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Representing interconversions among volatile fatty acids in the Molly cow model

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ABSTRACT

The Molly cow model uses fixed stoichiometric coefficients for predicting volatile fatty acid (VFA) production from the fermented individual dietary nutrient fractions of forage and concentrate. We previously showed that predictions of VFA production had large errors and hypothesized that it was due to a lack of representation of carbon exchange among VFA. The objectives of the present study were to add VFA interconversion equations based on thermodynamics to the Molly cow model and evaluate the effect of these additions on model accuracy and precision of VFA predictions. Previously described thermodynamic equations were introduced to represent interconversions among VFA. The model was further modified to predict de novo acetate, propionate, and butyrate production coefficients based on forage-to-concentrate ratios rather than discrete, fixed sets of coefficients for forage-based, concentrate-based, and mixed diets. Both the original model and the modified one were reparameterized and evaluated against a common data set containing 8 studies reporting pH, VFA concentration, and VFA production rates using isotope dilution techniques and 62 studies reporting VFA concentrations and pH. Evaluations after parameter estimation revealed that predictions of VFA production rates were not improved, with root mean squared prediction errors (RMSPE) of 77, 60, and 51% for acetate, propionate, and butyrate, respectively, for the revised model versus 75, 63, and 55, respectively, for the original model. The RMSPE for predictions of VFA concentrations were reduced from 28, 46, and 40% to 22, 31, and 26% for acetate, propionate, and butyrate, respectively, simply by rederiving the VFA coefficients, but minimal further improvement was achieved with the addition of thermodynamically driven interconversion equations (RMSPE of 21, 32, and 27% for acetate, propionate, and butyrate, respectively). Thus, the results indicate that thermodynamically driven interchanges among VFA, as represented in this study, may not be a primary determinant for the accuracy of predictions of net production rates. Including the effect of pH on VFA absorption reduced the mean bias of propionate production and slope bias of acetate production, but not the overall RMSPE. The larger prediction errors for VFA production as compared with concentrations suggest the data quality may not be high, or that our representation of VFA production and absorption as well as ruminal digestion is inadequate. Additional data are required to discriminate among these hypotheses.
Key words: volatile fatty acids, Molly, isotope dilution

INTRODUCTION

Accurate predictions of VFA production in the rumen are important in representing ruminal function, ruminal efficiency, and environmental impact of ruminants. The Molly cow model (Baldwin, 1995) is a dynamic, mechanistic model that represents nutrient digestion, metabolism, and production of a cow. The VFA predictions therein are based on stoichiometric coefficients described by Murphy et al. (1982) and Argyle and Baldwin (1988). The coefficients represent the fractional mass conversions of fermented substrate to each VFA, and were derived for each of 5 nutrient classes, starch, soluble carbohydrate, cellulose, hemicellulose, and protein, for each of 2 diet types, high-forage and high-concentrate feeding programs. These coefficients are based on the assumptions that the substrate supply is a primary determinant of VFA production rates and VFA interconversions either do not exist or are proportionally constant across diet types. However, our recent study demonstrated that predictions of the VFA production rates, with the use of these coefficients, are associated with large errors (Ghimire et al., 2014).

Dietary changes not only modify the available substrates, but also elements of the ruminal environment including pH, hydrogen partial pressure, and

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VFA concentrations, which will affect thermodynamic states and, thus, the metabolic pathways used by the microbes. Kohn and Boston (2000) estimated that the change in free energy ($\mathbf{\Delta G}$) when glucose is converted to acetate, propionate, and butyrate was −140.2, −144.3, and −178.9, respectively. Such large changes in free energy indicate that glucose production from VFA is unlikely without significant energy consumption, and thus reactions can be expected to proceed in the forward direction rather than the reverse. However, similar ΔG for these reactions also indicates that ΔG of VFA interconversion is close to zero at a fixed ruminal environment, and the rate of interconversion should be governed by thermodynamic changes in the rumen (Ungerfeld and Kohn, 2006). For example, Ungerfeld and Kohn (2006) calculated that acetate to propionate and acetate to butyrate interconversions were −11.2 and 4.2 kJ/mol, respectively, for a high-roughage diet and −12.5 and −2.8 kJ/mol for a high-concentrate diet, with some assumptions on unmeasured reactant and product concentrations. Such small changes in free energy make these reactions more susceptible to changes in dietary composition, which affects the ruminal environment, and changes in VFA interconversion have been reported by Sutton et al. (2003) for those dietary changes.

Thus, the stoichiometric coefficients may not capture the potential variation in net production rates caused by variable interconversions among VFA. Furthermore, the lack of a representation of the effect of pH on VFA absorption in the model may cause inaccurate estimation of VFA concentrations and can affect production rate predictions (Dijkstra et al., 1993; Bannink et al., 1997).

We hypothesized that representing carbon exchange among VFA can improve predictions of VFA production by the Molly cow model. The objective of our study was to introduce thermodynamically driven VFA interconversion equations into the Molly cow model, refit the coefficients describing VFA synthesis and absorption to a literature data set, and evaluate the effect of the changes on prediction errors of VFA production rates and concentrations.

MATERIALS AND METHODS

Model Description

The Molly cow model described by Baldwin (1995) with modifications (Hanigan et al., 2006, 2009, 2013) was used as a starting point (**M13 model**). Simulations were conducted using the acslX software package (V3.0, Aegis Technologies Group Inc., Huntsville, AL). Model simulations of each scenario were run for 10 d of model time to ensure steady state had been achieved, and model results from the last day of the run were compared with observed values.

