to butyrate (mol/d) in the hindgut; and f_{FermDM} represents the proportion of fermentable DM in the rumen ($F_{FermDM,Rum}$) to fermentable DM in the hindgut ($F_{FermDM,Int}$).

The model was compiled in ACSLX (version 3.1.4.2, Aegis Technologies Group Inc., Huntsville, AL) and solved using a fourth-order, variable step Runge-Kutta integration algorithm with a maximum step size of 0.005 d. The model was set to simulate 14 d, and val-

ues from the last day were collected and aligned with observed values to determine residual errors. This was determined to ensure that the model had reached steady state.

The parameters listed in Table 2 were estimated simultaneously using the Direction Set algorithm to maximize the log-likelihood function (Press et al., 2007). Use of a log-likelihood as the objective function minimizes the influence of extreme values on parameter estimation (Nocedal and Wright, 2006). Numerical bounds were placed on parameters during estimation to avoid unstable solutions. However, if the optimized values were at a bound, the minimal or maximal values were adjusted to expand the ranges, to ensure that the final optimized values were not resting on a bound. The accuracy and precision of model predictions before and after parameterization were assessed by calculation of root mean squared errors (**RMSE**), mean bias, and slope bias as described by Bibby and Toutenburg

Table 2. Model parameter estimates when the model was fitted to the observed data summarized in Table 1

Model parameter	Description	Previous value	Optimized value	SD ¹
Ruminal				
$K_{liquid,Rum}$	Rate constant for ruminal liquid outflow (per d)	2.66	3.29	0.01
$K_{RUSI,Rum}$	Intercept scalar to adjust in situ determined ruminal starch degradation rates (per mol)	0.15	0.16	0.02
$K_{slpRUSI,Rum}$	Slope scalar to adjust in situ determined ruminal starch degradation rates (per mol)	0.18	1.38	0.06
$K_{RUADF,Rum}$	Intercept scalar to adjust in situ determined ruminal cellulose degradation rates (per mol)	2.70	3.16	0.11
$K_{slpRUADF,Rum}$	Slope scalar to adjust in situ determined ruminal cellulose degradation rates (per mol)	0.48	1.68	0.02
$K_{HcCsI,Rum}$	Scalar to calculate the hemicellulose degradation rate constant from the cellulose rate constant (per mol)	0.69	0.40	0.01
$K_{RUP,Rum}$	Intercept scalar to adjust in situ determined ruminal protein degradation rates (per mol)	1.81	1.95	0.02
$K_{slpRUP,Rum}$	Slope scalar to adjust in situ determined ruminal protein degradation rates (per mol)	0.16	4.0×10^{-4}	3.1×10^{-5}
$K_{AmMi,Rum}$	Rate constant for the effect of ammonia on microbial growth (L/mol)	7.1×10^{-4}	3.7×10^{-4}	2.1×10^{-4}
$K_{FatMi,Rum}$	Constant to calculate the effect of fat on microbial growth (mol of DM/mol of dietary fat)	0.07	1.0×10^{-4}	1.1×10^{-5}
$K_{YATP,Rum}$	Efficiency constant defining the yield of microbial DM per mole of ATP (g/mol)	0.01	0.03	5.0×10^{-4}
X_{EAm}	Exponential factor to adjust the effect of ammonia on microbial growth (mol/mol)	8.85	1.0	0.2
$K_{AmpH,Rum}$	Slope for ammonia effect on pH (per mol)	NA ²	10.0	2.0×10^{-3}
$K_{pH,Rum}$	Intercept for pH prediction	6.52	7.16	4.0×10^{-3}
$K_{VFApH,Rum}$	Slope for total VFA effect on pH (per mol)	3.94	10.51	0.03
$K_{Am,RumBld}$	Rate constant for ammonia absorption (per d)	5.01	12.3	0.15
$K_{Ac,RumBld}$	Rate constant for acetate absorption (per d)	4.20	5.02	0.05
$K_{Pr,RumBld}$	Rate constant for propionate absorption (per d)	6.45	7.69	1.0×10^{-3}
$K_{Bu,RumBld}$	Rate constant for butyrate absorption (per d)	6.45	6.80	0.11
$K_{Ur,BldUrin}$	Rate constant for urea excretion by the kidney (per mol)	1.86	0.42	5.0×10^{-3}
Post-ruminal				
$K_{Protein,IntFec}$	Intestinal digestion coefficient for protein (g/g)	0.79	0.71	0.03
$K_{Starch,IntFec}$	Intestinal digestion coefficient for starch (g/g)	0.81	0.75	0.01
$K_{Fiber,IntFec}$	Intestinal digestion coefficient for fiber (g/g)	0.12	0.11	2.0×10^{-3}
$K_{Lipid,IntFec}$	Intestinal digestion coefficient for lipid (g/g)	0.71	0.70	2.0×10^{-3}

¹SD of the optimized value.

²NA = not applicable.

(1977); and concordance correlation coefficients (CCC) as described by Lin (1989).

Efficiency Simulations

Global sensitivity tests are helpful to identify the importance of model parameters, especially for the newly developed model. Hanigan et al. (2013) previously conducted global sensitivity tests for the model. As an alternative, model simulation is an effective method to test model performance. The model simulation results can provide practical feedback and determine the correctness and efficiency of the model before the model is applied in the real world. We hypothesized that the modified model would better represent ruminal reactions involving nitrogen and energy metabolism. To examine the effects of nutritional changes on nitrogen and energy efficiency, 4 isonitrogenous and isocaloric diets were designed to standardize nitrogen and energy intake. The 4 diets represented a 2×2 factorial arrangement of RDP at 40 or 60% of CP, and ruminally degraded starch (RDSt) at 50 versus 75% of total starch. All diets contained the same concentrations of 6% fat, 30% starch, 16% CP, 30% NDF, 20% ADF, and 6% ash. The animal was assumed to be a Holstein dairy cow weighing 600 kg and 70 DIM. All simulations were performed at an intake of 20 kg of DMI per day. Model values from d 14 of the simulation were used to represent steady state values. Given that the model has been fitted to a large number of observations from the literature, it is assumed that the model represents an average cow from the research herd population. There was no replication for the experimental unit, so no statistical analyses were conducted for the simulation results. All evaluations of nitrogen and energy metabolism were assessed based on numeric differences.