Murphy et al. (1982) devised VFA stoichiometric coefficients based on the Koong et al. (1975) model and defined the coefficients separately for forage and concentrate based diets using VFA concentration data. Argyle and Baldwin (1988) later added coefficients for a mixed diet set, which was the average of forage and concentrate coefficient sets (Baldwin, 1995). Thus, in the M13 model 1 of the 3 sets of coefficients was used for each diet depending on its forage content (% of dietary DM): forage set when more than 80%, concentrate set when less than 20% and mixed set for the remainder. However, using discrete sets of coefficients introduces discontinuities, which generally are not well tolerated in optimization problems (Floudas and Pardalos, 2008). Therefore, the M13 model was modified to represent de novo VFA stoichiometric coefficients for the mixed diet as a linear interpolation of the concentrate and forage sets using fractional proportion of forage in the diet (f_{For}; 0 to 1) to weight the forage and concentrate coefficients:

$$f_{i,j} = f_{i,j,For} \times f_{For} + f_{i,j,Con} \times (1 - f_{For}), \qquad [1]$$

where $f_{i,j}$ represents the stoichiometric coefficient defining production of each VFA (j = acetate, propionate, or butyrate, mol/mol of hexose equivalent) from each dietary substrate i (i = cellulose, hemicellulose, starch, and soluble carbohydrate). The *For* and *Con* coefficient sets were defined as those derived from diets with 100% forage and 100% concentrate (% of dietary DM), respectively. Based on this abbreviation scheme, for example, *fScPrFor* denotes the coefficient for production of propionate (mol/mol hexose equivalent) from the fermentation of soluble carbohydrate in the forage portion of the diet. The difference in this approach is that, for each diet, both forage and concentrate parameters of each substrate are used to yield a new parameter based on fraction of forage in that diet, whereas in (Baldwin, 1995) discrete sets are used for a diet depending on whether the diet is categorized as forage-based, concentrate-based, or a mixed diet. The model updated with Eq. 1 was denoted as the **M16 model**.

The following equations, described by Ungerfeld and Kohn (2006), were introduced into the M16 model to represent carbon interchange among the VFA (**M16VFA model**):

$$F_{A,P} = K_{A,P}[A][CO_2]P_{H_2}^3\left([ADP][P_i]\left[H^+\right]\right)^n; \qquad [2]$$

$$F_{P,A} = K_{P,A}[P][H_2O]^2([ATP][H_2O])^n; \quad [3]$$

$$F_{A,B} = K_{A,B}\left[A\right]^2 P_{H_2}^2 \left(\left[ADP\right]\left[P_i\right]\left[H^+\right]\right)^n; \quad [4]$$

$$F_{B,A} = K_{B,A}[B][H_2O]^2([ATP][H_2O])^n; \quad [5]$$

$$F_{P,B} = K_{P,B}\left[P\right]\left[CO_2\right]P_{H_2}^3 \left(\left[ADP\right]\left[P_i\right]\left[H^+\right]\right)^n; \quad [6]$$

and

$$F_{B,P} = K_{B,P}[B][H_2O]^2([ATP][H_2O])^n; \quad [7]$$

where $F_{i,j}$ represented the flux of i to j (mol/d), with i and j each representing acetate (A), propionate (P), or butyrate (B). The interconversion rate constants for each flux are denoted by $K_{i,j}$. The square brackets indicate concentrations, and n represents the number of ATP or ADP converted. The rate constants for these equations ($K_{i,j}$) were calculated separately for each of 10 treatment means from the studies that reported pH, VFA interconversion, VFA net production, and VFA concentrations (Sharp et al., 1982; Seal and Parker, 1994; Sutton et al., 2003; Markantonatos et al., 2009) by rearrangement of Eq. 2 through 7. The mean of the calculated rate constants from these studies was used in the VFA interconversion equations (Eq. 2 through 7). The coefficient of variation of the rate constants ranged from 20 to 100%. Concentrations (mol/L) of CO_2, H_2O, ADP, ATP, P_i (inorganic phosphorus), and the partial pressure of hydrogen (P_{H_2}; atm) were assumed constant and set to the values used by Ungerfeld and Kohn (2006). The moles of ATP (n) was set at 0.6 for $F_{A,P}$ and $F_{P,A}$; 0.47 for $F_{P,B}$ and $F_{B,P}$; and 0.13 for $F_{A,B}$ and $F_{B,A}$, as reported by Ungerfeld and Kohn (2006).

After an initial round of VFA stoichiometric coefficient estimation, slope bias was determined for VFA concentration and production predictions with respect to predicted ruminal pH. This problem has been previously described by Dijkstra et al. (1992). To address this issue, the M16 and M16VFA models were updated using the equation of Dijkstra et al. (1992):

$$F_{Abs_i} = \frac{V_{max} \times RumVol^{0.75}}{\left(1+\frac{0.338}{C_i}\right)\left[1+\left(\frac{pH}{6.45}\right)^{6.48}\right]}, \quad [8]$$

where F_{Abs_i} is the absorption rate of the ith VFA (i = acetate, propionate, or butyrate, mol/d); V_{max} is the maximum potential absorption of VFA (mol/d); *RumVol* (L) is the ruminal liquid volume; C_i is the concentration of the ith VFA (M); and pH is ruminal pH. The V_{max} was derived along with the VFA production and absorption parameters by fitting the model to observed ruminal pH, VFA concentrations, and de novo or net VFA production rates. The models containing this updated absorption equation were denoted as **M16PH** and **M16VFAPH**.

Model Evaluations and Parameterization

The VFA production data set described by Ghimire et al. (2014) was combined with the one described by Hanigan et al. (2013), which contained observations of ruminal nutrient digestion, VFA concentrations, and pH. The latter was a subset of those used by the NRC committee to formulate the 2001 nutrient requirement model (NRC, 2001). As Hanigan et al. (2013) previously used the latter data to parameterize nutrient digestibility in the model, fermentable substrate supplies predicted by the model were generally well matched to observed values, with ruminal nutrient outflow prediction errors ranging from 15 to 41%. Starch and RUP flows had errors over 35%, however, making those measurements is problematic. A key observation is that almost all of the residual error was random. Mean and slope bias was below 10% for all nutrients, except lipid where slope bias was 25% of mean squared prediction error. Thus, the model generally provided unbiased estimates of nutrient digestion in the rumen for this data set. The final data set contained 193 treatment means for acetate, propionate, and butyrate concentrations; 23, 23, and 21 treatment means for acetate, propionate, and butyrate net production rates, respectively, and 14, 14, and 12 treatment means for acetate, propionate, and butyrate de novo production rates, respectively.

An evaluation of the M13 model with the above data set served as a reference point. Parameter estimation and evaluation was subsequently undertaken using the same data set for each of the 4 model revisions (M16, M16VFA, M16PH, and M16VFAPH). For parameterization, the coefficients describing VFA production from each nutrient fraction were derived directly for propionate and butyrate and by difference for acetate based on molar carbon balance as described by Baldwin (1995). The VFA production coefficients ($f_{j,VFA,Diet}$, mol of VFA/mol of hexose equivalent fermented) were expressed assuming a stoichiometry of 2 moles of acetate or propionate or one mole of butyrate per mole of hexose equivalent converted to the respective VFA. The propionate and butyrate coefficients were fit directly and the acetate production coefficients were derived as

$$f_{j,Ac,Diet} = \left(1-\frac{f_{j,Pr,Diet}}{2}-f_{j,Bu,Diet}\right)\times 2, \quad [9]$$

where $f_{j,Ac,Diet}$ represents the acetate production coefficient (mol/mol of hexose equivalent) for conversion of each dietary substrate j (j = cellulose, hemicellulose, starch, and soluble carbohydrate) within diet type (*Diet* = forage, concentrate), $f_{j,PrDiet}$ is the coefficient for production of propionate (mol/mol of hexose equivalent), and $f_{j,Bu,Diet}$ is the coefficient for production of butyrate (mol/mol of hexose equivalent).

The coefficients for propionate and butyrate production were bounded to ensure that the acetate coefficients did not assume negative values. A single set of coefficients were also derived for fermentation of protein to VFA, as no evidence exists of diet-driven shifts in VFA production as for carbohydrate. The 3 existing rate parameters and the introduced *Vmax* for VFA absorption were derived concurrently with the production rate parameters to help ensure that ruminal VFA concentrations were not biased.

The Quasi-Newton optimization algorithm (Zhu et al., 1997) within acslX was used to derive parameters that maximized the log-likelihood function (**LLF**). Observed data used included ruminal pH, VFA concentrations, and de novo and net VFA production rates (referred to as production). Minimum and maximum bounds were initially set to 50 and 150% of the original parameters and were later expanded to 25 and 175%. At the latter settings, none of the final parameter estimates rested on a bound. Standard errors of the parameter estimates were derived using a bootstrap method as previously described (Efron and Tibshirani, 1986; Gregorini et al., 2015).

Differences in model fits were tested for significance using a χ^2 test of the likelihood ratio with the difference in the number of parameters between the alternative and null models representing the degrees of freedom (Wilks, 1938; Huelsenbeck and Crandall, 1997):

$$\chi^2 = 2(\mathrm{LLF}_{\text{Alternative model}} - \mathrm{LLF}_{\text{Null model}}). \quad [10]$$

By fitting both the M16 and M16VFA versions of the models to the data, one ensures that any improvements in model performance are due solely to the equations that have been added and not to improvements in model parameter estimates as compared with the original parameters.

Residual errors of prediction were used to calculate root mean squared prediction errors (**RMSPE**), which were expressed as percentage of the mean, and mean squared prediction errors were partitioned into mean bias, slope bias, and dispersion (Bibby and Toutenberg, 1977). Residuals for VFA production and concentration were also regressed on predicted VFA interconversion and ruminal pH to determine if the underlying structure of the model was contributing to unexplained variation.

Global sensitivity analysis of net VFA production to the VFA stoichiometry coefficients and absorption rate constants was also performed. The Fourier amplitude sensitivity test provided in acslX (Ver. 3.1; Aegis Technologies Group), as described by Saltelli et al. (1999), was used for the analysis. In this analysis, the sensitivity coefficient reflects the fraction of the total variation in model outputs due to the parameter of interest. Parameter sampling boundaries were set to 70 and 130% of final parameter estimates. Resampling and the interference factor (Saltelli et al., 1999) were set to 4. The resultant population of predictions using these settings was of 792,372, which were used to derive the global sensitivity coefficients.

RESULTS AND DISCUSSION

Recalculation and Rederivation of Stoichiometric Coefficients

As previously observed (Ghimire et al., 2014), the M13 model had significant prediction errors for acetate, propionate and butyrate concentrations (RMSPE of 28, 45, and 40%, respectively) with more than 40% of the error partitioning into mean bias and 5 to 20% of the error partitioning into slope bias (Table 1). The RMSPE for VFA production rates were even greater, at 69, 58, and 50% for acetate, propionate, and butyrate, respectively, with large mean bias for propionate and large slope bias for acetate and butyrate. The VFA production errors were slightly larger for acetate and lower for propionate than those reported by Ghimire et al. (2014). This could be because Ghimire et al. (2014) used discrete set of coefficients for mixed diets, whereas in our study a linear interpolation of the forage and concentrate sets was used. Based on the fit statistics in this comparison of models, it does not appear that the shift from a discrete representation of forage-to-concentrate ratio to a continuous representation provided any benefit or detriment as compared with the M13 model. Refitting of parameters with no additional changes in structure (M16 model) resulted in a reduction of RMSPE for VFA concentrations, which was almost entirely due to elimination of mean bias. The RMSPE of acetate, propionate, and butyrate production, however, were not improved (Table 1).