RESULTS AND DISCUSSIONS

Residual error analyses for the model predictions before and after reparameterization are presented in Table 3. In this comparison, the initial model refers to the Molly cow model after improved representation of urea and ammonia N recycling, as described in Li et al. (2019b), and the modified model is the model after revising equations for ruminal volume pool size, ruminal pH, proportion of fermentable DM, and reparameterization herein. In general, except for BW, ruminal pH, ruminal ammonia concentrations, and fecal protein output predictions for the initial model, both the initial and modified models had minor slope bias (less than 10% of mean squared error), suggesting that the encoded mechanisms representing ruminal metabolism and nutrient digestion were appropriately

conceived and parameterized. The combination of updating the ruminal pH and initial rumen volume equations and refitting model parameters listed in Table 2 to the data significantly increased the accuracy and precision of model predictions for BW, rumen DM and liquid pool size, ruminal nutrient outflow [ADF, NDF, starch, microbial N, nonammonia N, and nonammonia nonmicrobial N (NANMN)], ammonia and blood urea concentrations, and fecal nutrient outflow (DM, protein, NDF, and starch), primarily due to decreased mean bias. Improvements in ruminal and fecal outflow of DM and fat were negligible. Minor mean and slope bias remained for predicted ruminal pH and VFA concentrations in the modified model. However, the small CCC values suggest that the model fails to capture a large proportion of the observed variation. In some cases, as for ruminal pH and VFA concentrations, this may reflect variation in sampling time with respect to a meal and sample location, neither of which were captured in the data. Therefore, it is unclear whether the model truly fails to represent the underlying processes or whether the unexplained variation is a function of sampling and analytical error.

BW and Rumen Pool Size

As shown in Figure 1, the initial model tended to overpredict BW with a moderate slope bias (19.3% of RMSE with a mean bias of 76.0% and a slope bias of 17.2%; Table 3). In the model, initial BW is taken as an input and used to calculate initial pool sizes for most elements in the model. Predicted BW is subsequently represented as a sum of empty BW and gut contents, with both being the sum of the pools within those compartments. Initial rumen DM and liquid pool sizes were being overestimated at model initialization, and the overprediction of BW derived from this initialization problem. Previously, initial rumen volume was a proportion of DMI (Baldwin, 1995), which could inflate to increase the stability of rumen pool. However, this is problematic when DMI is being predicted and is not known at model initialization. Therefore, BW was used to initialize rumen volume, and the rate constant for ruminal liquid outflow ($K_{liquid, Rumen}$) was optimized by refitting to observations of ruminal DM and liquid pool size. These changes resulted in a decrease in BW prediction errors from 19.3 to 6.2%, with decreased mean bias (from 76.0 to 11.5%) and slope bias (from 17.2 to 7.7%). The CCC was increased from 0.58 to 0.91, suggesting that the revised representation was an improvement. However, rumen DM and liquid pool sizes were still overestimated. Rumen DM is determined from the balance of DM intake, passage, and degradation with passage as function of solubility, particle size,

Table 3. Residual error analyses for Molly predictions before and after reparameterizations¹

Item	n	Observed mean	Predicted mean		RMSE, % of mean		Mean bias, % of MSE		Slope bias, % of MSE		CCC	
			Initial ²	Modified ³	Initial	Modified	Initial	Modified	Initial	Modified	Initial	Modified
BW, kg	1,154	583	682	571	19.3	6.2	76.0*	11.5*	17.2*	7.7*	0.58	0.91
Rumen pool size, kg												
DM	94	12.0	18.7	15.6	73.7	54.4	57.7*	31.2*	7.8*	6.4*	0.26	0.36
Liquid	66	57.4	118	90.5	113	67.4	87.5*	73.2*	5.9*	8.1*	0.05	0.09
Ruminal outflow, kg/d												
DM	176	14.1	14.0	13.3	14.4	15.0	17.4*	16.2*	2.0	1.5	0.79	0.79
ADF	229	2.3	2.5	2.2	22.4	20.9	17.0*	3.2	3.5*	1.9	0.75	0.76
NDF	337	3.6	4.2	3.9	27.6	23.7	29.6*	10.2*	2.5	3.3	0.66	0.73
Starch	206	2.3	2.4	2.3	50.7	48.3	10.0*	11.3*	0.2	0.1	0.29	0.37
Fat	39	1.0	1.0	1.0	20.0	20.3	0.001	7.4*	1.1	0.6	0.85	0.85
Total N, g/d	508	497	498	508	19.2	19.0	0.02	1.4	0.7	0.3	0.80	0.80
Microbial N, g/d	629	272	191	276	41.1	27.6	52.5*	0.3	5.1*	0.8	0.33	0.59
NAN, g/d	598	486	433	487	22.7	19.5	23.8*	0.01	1.1	0.2	0.65	0.74
NANMN, ⁴ g/d	599	208	238	205	38.0	34.5	14.6*	0.1	8.9*	5.2*	0.51	0.52
Ruminal fermentation												
pH	593	6.2	6.2	6.1	4.6	3.7	5.0*	5.7*	14.9*	10.5*	-0.08	0.11
Ammonia, mM	536	7.2	5.2	7.0	86.9	33.8	64.5*	3.2*	19.9*	16.7*	0.22	0.47
Total VFA, mM	575	112	95	108	25.3	20.5	34.6*	2.6	9.2*	9.2*	0.09	0.09
Acetate, mM	562	68.2	61.9	68.9	22.3	20.1	17.3*	0.2	5.3*	2.6*	0.21	0.19
Propionate, mM	562	25.7	22.5	25.8	40.2	38.1	9.9*	0.004	8.7*	9.8*	0.01	0.04
Butyrate, mM	562	13.3	10.6	13.2	34.9	28.6	35.4*	0.1	4.4*	5.3*	0.07	0.10
Blood urea N, mg/dL	117	12.8	8.0	12.3	78.9	34.2	86.3*	1.1	0.03	7.5*	0.02	0.29
Fecal outflow, kg/d												
DM	375	6.1	5.4	5.7	19.7	16.2	31.8*	14.8*	8.3*	3.5*	0.81	0.88
Protein	509	1.0	0.8	1.1	36.3	19.2	57.0*	1.9	16.9*	4.1*	0.44	0.81
ADF	257	2.1	2.3	2.0	17.8	17.9	21.6*	6.4*	0.0	1.4	0.88	0.87
NDF	413	3.1	3.7	3.5	27.7	24.0	41.3*	19.6*	2.2	2.8	0.75	0.80
Fat	142	0.3	0.3	0.3	36.7	36.4	0.2	5.3*	4.6*	2.8	0.76	0.76
Starch	242	0.4	0.4	0.4	85.4	83.4	2.2	0.1	3.6*	3.7*	0.31	0.37

¹RMSE = root mean squared error; MSE = mean squared error; CCC = concordance correlation coefficient.²Initial model refers to the Molly cow model after improved representation of urea and ammonia N recycling, as described in Li et al. (2019b).³Modified model is the model after revising equations for ruminal volume pool size, ruminal pH, proportion of fermentable DM, and reparameterization.⁴Nonammonia nonmicrobial N.*Model bias significantly different from 0 ($P < 0.05$).

and liquid outflow. Water entry to the rumen is driven by osmotic balance in the rumen, and liquid outflow is a mass-action function of the liquid pool size. An increase in the passage rate constant ($K_{liquid,Rum}$) will generally decrease the digesta retention time, leading to decreased extent of nutrient degradation. Because all of the parameters related to nutrient degradation rate were refitted when $K_{liquid,Rum}$ was derived, the bias in rumen pool size suggests a compromise among the parameters to maximize the log-likelihood function for the given data, and additional gains in accuracy may be achievable with additional work. A significant factor in the prediction scheme is osmolality. Much of the osmotic potential derives from minerals, but mineral solubility is rarely characterized and reported. Further progress in this area will likely require additional experiments, in which dietary ash content, solubility, and osmolality; rumen volume, composition, and osmolality; and ruminal liquid and particulate outflow, including particle size, have been measured under conditions of varying dietary osmotic potential.

Nutrient Outflow from the Rumen

Altering the model structure and refitting intercept scalar $K_{RUADF,Rum}$ and slope scalar ($K_{slpRUADF,Rum}$) to adjust in situ determined ruminal cellulose degradation rate, and scalar to calculate the hemicellulose degra-

dation rate constant from the cellulose rate constant ($K_{HcCs1,Rum}$), resulted in reductions in prediction errors. Root mean squared errors for ruminal outflow of ADF were 22.4 and 20.9% for the initial and modified versions, respectively, and 27.6 and 23.7%, respectively, for NDF outflow. Mean bias decreased from 17.0 to 3.2% for ADF and from 29.6 to 10.2% for NDF; and slope bias was mixed. The CCC increased from 0.75 to 0.76 for ruminal ADF outflow and 0.66 to 0.73 for NDF outflow.

The descriptive summary of the observed data showed that roughly 40% of ADF and 44% of NDF were degraded in the rumen (Table 1). In the model, an estimate of undegraded ADF (% of ADF) is used as a model input for each feed. The intent of this input is to allow use of in situ data to set the intrinsic degradation characteristics of each feed, as is done for RUP. However, the available data for feed ingredients is not as extensive as for protein degradability, and thus, in practice, this input is often set by diet using observed ruminal digestibility values. In the current study, rumen ADF degradation was estimated from dietary starch concentration and DMI using the same data and statistical approach as reported by White et al. (2017). Thus the predictions of ruminal outflow are not completely independent of the data, but more so than using the observed fractional degradation directly. Although use of in situ determined degradation rate

Figure 1. Residual analyses for BW before (A) and after (B) modification of rumen volume equations. The solid line represents the line of unity. RMSE = root mean squared error; MSE = mean squared error; CCC = concordance correlation coefficient.

as an input provides independence, it is likely that those rates are biased compared with measurements of duodenal or omasal fiber flows. The model calculates a prevailing rate of degradation based on microbial pool size, ruminal pH, and the specified potential degradation, and uses this with outflow predictions to calculate the extent of degradation. The intercept ($K_{RUADF,Rum}$) and slope scalars ($K_{slpRUADF,Rum}$) provide any needed bias adjustment to align in situ determined ruminal cellulose degradation rate constants for the feed with the observed outflow data (Hanigan et al., 2013; Gregorini et al., 2015). The rate constant for hemicellulose degradation was calculated as a proportion ($K_{HcCs1,Rum}$) of the cellulose rate constant (Baldwin, 1995).

Ruminal starch outflow exhibited a RMSE of 48.3% and a CCC of 0.37 in the modified model, suggesting that starch degradation was still poorly estimated. However, 90% of the error segregated as random, indicating that the model mechanisms were properly configured and parameterized, yielding unbiased estimates. Similar results were also reported in previous studies (Hanigan et al., 2013; Gregorini et al., 2015; Li et al., 2018). Depending on the diet type, approximately $66.5 \pm 18.8\%$ of starch is degraded in the rumen. Although an official AOAC method for determining starch content exists, the interlaboratory variation in starch analysis was very high (Mills et al., 1999; Firkins et al., 2001). Because the proportions of starch within the various particle sizes were different (Galyean et al., 1981), large variations are likely introduced when collecting digesta samples from duodenal or omasal cannula due to sampling bias. Additionally, factors that affect starch degradability, such as grain type (Firkins et al., 2001), processing methods (Svihus et al., 2005), and animal genetic variation (Channon et al., 2004), were not represented in the model. Therefore, refitting the intercept ($K_{RUST,Rum}$) and slope scalars ($K_{slpRUST,Rum}$), although this improved accuracy, did not improve the precision of model predictions for ruminal starch degradation.

Approximately 59% of dietary protein was degraded in the rumen. Similar to ruminally undegraded ADF and starch, RUP determined from in situ evaluations was a model input used to calculate the model rate parameter for protein degradation with mean ($K_{RUP,Rum}$) and slope bias scalars ($K_{slpRUP,Rum}$). Refitting the latter 2 parameters led to a slight RMSE decrease for ruminal NANMN outflow, from 38.0 to 34.5%, with a reduction in mean bias from 14.6 to 0.1%. As for starch flows, it is difficult to assess what proportion of this error is due to random animal, sampling, and analytical error, but the lack of mean and slope bias in the predictions suggests that the concepts incorporated into the model are unbiased representations of those mechanisms.

Microbial protein accounts for more than 50% of metabolizable protein. As originally described by Baldwin (1995), microbial growth is driven by ruminally available substrate (AA, peptides, and ammonia) and energy with greater efficiency if minimal amounts of preformed fatty acids were available, but inhibited by supplemental dietary fat. Improving the representations of ruminal pH and initial ruminal volume as well as refitting the rate constants for the effect of ammonia on microbial growth ($K_{AmMi,Rum}$), the yield of microbial DM per mole of ATP ($K_{YATP,Rum}$), and the inhibitory effect of fat on microbial growth ($K_{FatMi,Rum}$) significantly decreased mean bias (from 52.5 to 0.3%) and slope bias (from 5.1 to 0.8%) for ruminal microbial N outflow predictions, resulting in a reduction of RMSE from 41.1 to 27.6%. Because the modified model as described in Li et al. (2019b) was not fitted to the whole data set, it is more reasonable to compare the final model predictions with the model before we updated the N equations, as described by Gregorini et al. (2015). The precision was 30.9% (with 18.4% mean bias and 0.01% slope bias) in the model before modifications of the N equations (Li et al., 2018), implying that progress was slight, although the mean bias decreased. Because the microbial equation is very empirical in nature, further progress in prediction accuracy may require a more mechanistic approach. Additionally, the precision of measurement for the observed data is not great with current methods, which will limit the apparent model precision.

Ruminal Fermentation Parameters

Mean observed daily ruminal pH values ranged from 5.44 to 6.92. After modification and refitting to the observations, RMSE for ruminal pH was slightly decreased, from 4.6 to 3.7, due to decreased slope bias (from 14.9 to 10.5%). However, as shown in Figure 2, model performance before the modifications was abysmal, and although performance improved after modification, it remained far from a robust representation of the data. The range in predicted pH, although still less than observed, is much greater than for the prior representation, as reflected in the CCC increase from -0.08 to 0.11. Li et al. (2019a) developed a multiple linear regression model with DMI and dietary lignin and ash contents as independent variables and a neural network model to predict ruminal pH, and both models had a greater CCC (0.37 and 0.57) than the Molly model, suggesting that additional progress is possible.