Although one cannot rule out predictions of ruminal substrate as a contributor to VFA concentration and production prediction errors, the lack of systematic bias in the substrate supply predictions (Hanigan et

Table 1. Residual error analyses for predictions of ruminal pH, VFA concentrations, and VFA production rates by the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) before (M13) and after rederivation of parameters listed in Table 2, without (M16) and with (M16VFA) representation of thermodynamically driven VFA interconversions[1] when VFA absorption was driven by mass action without pH control or with a Michaelis-Menten absorption equation considering pH regulation (M16PH and M16VFAPH, respectively)

Item	Ruminal pH	Concentration (mmol/L)				Net production (mol/d)			De novo production (mol/d)		
		VFA	Ac	Pr	Bu	Ac	Pr	Bu	Ac	Pr	Bu
N[2]	198	196	193	193	193	23	23	21	14	14	12
Observed	6.14	107	65	25	13	22.8	10.7	6.1	27.3	12.5	5.6
Predicted											
M13	6.05	126	77	33	16	26.4	13.8	6.4	—	—	—
M16	6.12	104	66	26	13	29.4	14.2	6.7	—	—	—
M16VFA	6.12	104	67	26	13	28.3	14.5	5.6	37.2	13	7.2
M16PH[3]	6.12	104	66	26	13	31.8	12.9	6.6	—	—	—
M16VFAPH[4]	6.12	104	66	26	12	30.2	13	5.9	38.2	11.7	7.8
RMSPE[5] (% of observed mean)											
M13	4.5	28	28	46	40	69	58	50	—	—	—
M16	4.3	22	22	31	26	75	63	55	—	—	—
M16VFA	4.3	22	21	32	27	77	60	51	85	49	57
M16PH	4.3	20	20	31	26	80	53	53	—	—	—
M16VFAPH	4.3	20	19	31	26	77	53	52	86	48	66
Mean bias (% of MSPE)											
M13	12.9	39	44	44	41	5	25	1	—	—	—
M16	0.4	1.1	0.7	0.1	0.04	15	27	4	—	—	—
M16VFA	0.4	1.2	1.1	0.0	0.2	10	35	3	19	0.7	28
M16PH	0.4	1.6	0.6	0.1	0.1	25	14	2	—	—	—
M16VFAPH	0.4	1.5	0.6	0.2	0.3	18	17	1	22	2	37
Slope bias (% of MSPE)											
M13	2.4	12	6	13	20	42	2	56	—	—	—
M16	3.6	17	21	8	11	43	3	59	—	—	—
M16VFA	4.7	15	12	14	14	49	1	56	42	2	39
M16PH	7.1	4	3	3	11	38	1	59	—	—	—
M16VFAPH	7.3	4	2	3	12	42	0.2	59	40	0.4	37

[1]Ac = acetate, Pr = propionate, Bu = butyrate.

[2]N = number of treatment means used for the analyses.

[3]After including effect of pH on VFA absorption in M16 model.

[4]After including effect of pH on VFA absorption in M16VFA model.

[5]Root mean squared prediction error.

al., 2013) and the general commonality of observations in the 2 efforts argues against such a point. None of the studies reporting VFA production rates reported ruminal digestion variables; thus, a complete assessment of errors in ruminal digestion was not possible.

The derived parameter estimates for the M16 model are presented in Table 2. The model was well defined by the data with coefficient of variation of the parameter estimates being less than 15%. In general, the coefficients for production of propionate from each substrate increased compared with the original values in M13 for forage diets and decreased for concentrate diets. Changes in parameters associated with de novo acetate and butyrate production were also observed. Some difference in VFA production parameters in M16 as compared with M13 can be expected given the differences in representation, where the M13 coefficients were applied as 3 discrete sets based on diet type using rather arbitrary boundaries for diet type (Baldwin, 1995), as compared with the current approach using a linear interpolation based on the proportion of forage in the diet (see Eq. 1).

Addition of VFA Interconversion Equations and Rederivation of Stoichiometric Coefficients

Parameter estimates for the M16VFA model are presented in Table 2. The standard error of the estimates were slightly greater than for the M16 model, but not above 15%, indicating the model parameters were still well defined by the data. In contrast to the M16 model, coefficients for propionate production from all substrates were reduced as compared with the M13 model regardless of diet type, and butyrate production was increased from all substrates for forage diets and reduced from all substrates for concentrate diets. Butyrate yield from AA fermentation was also reduced. Acetate coefficients generally decreased in forage diets (except from the soluble CHO fraction) and increased for concentrate diets (Table 2). Acetate production

Table 2. Estimates of VFA stoichiometry and absorption parameters without (M16) and with (M16VFA) equations representing VFA interconversion in the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) when VFA absorption was driven by mass action without pH control or with a Michaelis-Menten absorption equation considering pH regulation (M16PH and M16VFAPH, respectively)