Although a negative correlation occurred between ruminal VFA concentrations and ruminal pH, the relationship appears to be weak (Allen, 1997). Ruminal pH fluctuations are responsive to meals and chewing behavior, resulting in significant variation within a day,

Figure 2. Residual analyses for ruminal pH before (A) and after (B) modification of pH equation and parameter estimations. Solid line represents the line of unity. Each point represents a treatment mean of ruminal pH. RMSE = root mean squared error; MSE = mean squared error; CCC = concordance correlation coefficient.

and such temporal variation is generally not captured in the reported data. Additional factors that affect pH, including particle sizes, chewing behavior, time after a meal, size of the meal, and sampling location, are rarely reported, but should be if we are to gain a better understanding of the problem. Despite these limitations, the model now clearly better represents changes in pH so that the effects of pH on digestion can be evaluated.

Volatile fatty acids are produced from carbohydrate and protein fermentation in ruminal microbes (France and Dijkstra, 2005). The vast majority of the resulting VFA are absorbed through the rumen wall (Sutton et al., 2003). Therefore, VFA concentrations are influenced by VFA production and absorption rates. Altering ruminal pH equation and initial ruminal volume along with the newly derived rate constants for acetate, propionate, and butyrate absorption ($K_{Ac,RumBld}$, $K_{Pr,RumBld}$, and $K_{Bu,RumBld}$) led to improved precision and accuracy of VFA concentrations. Root mean squared errors decreased from 25.3 to 20.5% for total VFA, 22.3 to 20.1% for acetate, 40.2 to 38.1% for propionate, and 34.5 to 28.6% for butyrate. Negligible mean bias (less than 3%) was observed, and from 5 to 12% of the errors were due to slope bias. Because bias was not able to be resolved through adjustment of the rate constants for absorption, its presence suggests other factors possibly affecting VFA concentrations, which are not adequately

represented. As described by Argyle and Baldwin (1988), VFA production rates are regulated by ruminal pH. Our previous work found significant correlations between observed pH and residuals for VFA concentrations, suggesting that the effects of pH on VFA production were not properly represented (Li et al., 2018). In the current study, correlations between VFA residuals and ruminal pH were not significant, implying that the improvements in pH precision contributed to improved predictions of VFA production, as originally described by Argyle and Baldwin (1988). However, the relatively weak performance for ruminal VFA concentration predictions suggests that additional work is required.

Ghimire et al. (2014) indicated that predicted VFA concentration bias was caused by inappropriate predictions of VFA production rates, which may be affected by thermodynamic state. Ghimire et al. (2017) subsequently introduced a representation of thermodynamic control of the interchanges among the VFA into Molly, based on the work of Ungerfeld and Kohn (2006), and derived new estimates of de novo VFA synthesis from the individual substrates according to the scheme of Murphy et al. (1982), by fitting to a large data set from the literature ($n = 198$). However, the changes did not provide any benefit in terms of model precision and only a very small improvement in bias for propionate concentration prediction. More recently, van Lingen et

al. (2019) developed a model of net VFA production using the predicted ratio of NAD^+/NADH based on thermodynamic control as a competitive inhibitor of net acetate production and an activator of net propionate production, assuming no interconversion among VFA. After fitting to a small data set ($n = 40$), they observed that RMSE for acetate, propionate, and butyrate concentrations after a meal were approximately 4, 12, and 13%, respectively. However, although bias and CCC were not assessed, the graphical representations of the data indicated moderate slope bias for acetate and propionate proportions and severe slope bias for butyrate. The model appeared to capture very little, if any, of the variation among diets within each time point after a meal. A wider-ranging test of this latter approach is required to assess its potential value. Work is also needed to assess the combination of thermodynamic control of de novo synthesis and interconversions among VFA. It is certainly possible that both are contributing variation, and, thus, neither appears to provide benefit when implemented in isolation. Unfortunately, these more complicated prediction schemes cannot be fully assessed using historical data because key measurements, such as the partial pressure of H_2 , were not generally reported in the past. Thus, new data will be required, and it must be emphasized that in addition to the normal measurements of substrate degradation, pH, and VFA concentrations, assessments of de novo VFA synthesis rates, interconversions among VFA, and the partial pressure of H_2 are needed. Additionally, it is critical to assess rates of eating if the animals are not frequently fed, report the exact times relative to feeding for sampling, and ensure that samples are truly representative of the rumen as a whole.

Both ruminal ammonia and blood urea concentrations were underestimated in the initial model, suggesting that excessive urea may have been excreted in the urine. Therefore the rate constant for urea excretion by the kidney ($K_{Ur,BldUrin}$ per mol) was fitted to align urinary urea excretion. Parameter estimates for $K_{Ur,BldUrin}$ decreased from 1.86 to 0.42, resulting in increased blood urea concentrations. Because the parameter defining entry into the gut was unaltered, this resulted in a greater proportion of synthesized urea being recycled back to the gut. Estimates of $K_{Ur,BldUrin}$ were also affected by alteration in ruminal ammonia concentrations due to the altered rates of urea entry into the rumen and ammonia absorption ($K_{Am,RumBld}$ per d). Estimates of the latter rate constant increased from 5.01 to 12.3, reflecting greater ammonia absorption and causing a reduction in ruminal ammonia concentrations. The increased N cycling across the rumen wall dampened ruminal ammonia concentration responses, leaving concentrations more stable. Nolan (1975) found

that the ruminal ammonia pool completely turned over in 2 h, which was consistent with our current results. After refitting to the observed values, the RMSE for predicted ammonia and blood urea concentrations significantly decreased, from 86.9 to 33.8% and from 78.9 to 34.2%. The decreased prediction error was primarily due to decreased mean bias, and the CCC for both were increased, suggesting that the accuracy and precision for ammonia and blood urea concentrations were improved.

Nutrient Digestion

The RMSE for fecal outflow of DM, protein, and NDF were decreased from 19.7 to 16.2%, 36.3 to 19.2%, and 27.7 to 24.0%, respectively. Given that intestinal digestion is represented using simple mass-action functions that were fitted to the data, the improved precision for predictions of fecal output of protein, starch, ADF, and fat must derive from improved ruminal predictions.

The RMSE for fecal outflow of ADF, fat, and starch were essentially the same as for the prior model. A small proportion of the fecal starch output prediction error was segregated as mean and slope bias. Additionally, the overall RMSE for fecal starch outflow was relatively large at 83%, but the absolute error was less than 8% of that consumed, reflecting the large proportion of digested starch and, thus, the difficulty in predicting a small residual fraction.

The newly derived intestinal digestion coefficient for protein was 0.71 g/g, which falls within the range of apparent digestibilities of 0.67 to 0.77 g/g reported by Santos et al. (1984) for dairy cows. However, we anticipated greater digestibility, given that the model considers urea N entry into the terminal intestine, whereas apparent digestibility measures do not. The model value does not reflect fecal endogenous losses and thus is not fully a true digestibility estimate. Digestibilities ranging from 0.75 to 0.86 g/g have been reported for rats, growing pigs, and broiler chickens (Donkoh and Moughan, 1994; Hess et al., 2000; Gabriel et al., 2008).

The derived postruminal fiber digestion coefficient was 0.11 g/g, which was slightly greater than reported in previous studies. Pantoja et al. (1994) reported that intestinal NDF digestibility ranged from 0.054 to 0.16. Jensen et al. (2005) showed that hindgut NDF digestibility was around 0.08. The reparametrized starch digestion coefficient was 0.75 g/g, which was consistent with an average starch digestibility of 0.78 g/g reported by Moharrery et al. (2014). Boerman et al. (2015) reported that the apparent intestinal digestibility of total fatty acids was around 0.75 g/g in lactating dairy cows using a meta-analysis, which is close to the value of 0.70 g/g derived in the current study.

Table 4. Using Molly to simulate the effects of RDP and rumen-degraded starch (RDSt) on nitrogen fluxes in a dairy cow¹

Item	40% RDP		60% RDP	
	50% RDSt	75% RDSt	50% RDSt	75% RDSt
N balance, g of N/d				
N intake	512	512	512	512
Fecal N	198	197	191	191
Urinary N	180	187	189	196
Retained N	−37	−41	−38	−42
Milk N	208	206	208	206
Ruminal N fluxes, g of N/d				
Degraded protein	153	165	179	190
Deaminated AA	87	95	106	114
AA captured in microbes	72	75	78	82
AA outflow	1.7	1.6	1.9	1.8
Ammonia captured in microbes	139	150	147	157
Ammonia outflow	16	16	19	18
Nonammonia nonmicrobial N outflow	310	298	285	274
Microbial N outflow	215	230	230	244
Total N outflow	541	544	533	536
Absorbed ammonia N	60	59	69	68
Postrumen, g of N/d				
Digested microbial protein	139	149	149	158
Digested RUP	190	183	174	168
Absorbed AA	332	334	325	328
Recycled urea captured in microbes	14	14	14	14
Urea recycling, g of N/d				
Blood urea entry	287	295	295	302
Ruminal urea entry	74	76	75	77
Intestinal urea entry	55	56	55	56
Urinary urea excretion	158	163	164	169

¹Isonitrogenous and isocaloric diets contained fat, starch, protein, NDF, ADF, and ash concentrations of 6, 30, 16, 30, 20, and 6% of DM, respectively, designed to feed a Holstein dairy cow weighing 600 kg at 70 d of lactation; 20 kg DMI was consumed by the animal.

Model Simulations

Protein Fluxes. The results from simulations of high and low RDP and high and low RDSt are presented in Table 4. A nitrogen flux (g of N per d) diagram displaying the model simulation results for a diet with 60% RDP and 75% RDSt is provided in Figure 3. As anticipated, the diets with 60% RDP were predicted to have greater protein degradation and ammonia production rates than the diets with 40% RDP, which led to reductions in NANMN outflow, slight increases in ammonia and AA outflow, and increases in ammonia absorption rate. Because of the positive effect of fermentable energy supply on microbial growth and activity, the diets containing 75% RDSt were predicted to have increased protein degradation rates and ammonia production rates compared with the diets with 50% RDSt. An interaction occurred between RDP and RDSt on microbial protein synthesis. High-RDP diets provided more substrate for microbial protein synthesis, but because microbial protein synthesis is an energy-dependent process, greater RDSt diets produced more ATP, which facilitated microbial growth. Consequently, the greatest capture of AA and ammo-

nia by rumen microbes was predicted to occur for the 60% RDP and 75% RDSt diet, which had 5% more synthesized microbial protein than the 40% RDP and 50% RDSt diet.

Consistent with the ruminal N outflow, diets with greater RDSt were predicted to have more digested microbial protein, whereas diets with greater RDP had less digested protein in the intestines. Overall, the diets with 40% RDP had greater AA absorption than diets with 60% RDP. Within RDP diets, those with greater RDSt had greater AA absorption rates. Compared with the diet with 60% RDP and 50% RDSt, the diet with 40% RDP and 75% RDSt had 3% greater absorbed AA.

Blood urea entry accounts for approximately 57% of N intake, which is consistent with previous studies (Lapierre and Lobley, 2001; Reynolds and Kristensen, 2008; Bailey et al., 2012). Diets with 75% RDSt had increased blood urea entry, primarily due to increased AA absorption. As a consequence of the increased blood urea entry, the diets with 75% RDSt had increased ruminal urea entry. Because 25% of intestinal urea entry was assumed to be captured in microbial N, intestinal urea entry was not increased in the diets with 50% RDSt.

Figure 3. Nitrogen flux diagram derived from the model simulation. Solid boxes represent pools, and boxes with dashed outlines represent compartments. Numbers indicate nitrogen fluxes. The unit is g of N/d. A diet containing fat, starch, protein, NDF, ADF, and ash concentrations of 6, 30, 16, 30, 20, and 6% of DM, respectively, was designed to feed a Holstein dairy cow weighing 600 kg at 70 d of lactation. The simulated diet contained 60% RDP and 75% rumen-degraded starch. The animal consumed 20 kg DMI. NANMN = nonammonia nonmicrobial N. NA = nucleic acids.

Considering both ruminal N degradation and postrumen N digestion, diets with 40% RDP had greater fecal N outflow. Greater urinary urea excretion was observed in the diets with 60% RDP. Because urea is the major nitrogenous component in urine, similar results were observed for urinary N excretion. Negative retained N was observed, suggesting that body protein was mobilized to support high milk production, and indicating an area of the model potentially needing further work. Such work is aided by the changes undertaken to date to improve estimates of AA and energy supplies to the animal, as those supplies can be predicted with some confidence for studies that measured BW change without measuring diet digestibility. Although the difference on milk N was negligible (less than 3 g/d), the diet with 40% RDP and 50% RDSt exhibited the highest milk N, and the diet with 60% RDP and 75% RDSt had the lowest milk N.

Energy Fluxes. The results from simulations of varying RDP and RDSt diets on energy metabolism are presented in Table 5. An energy metabolism diagram derived from the model simulation results is shown in Figure 4 for a diet with 60% RDP and 75% RDSt. The

diets with 40% RDP tended to increase fecal energy compared with the diets with 60% RDP; thus an opposite direction was observed for the digestible energy. No difference was observed on urinary energy, and a small difference was detectable on CH₄ energy among the 4 diets. Consequently, the metabolizable energy was very similar. Although total absorbed energy was similar, the energy profile was different. The diets with 50% RDSt had greater absorbed energy from glucose than the diets with 75% RDSt, and the high-RDP diets had slightly decreased absorbed energy from AA compared with the low-RDP diets. Absorbed VFA energy accounted for 45 to 53% of total absorbed energy. The high-RDSt diets had greater absorbed energy from acetate, propionate, and butyrate than did the low-RDSt diets. Overall, diets with 75% RDSt had 0.6 Mcal greater milk energy output than did the 50% RDSt diets.