Model parameter	Description	Initial value	M16		M16PH		M16VFA		M16VFAPH	
			Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
VFA stoichiometry coefficient[1]										
fScPrFor	Fermentation of soluble CHO in forage to propionate	0.4	0.44	0.022	0.40	0.031	0.23	0.025	0.25	0.019
fStPrFor	Fermentation of starch in forage to propionate	0.34	0.36	0.025	0.22	0.007	0.33	0.031	0.14	0.015
fHcPrFor	Fermentation of hemicellulose in forage to propionate	0.4	0.39	0.023	0.55	0.072	0.3	0.025	0.59	0.046
fCePrFor	Fermentation of cellulose in forage to propionate	0.2	0.28	0.022	0.18	0.012	0.15	0.016	0.09	0.01
fScPrCon	Fermentation of soluble CHO in concentrate to propionate	0.46	0.3	0.017	0.44	0.107	0.35	0.033	0.33	0.735
fStPrCon	Fermentation of starch in concentrate to propionate	0.7	0.64	0.047	0.66	0.097	0.58	0.046	0.6	0.20
fHcPrCon	Fermentation of hemicellulose in concentrate to propionate	0.58	0.3	0.018	0.6	0.35	0.4	0.029	0.4	0.4
fCePrCon	Fermentation of cellulose in concentrate to propionate	0.2	0.11	0.010	0.11	0.008	0.14	0.012	0.09	0.227
fScBuFor	Fermentation of soluble CHO in forage to butyrate	0.11	0.12	0.017	0.12	0.012	0.17	0.015	0.11	0.007
fStBuFor	Fermentation of starch in forage to butyrate	0.23	0.26	0.023	0.24	0.012	0.35	0.025	0.23	0.015
fHcBuFor	Fermentation of hemicellulose in forage to butyrate	0.23	0.32	0.023	0.27	0.014	0.37	0.026	0.19	0.019
fCeBuFor	Fermentation of cellulose in forage to butyrate	0.24	0.4	0.012	0.31	0.026	0.33	0.030	0.41	0.021
fScBuCon	Fermentation of soluble CHO in concentrate to butyrate	0.32	0.18	0.013	0.35	0.081	0.16	0.017	0.16	0.205
fStBuCon	Fermentation of starch in concentrate to butyrate	0.25	0.21	0.018	0.16	0.013	0.14	0.018	0.22	0.081
fHcBuCon	Fermentation of hemicellulose in concentrate to butyrate	0.15	0.16	0.013	0.08	0.026	0.1	0.011	0.13	0.358
fCeBuCon	Fermentation of cellulose in concentrate to butyrate	0.11	0.16	0.011	0.07	0.014	0.1	0.013	0.10	0.335
fAaPr	Fermentation of AA to propionate	0.6	0.71	0.042	0.44	0.03	0.49	0.041	0.36	0.021
fAaBu	Fermentation of AA to butyrate	0.25	0.23	0.020	0.30	0.007	0.2	0.022	0.30	0.029
fScAcFor	Fermentation of soluble CHO in forage to acetate	1.38	1.32	—	1.36	—	1.42	—	1.53	—
fStAcFor	Fermentation of starch in forage to acetate	1.2	1.12	—	1.31	—	0.97	—	1.40	—
fHcAcFor	Fermentation of hemicellulose in forage to acetate	1.14	0.97	—	0.92	—	0.97	—	1.02	—
fCeAcFor	Fermentation of cellulose in forage to acetate	1.32	0.93	—	1.20	—	1.19	—	1.09	—
fScAcCon	Fermentation of soluble CHO in concentrate to acetate	0.9	1.34	—	0.86	—	1.34	—	1.35	—
fStAcCon	Fermentation of starch in concentrate to acetate	0.8	0.94	—	1.01	—	1.15	—	0.98	—
fHcAcCon	Fermentation of hemicellulose in concentrate to acetate	1.12	1.38	—	1.23	—	1.41	—	1.38	—
fCeAcCon	Fermentation of cellulose in concentrate to acetate	1.58	1.58	—	1.74	—	1.66	—	1.71	—
fAaAc	Fermentation of AA to acetate	0.67	0.6	—	0.75	—	0.86	—	0.82	—
Rate constant for absorption										
KabsAc	Rate constant for acetate absorption	5.9	9.5	0.17	—	—	9.3	0.26	—	—
KabsPr	Rate constant for propionate absorption	8.8	13.5	0.18	—	—	13.3	0.25	—	—
KabsBu	Rate constant for butyrate absorption	7.96	12.8	0.2	—	—	10.8	0.27	—	—
V_{max}	Maximum total VFA absorption potential (mol/d)	—	—	—	18.5	0.306	—	—	17.6	0.33

[1]Value represents the stoichiometric coefficient defining production of each VFA (*Ac* = acetate, *Pr* = propionate, or *Bu* = butyrate; mol/mol of hexose equivalent) from each dietary substrate (*Ce* = cellulose, *Hc* = hemicellulose, *St* = starch, and *Sc* = soluble carbohydrate). The *For* and *Con* coefficient sets were defined as those derived from diets with >80% forage and >80% concentrate (% of dietary DM), respectively, for initial values and with 100% forage and 100% concentrate, respectively, for the rest of the model coefficients. Parameters were bound to ±75% of the previous value. The value for acetate was calculated from difference.

from soluble CHO and starch changed the most for concentrate diets by 44 and 49%, respectively.

The rate constants for absorption of acetate, propionate, and butyrate (*KabsAc*, *KabsPr*, and *KabsBu*, respectively) increased by more than 50%. This was also expected given the overprediction of VFA concentrations by the M13 model.

Despite the large changes in parameters, the addition of VFA interconversion equations (M16VFA model) to the M16 model, however, resulted in very small changes in RMSPE for acetate, propionate, and butyrate concentrations (Table 1). The mean predicted VFA concentrations for both the M16 and M16VFA models were close to the observed values; however, we noted a modest slope bias for all VFA (8 to 21%) in the M16 model that was reduced in the M16VFA model for acetate, and increased for propionate and butyrate (Table 1). Inaccurate predictions of liquid passage rates can be a possible explanation for the slope bias errors for VFA concentrations. Passage is calculated as a constant fractional proportion of rumen liquid volume in the M16 model (Baldwin, 1995). Because dietary changes, particularly forage-to-concentrate ratios, affect VFA production rates, predicting inappropriate changes in liquid passage rate across diet types could lead to slope errors for all the VFA concentrations. Gregorini et al. (2015) recently updated the passage rate equations for liquid and solids in Molly and evaluated the model against most of the same VFA concentration data used herein. Those authors observed RMSPE of 19, 27, and 24% for acetate, propionate, and butyrate concentrations, respectively, with no mean and essentially no slope bias, suggesting that inaccurate representation of passage rate may be the cause of some of the slope errors herein.

Prediction errors for net production rates were slightly increased for acetate and reduced for propionate and butyrate for the M16VFA model relative to M16 model (Table 1). When the M16VFA model was compared with the M16 model using the LLF comparison, no significant improvement in model prediction was identified ($P > 0.05$), which contradicted our hypothesis.