Future Work. The predicted values were derived from the models and associated parameters, and are not independent of the observed data or parameter estimates. Thus, the RMSE represents the residual variation in the same way that the standard error from regression reflects unexplained variance. Cross-evaluation is the

Table 5. Using Molly to simulate the effects of RDP and rumen-degraded starch (RDSt) on energy metabolism in a dairy cow¹

Item	40% RDP		60% RDP	
	50% RDSt	75% RDSt	50% RDSt	75% RDSt
Gross energy, Mcal	91.4	91.4	91.4	91.4
Fecal energy, Mcal	30.4	30.3	29.9	29.9
Urinary energy, Mcal	0.9	0.9	0.9	0.9
CH ₄ energy, Mcal	2.9	3.0	2.9	3.1
Metabolizable energy, Mcal	57.3	57.2	57.7	57.6
Milk energy, Mcal	25.3	25.9	25.3	25.9
Absorbed energy, Mcal	53.9	53.4	54.2	53.7
Energy from absorbed AA, Mcal	11.2	11.3	11.0	11.1
Energy from absorbed glucose, Mcal	9.9	6.6	9.7	6.5
Energy from absorbed acetate, Mcal	10.4	10.9	10.5	11.1
Energy from absorbed propionate, Mcal	8.5	9.9	8.9	10.2
Energy from absorbed butyrate, Mcal	5.8	6.7	6.1	6.8

¹Isonitrogenous and isocaloric diets contained fat, starch, protein, NDF, ADF, and ash concentrations of 6, 30, 16, 30, 20, and 6% of DM, respectively, designed to feed a Holstein dairy cow weighing 600 kg at 70 d of lactation; 20 kg DMI was consumed by the animal.

most valid approach to evaluate model performance independent of the fitting data, which would be helpful in assessing the reliability of the predictions. However, such an effort is computationally expensive and not achievable in realistic time frames unless parallelized. Simulation of the 1,223 observations in the data set requires between 6 and 10 min on a single processing core; optimization typically requires between 500 and

10,000 iterations for convergence (Nocedal and Wright, 2006); and cross-evaluations involve repeating the process from 20 to 500 times (Stone, 1974). Multiplying the minimum of each yields an estimated computation time of 41 d on a single core. Parallel processing also provides an opportunity to make use of other Monte Carlo-based methods, which simulate population variance numerically rather than estimating analytically (Robert and Casella, 2013). The model has been re-coded in C++ and implemented in R, which supports parallel processing. The move to R also provides a potential opportunity to consider random effects when conducting parameter estimation using the nlmeODE package (Tornøe, 2007). Significant additional work is required to generate the additional code required to set up the problem and the model in R so that the process can be parallelized, and to move the code to a supercomputer.

CONCLUSIONS

Adding ammonia concentration as an independent variable to the pH equation, along with refitting model parameters, increased the precision of predicted ruminal pH and, thereby, the precision of predicted VFA concentrations due to an improved representation of pH regulation of VFA production rates. Although minor mean bias for pH and moderate mean bias and slope bias for VFA concentrations were observed in the modified model, the small CCC values suggest that considerable unexplained variation remains, despite these modifications. After reparameterizations, model predictions of ruminal outflow of ADF, NDF, fat, total N, NANMN, and microbial N had RMSE ranging from 15.0 to 34.5%, with essentially no slope bias and minor

Figure 4. Energy metabolism diagram derived from the model simulation. Solid boxes represent pools, and open boxes represent compartments. Numbers indicate energy fluxes. The unit is megacalorie per day. A diet containing fat, starch, protein, NDF, ADF, and ash concentrations of 6, 30, 16, 30, 20, and 6% of DM, respectively, was designed to feed a Holstein dairy cow weighing 600 kg at 70 d of lactation. The simulated diet contained 60% RDP and 75% rumen-degraded starch. The animal consumed 20 kg DMI. GE = gross energy; DE = digestible energy; FE = fecal energy; UE = urinary energy; NEp = net energy for production; HP = net energy for maintenance + heat increment.

mean bias. The combination of model changes described herein and prior work to improve the representation of N cycling across the rumen wall have contributed to increased accuracy and precision for predictions of ruminal pH, ruminal ammonia concentrations, microbial outflow from the rumen, and fecal excretion of protein, ADF, NDF, and fat. The results from simulations of diets with varying RDP and RDSt indicate that decreasing RDP and RDSt proportions in an isonitrogenous and isocaloric diet can slightly improve N efficiency, and increasing RDSt proportions can increase energy efficiency.

ACKNOWLEDGMENTS

This work was funded, in part, by the Virginia Agricultural Experiment Station (Glade Spring, VA), the Hatch Program of the National Institute of Food and Agriculture, US Department of Agriculture (Washington, DC), the College of Agriculture and Life Sciences Pratt Endowment at Virginia Tech (Blacksburg, VA), and the Alberta Livestock and Meat Agency Ltd. (Edmonton, Alberta, Canada; grant 2015F055R). The authors have no conflicts of interest to declare.