Several studies have shown that VFA interconversions are significant and can be variable across diets (Leng and Leonard, 1965; Seal and Parker, 1994; Kristensen, 2001; Sutton et al., 2003). Our hypothesis was that the lack of representation of interconversion in Molly was contributing to errors of predictions for VFA production (Ghimire et al., 2014), and that representing interconversion among VFA would reduce those errors. Such interconversions are proposed to be controlled or at least influenced by the thermodynamic state in the rumen, which is affected by the ruminal environment (Kohn and Boston, 2000; Ungerfeld and Kohn, 2006; Ghimire et al., 2014). Infusion of propionate into the rumen increases propionate concentrations, which represents a shift in thermodynamic state, and this has been observed to cause a shift in conversion of propionate to acetate as would be predicted (Seal and Parker, 1994). This indicates that our approach of representing interconversions should, in theory, capture the shift in net VFA production. Therefore, large errors of prediction in our study, even when using the M16VFA model, could be because several other factors that define thermodynamic state had to be assumed, and this may have resulted in inaccurate derivations of the interconversion rate constants. It is also possible that the reported VFA production data used for the work were not representative of the true rates. Finally, it is possible that the models representation of the effect of pH on the VFA stoichiometric coefficients was inappropriate.

Assumed Constants in the Interconversion Equations. The reactant and product concentrations that were assumed constant in the thermodynamic equations used in our study could vary depending upon the nature of the diet (Hegarty and Gerdes, 1999; Janssen, 2010); however, the data needed to represent this variation is scant in the literature. Similarly, the effect of pH on VFA stoichiometry coefficients in the Molly cow model is a discontinuous one (Baldwin, 1995). A continuous equation derived from a wide range of pH on various diet types may better explain the effect of pH on the stoichiometry coefficients. The data set used for our study was mostly from lactating cows, which are typically not fed wide ranges of dietary concentrate as compared with beef cattle. This could have caused less robust representation of pH effect in our study.

Variability in Reported VFA Production Rates. It would have been preferable to derive the VFA interconversion constants ($K_{i,j}$) simultaneously with the de novo synthesis rates rather than calculating average rate constants before parameter fitting. Such an integrated fitting approach has a better chance of ensuring appropriate transfer rates regardless of the production rates. However, we were unsuccessful in those attempts due to the limited number of de novo synthesis observations and large variance among studies in those measurements. The variance is demonstrated in Figure 1. Acetate production ranges from a low of less than 1 mol of acetate/kg of DMI to a high of more than 5 mol of acetate/kg of DMI. Similar ranges exist for propionate and butyrate production, and the expected increase in propionate and decrease in acetate and butyrate production rates associated with dietary forage percent were not apparent. Although patterns in the data were not clearly evident, the studies tended to cluster with 9 of 23 treatment means having average production rates

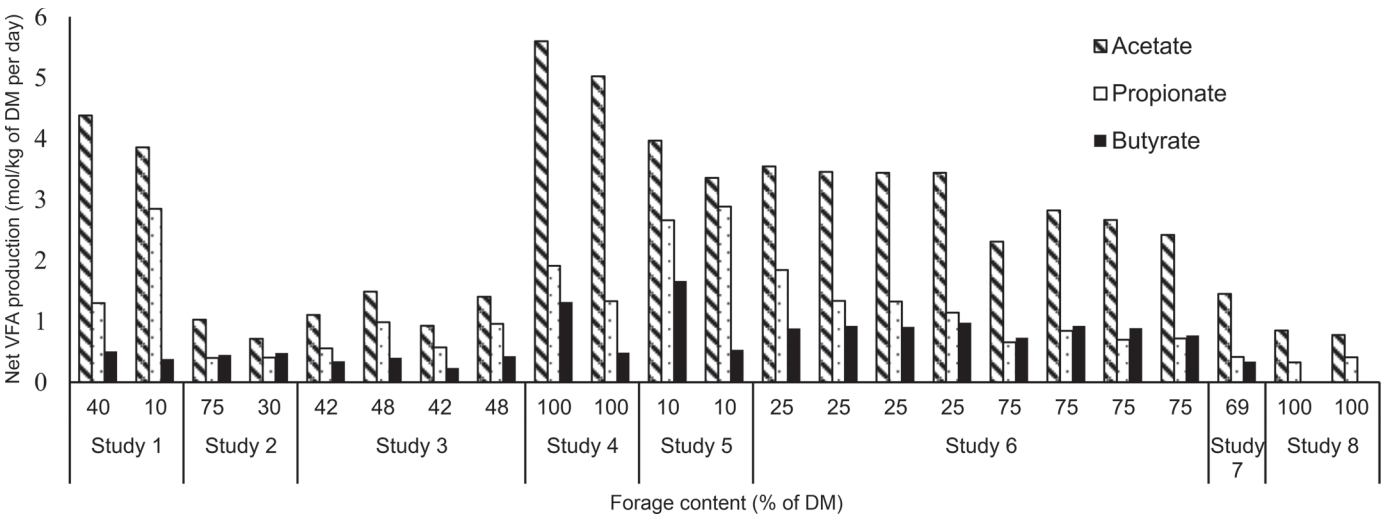

Figure 1. Reported net VFA production (mol/kg of DMI per day) and dietary forage content (% DM) in each treatment of study 1 through 8 (Sutton et al., 2003; Markantonatos et al., 2008; Markantonatos et al., 2009; Esdale et al., 1968; Sharp et al., 1982; Rogers and Davis 1982; Armentano and Young, 1983; Seal and Parker, 1994, respectively) used in the data set.

of 1.08 ± 0.30, 0.56 ± 0.25, and 0.30 ± 0.18 mol/d of acetate, propionate, and butyrate, respectively, compared with 3.59 ± 0.94, 1.54 ± 0.78, and 0.85 ± 0.34 mol/d of acetate, propionate, and butyrate, respectively, for the remainder of the treatments. The effect of sampling locations on VFA production estimates in this data set has been discussed previously, and clear evidence that the divergent sampling locations in the data set affected the estimates is not evident (Ghimire et al., 2014).