REFERENCES

- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80:1447–1462. [https://doi.org/10.3168/jds.S0022-0302\(97\)76074-0](https://doi.org/10.3168/jds.S0022-0302(97)76074-0).
- Argyle, J. L., and R. L. Baldwin. 1988. Modeling of rumen water kinetics and effects of rumen pH changes. *J. Dairy Sci.* 71:1178–1188. [https://doi.org/10.3168/jds.S0022-0302\(88\)79672-1](https://doi.org/10.3168/jds.S0022-0302(88)79672-1).
- Bailey, E. A., E. C. Titgemeyer, K. C. Olson, D. W. Brake, M. L. Jones, and D. E. Anderson. 2012. Effects of supplemental energy and protein on forage digestion and urea kinetics in growing beef cattle. *J. Anim. Sci.* 90:3492–3504. <https://doi.org/10.2527/jas.2011-4458>.
- Baldwin, R. 1995. Modeling ruminant digestion and metabolism. Springer Science and Business Media, Heidelberg, Germany.
- Baldwin, R. L., J. France, D. E. Beever, M. Gill, and J. H. Thornley. 1987a. Metabolism of the lactating cow: III. Properties of mechanistic models suitable for evaluation of energetic relationships and factors involved in the partition of nutrients. *J. Dairy Res.* 54:133–145. <https://doi.org/10.1017/S0022029900025243>.
- Baldwin, R. L., J. France, and M. Gill. 1987b. Metabolism of the lactating cow: I. Animal elements of a mechanistic model. *J. Dairy Res.* 54:77–105. <https://doi.org/10.1017/S002202990002522X>.
- Baldwin, R. L., J. H. Thornley, and D. E. Beever. 1987c. Metabolism of the lactating cow: II. Digestive elements of a mechanistic model. *J. Dairy Res.* 54:107–131. <https://doi.org/10.1017/S0022029900025231>.
- Bibby, J., and H. Toutenburg. 1977. Prediction and improved estimation in linear models. Wiley, Hoboken, NJ.
- Boerman, J. P., J. L. Firkins, N. R. St-Pierre, and A. L. Lock. 2015. Intestinal digestibility of long-chain fatty acids in lactating dairy cows: A meta-analysis and meta-regression. *J. Dairy Sci.* 98:8889–8903. <https://doi.org/10.3168/jds.2015-9592>.
- Briggs, P., J. Hogan, and R. Reid. 1957. Effect of volatile fatty acids, lactic acid and ammonia on rumen pH in sheep. *Crop Pasture Sci.* 8:674–690. <https://doi.org/10.1071/AR9570674>.
- Channon, A., J. Rowe, and R. Herd. 2004. Genetic variation in starch digestion in feedlot cattle and its association with residual feed intake. *Aust. J. Exp. Agric.* 44:469–474. <https://doi.org/10.1071/EA02065>.
- Donkoh, A., and P. Moughan. 1994. The effect of dietary crude protein content on apparent and true ileal nitrogen and amino acid digestibilities. *Br. J. Nutr.* 72:59–68. <https://doi.org/10.1079/BJN19940009>.
- Firkins, J., M. Eastridge, N. St-Pierre, and S. Noftsker. 2001. Effects of grain variability and processing on starch utilization by lactating dairy cattle. *J. Anim. Sci.* 79(E Suppl.):E218–E238.
- Fleming, A. J., H. Lapiere, R. Martineau, R. R. White, and M. D. Hanigan. 2019. Modeling portal-drained viscera and liver fluxes of essential amino acids in dairy cows. *J. Dairy Sci.* 102:10964–10982. <https://doi.org/10.3168/jds.2019-16302>.
- Fox, D. G., C. J. Sniffen, J. D. O'Connor, J. B. Russell, and P. J. Van Soest. 1992. A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. *J. Anim. Sci.* 70:3578–3596. <https://doi.org/10.2527/1992.70113578x>.
- France, J., and J. Dijkstra. 2005. Volatile fatty acid production. *Quantitative Aspects of Ruminant Digestion and Metabolism* 2:157–175.
- Gabriel, I., M. Lessire, H. Juin, J. Burstin, G. Duc, L. Quillien, J. Thibault, M. Leconte, J. Hallouis, P. Ganier, N. Mézière, and B. Sève. 2008. Variation in seed protein digestion of different pea (*Pisum sativum* L.) genotypes by cecectomized broiler chickens: 1. Endogenous amino acid losses, true digestibility and in vitro hydrolysis of proteins. *Livest. Sci.* 113:251–261. <https://doi.org/10.1016/j.livsci.2007.04.002>.
- Galyean, M., D. Wagner, and F. Owens. 1981. Dry matter and starch disappearance of corn and sorghum as influenced by particle size and processing. *J. Dairy Sci.* 64:1804–1812. [https://doi.org/10.3168/jds.S0022-0302\(81\)82769-5](https://doi.org/10.3168/jds.S0022-0302(81)82769-5).
- Ghimire, S., P. Gregorini, and M. D. Hanigan. 2014. Evaluation of predictions of volatile fatty acid production rates by the Molly cow model. *J. Dairy Sci.* 97:354–362. <https://doi.org/10.3168/jds.2012-6199>.
- Ghimire, S., R. A. Kohn, P. Gregorini, R. R. White, and M. D. Hanigan. 2017. Representing interconversions among volatile fatty acids in the Molly cow model. *J. Dairy Sci.* 100:3658–3671. <https://doi.org/10.3168/jds.2016-11858>.
- Gregorini, P., P. Beukes, G. Waghorn, D. Pacheco, and M. Hanigan. 2015. Development of an improved representation of rumen digesta outflow in a mechanistic and dynamic model of a dairy cow, Molly. *Ecol. Modell.* 313:293–306. <https://doi.org/10.1016/j.ecolmodel.2015.06.042>.
- Hanigan, M. D., H. G. Bateman, J. G. Fadel, and J. P. McNamara. 2006. Metabolic models of ruminant metabolism: recent improvements and current status. *J. Dairy Sci.* 89:E52–E64. [https://doi.org/10.3168/jds.S0022-0302\(06\)72363-3](https://doi.org/10.3168/jds.S0022-0302(06)72363-3).
- Hanigan, M. D., J. A. Appuhamy, and P. Gregorini. 2013. Revised digestive parameter estimates for the Molly cow model. *J. Dairy Sci.* 96:3867–3885. <https://doi.org/10.3168/jds.2012-6183>.
- Hanigan, M. D., C. C. Palliser, and P. Gregorini. 2009. Altering the representation of hormones and adding consideration of gestational metabolism in a metabolic cow model reduced prediction errors. *J. Dairy Sci.* 92:5043–5056. <https://doi.org/10.3168/jds.2008-1922>.
- Hanigan, M. D., A. G. Rius, E. S. Kolver, and C. C. Palliser. 2007. A redefinition of the representation of mammary cells and enzyme activities in a lactating dairy cow model. *J. Dairy Sci.* 90:3816–3830. <https://doi.org/10.3168/jds.2007-0028>.
- Hess, V., P. Ganier, J.-N. Thibault, and B. Seve. 2000. Comparison of the isotope dilution method for determination of the ileal endogenous amino acid losses with labelled diet and labelled pigs. *Br. J. Nutr.* 83:123–130. <https://doi.org/10.1017/S0007114500000167>.
- Hollmann, M., M. Allen, and D. Beede. 2011. Dietary protein quality and quantity affect lactational responses to corn distillers grains: A meta-analysis. *J. Dairy Sci.* 94:2022–2030. <https://doi.org/10.3168/jds.2010-3712>.
- Huhtanen, P., and A. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein

- yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92:3222–3232. <https://doi.org/10.3168/jds.2008-1352>.
- Jensen, C., M. R. Weisbjerg, P. Nørgaard, and T. Hvelplund. 2005. Effect of maize silage maturity on site of starch and NDF digestion in lactating dairy cows. *Anim. Feed Sci. Technol.* 118:279–294. <https://doi.org/10.1016/j.anifeedsci.2004.10.011>.
- Lapierre, H., and G. Lobley. 2001. Nitrogen recycling in the ruminant: A review. *J. Dairy Sci.* 84:E223–E236. [https://doi.org/10.3168/jds.S0022-0302\(01\)70222-6](https://doi.org/10.3168/jds.S0022-0302(01)70222-6).
- Leduc, M., M.-P. Létourneau-Montminy, R. Gervais, and P. Chouinard. 2017. Effect of dietary flax seed and oil on milk yield, gross composition, and fatty acid profile in dairy cows: A meta-analysis and meta-regression. *J. Dairy Sci.* 100:8906–8927. <https://doi.org/10.3168/jds.2017-12637>.
- Li, M. M., S. Sengupta, and M. D. Hanigan. 2019a. Using artificial neural networks to predict pH, ammonia, and volatile fatty acid concentrations in the rumen. *J. Dairy Sci.* 102:8850–8861. <https://doi.org/10.3168/jds.2018-15964>.
- Li, M. M., E. Titgemeyer, and M. D. Hanigan. 2019b. A revised representation of urea and ammonia nitrogen recycling and use in the Molly cow model. *J. Dairy Sci.* 102:5109–5129. <https://doi.org/10.3168/jds.2018-15947>.
- Li, M. M., R. R. White, and M. D. Hanigan. 2018. An evaluation of Molly cow model predictions of ruminal metabolism and nutrient digestion for dairy and beef diets. *J. Dairy Sci.* 101:9747–9767. <https://doi.org/10.3168/jds.2017-14182>.
- Lin, L. I.-K. 1989. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 45:255–268. <https://doi.org/10.2307/2532051>.
- Mills, J., J. France, and J. Dijkstra. 1999. A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 1. Dietary starch characterisation and ruminal starch digestion. *J. Anim. Feed Sci.* 8:291–340. <https://doi.org/10.22358/jafs/68938/1999>.
- Moharrery, A., M. Larsen, and M. R. Weisbjerg. 2014. Starch digestion in the rumen, small intestine, and hind gut of dairy cows—A meta-analysis. *Anim. Feed Sci. Technol.* 192:1–14. <https://doi.org/10.1016/j.anifeedsci.2014.03.001>.
- Murphy, M. R., R. L. Baldwin, and L. J. Koong. 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *J. Anim. Sci.* 55:411–421. <https://doi.org/10.2527/jas1982.552411x>.
- Nocedal, J., and S. Wright. 2006. *Numerical Optimization*. Springer Science and Business Media, Heidelberg, Germany.
- Nolan, J. 1975. *Quantitative models of nitrogen metabolism in sheep. Digestion and Metabolism in the Ruminant*. University of New England Publishing Unit, Armidale, Australia.
- NRC (National Research Council). 2001. *Nutrient Requirements of Dairy Cattle*. 7th ed. National Academy Press, Washington, DC.
- O'Connor, J. D., C. J. Sniffen, D. G. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *J. Anim. Sci.* 71:1298–1311. <https://doi.org/10.2527/1993.7151298x>.
- Pantoja, J., J. Firkins, M. Eastridge, and B. Hull. 1994. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows. *J. Dairy Sci.* 77:2341–2356. [https://doi.org/10.3168/jds.S0022-0302\(94\)77177-0](https://doi.org/10.3168/jds.S0022-0302(94)77177-0).
- Press, W. H., S. A. Teukolsky, W. T. Vetterling, and B. P. Flannery. 2007. *Numerical Recipes, 3rd Edition: The Art of Scientific Computing*. Cambridge University Press, Cambridge, UK.
- Reynolds, C., and N. B. Kristensen. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. *J. Anim. Sci.* 86(Suppl. 14):E293–E305.
- Robert, C., and G. Casella. 2013. *Monte Carlo Statistical Methods*. Springer Science and Business Media, Heidelberg, Germany.
- Russell, J. B., J. O'Connor, D. Fox, P. Van Soest, and C. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551–3561. <https://doi.org/10.2527/1992.70113551x>.
- Santos, K. A., M. D. Stern, and L. D. Satter. 1984. Protein degradation in the rumen and amino acid absorption in the small intestine of lactating dairy cattle fed various protein sources. *J. Anim. Sci.* 58:244–255. <https://doi.org/10.2527/jas1984.581244x>.
- Sauvant, D., L. Delaby, and P. Nozière. 2017. *INRA Feeding System for Ruminants*. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Short, S. D. 2004. Characteristics and production costs of US dairy operations. US Department of Agriculture, Economic Research Service. Statistical Bulletin No. SB974-6. Feb. 2004.
- Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562–3577. <https://doi.org/10.2527/1992.70113562x>.
- St-Pierre, N. R., and D. Glamocic. 2000. Estimating unit costs of nutrients from market prices of feedstuffs. *J. Dairy Sci.* 83:1402–1411. [https://doi.org/10.3168/jds.S0022-0302\(00\)75009-0](https://doi.org/10.3168/jds.S0022-0302(00)75009-0).
- St-Pierre, N. R., and C. S. Thraen. 1999. Animal grouping strategies, sources of variation, and economic factors affecting nutrient balance on dairy farms. *J. Anim. Sci.* 77(Suppl. 2):72–83.
- Stone, M. 1974. Cross-validatory choice and assessment of statistical predictions. *J. R. Stat. Soc. Series B Stat. Methodol.* 36:111–133. <https://doi.org/10.1111/j.2517-6161.1974.tb00994.x>.
- Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *J. Dairy Sci.* 86:3620–3633. [https://doi.org/10.3168/jds.S0022-0302\(03\)73968-X](https://doi.org/10.3168/jds.S0022-0302(03)73968-X).
- Svihus, B., A. K. Uhlen, and O. M. Harstad. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Anim. Feed Sci. Technol.* 122:303–320. <https://doi.org/10.1016/j.anifeedsci.2005.02.025>.
- Tornøe, C. 2007. nlmeODE: Non-linear mixed-effects modelling in nlme using differential equations. R package version 0.3-1. Accessed Oct. 27, 2012. <http://CRAN.R-project.org/package=nlmeODE>.
- Ungerfeld, E. and R. Kohn. 2006. The role of thermodynamics in the control of ruminal fermentation. Pages 55–85 in *Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress*. K. Sejrsen, T. Hvelplund, and M. O. Nielsen, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- van Lingen, H. J., J. G. Fadel, L. E. Moraes, A. Bannink, and J. Dijkstra. 2019. Bayesian mechanistic modeling of thermodynamically controlled volatile fatty acid, hydrogen and methane production in the bovine rumen. *J. Theor. Biol.* 480:150–165. <https://doi.org/10.1016/j.jtbi.2019.08.008>.
- Volden, H. 2011. *NorFor—The Nordic Feed Evaluation System*. EAAP Publication No. 130. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Vyas, D., and R. Erdman. 2009. Meta-analysis of milk protein yield responses to lysine and methionine supplementation. *J. Dairy Sci.* 92:5011–5018. <https://doi.org/10.3168/jds.2008-1769>.
- White, R. R., Y. Roman-Garcia, J. L. Firkins, M. J. VandeHaar, L. E. Armentano, W. P. Weiss, T. McGill, R. Garnett, and M. D. Hanigan. 2017. Evaluation of the National Research Council (2001) dairy model and derivation of new prediction equations. 1. Digestibility of fiber, fat, protein, and nonfiber carbohydrate. *J. Dairy Sci.* 100:3591–3610. <https://doi.org/10.3168/jds.2015-10800>.

ORCIDS

Meng M. Li <https://orcid.org/0000-0001-5958-0941>

Mark D. Hanigan <https://orcid.org/0000-0002-5639-9677>