A portion of the variance may be due to the choice of isotope used to derive VFA production rates. Ideally, one would use VFA labeled in the 2 position, as that carbon is generally not lost from the molecule unless the VFA is completely converted to CO_2, whereas the carboxyl carbon is exchanged much more readily with the CO_2 pool, which will result in an underestimation of production rates due to removal of the label from the system (Kristensen, 2001; France and Dijkstra, 2005). Label entering the CO_2 pool can subsequently be introduced into other compounds including microbes and VFA, the latter yielding an overestimate of the transfer rates. Markantonatos et al. (2008, 2009; n = 6) used VFA labeled on the first carbon, resulting in acetate production rates less than 1.5 mol/kg of DMI. In contrast, Sutton et al. (2003; n = 2) observed acetate production rates of 4.39 mol/kg of DMI for the normal forage diet and 3.84 mol/kg of DMI for the low-roughage diet when using VFA labeled in the 2 position only. Seal and Parker (1994; n = 2) also used VFA labeled in the 2 position, but the reported VFA production rates were half of those reported by Sutton et al. (2003). It is unclear what may have caused this, but the reported DMI in Seal and Parker (1994) is much greater (6.7% of BW) than what the animals could probably consume, and thus likely an error. Armentano and Young (1983; n = 1) used carboxyl-labeled propionate and uniformly labeled acetate and butyrate. Acetate and butyrate were possibly slightly underestimated as one-half and three-quarters of the carbon would not have exchanged with CO_2, but the transfer among VFA may have been overestimated. Thus, potential reasons exist for the cluster of low values, but the overall data are so limited in number, meaning it is not possible to make well-supported data exclusion decisions; thus, we retained all the data. As more data are generated using VFA labeled in the 2 position, it may be possible to refine the older data with more confidence and to derive both the interchange and de novo synthesis constants simultaneously.

Assumption of Steady State for VFA Production Estimates. As the attainment of steady state is the basis of production rate estimates in almost all of the studies, some of the reported variation in VFA production rates could have been caused by biased estimates of production rates. Moreover, with the models used for VFA production studies, a buffer period is generally required to avoid carry over effects of one isotope into the next labeling period. Failure to achieve true steady state might also indicate significant carryover of each isotope to the subsequent labeling period further biasing production estimates. For example, Markantonatos et al. (2009) infused acetate and propionate in 1 d, followed by butyrate the next day. If the exchange flux between acetate and propionate were different, the results could be biased. Furthermore, any carryover of

acetate isotope would overestimate flux of butyrate to acetate and underestimate production of butyrate.

Effect of pH on VFA Concentration and Production Estimates. The residuals for acetate concentration and acetate and propionate production were also found to be correlated with dietary NDF and starch ($P < 0.05$) in both the M16 and M16VFA models. The slopes of the NDF and starch correlations suggested that the errors might also be correlated with pH, which was indeed the case for concentrations of all VFA (Figure 2), and production of acetate and propionate (Figure 3; $P < 0.05$) in both the M16 and M16VFA models. Given that concentrations were under predicted at high pH, it was hypothesized that the concentration errors were due to the lack of representation of the effects of pH on VFA absorption in the models. Absorption of VFA has been known to be affected by ruminal pH (France and Dijkstra 2005; Penner et al., 2009; Dijkstra et al., 2012; Laarman et al., 2013). The lack of representation of a pH effect could have also caused the optimizer to choose larger coefficients for VFA production to minimize differences between observed and predicted concentration values.

To represent the effect of pH on VFA absorption, the original mass action absorption equations in both the M16 and M16VFA models were replaced with the Michaelis-Menten equation (Eq. 8) of Dijkstra et al. (1992) resulting in models denoted as M16PH and M16VFAPH, respectively. The V_{max} was rederived along with the VFA production parameters (Table 2). The overall model improvement was significant in both M16PH and M16VFAPH models ($P < 0.05$) compared with M16 and M16VFA models, respectively (Table 1). This indicates that the altered equation for VFA absorption resulted in slight improvements over the original mass action equations. However, the errors of prediction for production rates were still quite large, being over 50% for each. Furthermore, the M16VFAPH model was not significantly better than the M16PH model ($P > 0.05$), which provides additional evidence that the thermodynamic relationships incorporated in our study did not improve model fit. Changes in prediction errors for VFA concentration and production were below 1%, except for a marginal improvement in acetate production in the M16VFAPH model as compared with the M16PH model (Table 1). The slight improvement in prediction errors for acetate production was associated with a decrease in proportion of mean bias and slight increase in slope bias. The proportions of VFA production errors segregating into mean and slope bias was very similar between the 2 models. These results are consistent with the earlier results from the comparison between M16 and M16VFA models and indicate that the addition of VFA interconversion equations did not improve model predictions. It is unclear if this would also be the conclusion if a broader range of dietary conditions were included in the evaluation data set. For example, inclusion of feedlot and low-quality grass forage-based diets and more measurements of interconversion rates under conditions of changing thermodynamic state may provide the additional range needed to better define the model parameters allowing potential thermodynamic effects to become apparent.

Sensitivity Analysis of Parameters

The results of global model sensitivity to the parameters of the M16, M16PH, M16VFA, and M16VFAPH models are presented in Table 3. The sensitivity coefficient for acetate production in the M16 model was the greatest for AA fermentation (*fAaPr*, 0.295) and the rate of acetate absorption (*KabsAc*, 0.215). Propionate production was most sensitive to AA fermentation (*fAaPr*, 0.553) and starch fermentation from concentrate (*fStPrCon,* 0.343). Butyrate production was most sensitive to AA fermentation (*fAaBu*, 0.418) and starch fermentation from concentrate (*fStBuCon*, 0.28). The sensitivity of acetate production to acetate absorption likely represents the effect of total VFA on ruminal pH predictions, which in turn inhibits microbial fermentation (Baldwin, 1995). As overall VFA concentrations are influenced to a lesser degree by propionate and butyrate due to their lower concentrations, the sensitivity to absorption of those VFA was less, but still present. The effect of AA fermentation on production of all 3 VFA was more surprising. Amino acid catabolism is much less than sugar fermentation, and thus the yield of VFA would be expected to be much less sensitive to AA parameters than to starch and fiber fermentation parameters. The relationship may be a reflection of the previously observed oversensitivity of microbial growth to ruminal ammonia concentrations (Hanigan et al., 2013). Microbial growth rates and mass would have increased as dietary RDP increased, which would have influenced the rate of VFA production and, thus, may explain the sensitivity to AA degradation. A recent evaluation of the microbial growth prediction equations in the Dairy NRC model found that observed microbial N predictions were less sensitivity than what would be predicted by the model (White et al., 2016). This oversensitivity is consistent with the overpredictions of microbial growth in response to ruminal ammonia concentrations observed by Hanigan et al. (2013), and is seemingly consistent with the sensitivity of VFA production to AA fermentation herein.

Acetate production predictions by the M16VFA model were also primarily sensitive to the absorption rate

of acetate (*KabsAc*, 0.835), whereas propionate production predictions were most sensitive to starch fermentation from concentrate (*fStPrCon*, 0.343) followed by AA fermentation (*fAaPr*, 0.353); butyrate production had the greatest sensitivity to rates of absorption of butyrate (*KabsBu*, 0.496) and acetate (*KabsAc*, 0.316). The sensitivity of all 3 VFA to the rate constants for absorption of each VFA at least partially reflects the effects of VFA concentrations on pH and the subsequent effects on production rates encoded in the original model (Baldwin, 1995) and the thermodynamically driven exchange among VFA. With the introduction

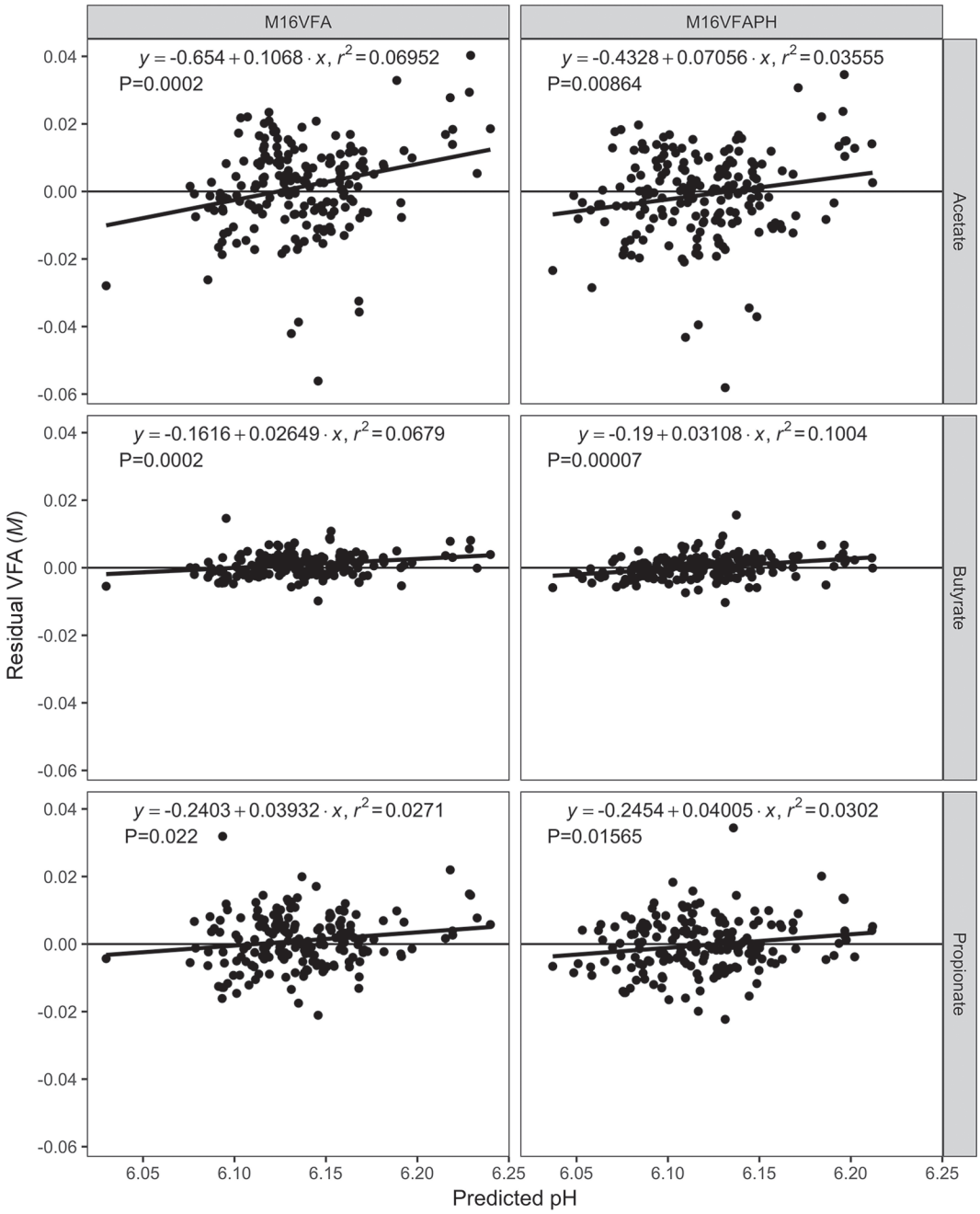

Figure 2. Residual errors of acetate, propionate, and butyrate concentration versus predicted pH using the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) after estimates of parameters listed in Table 3 and the representation of VFA interconversion without pH (M16VFA) or with (M16VFAPH) pH-regulated VFA absorption.

of pH-dependent VFA absorption, acetate production was most sensitive to the maximum absorption *Vmax* both in the M16PH model (0.35) and the M16VFAPH (0.86) models. As the former model does not represent thermodynamic control of interchanges, these results suggest that most of the effect of absorption on production is exerted through altered pH and the effects of pH on microbial fermentation of substrate. Propionate and butyrate production were most sensitive, respectively, to *fScPrCon* (0.7 and 0.6 in the M16PH and M16VFAPH models, respectively) and *fAaBu* (0.56 and 0.64 in the M16PH and M16VFAPH models, respectively).

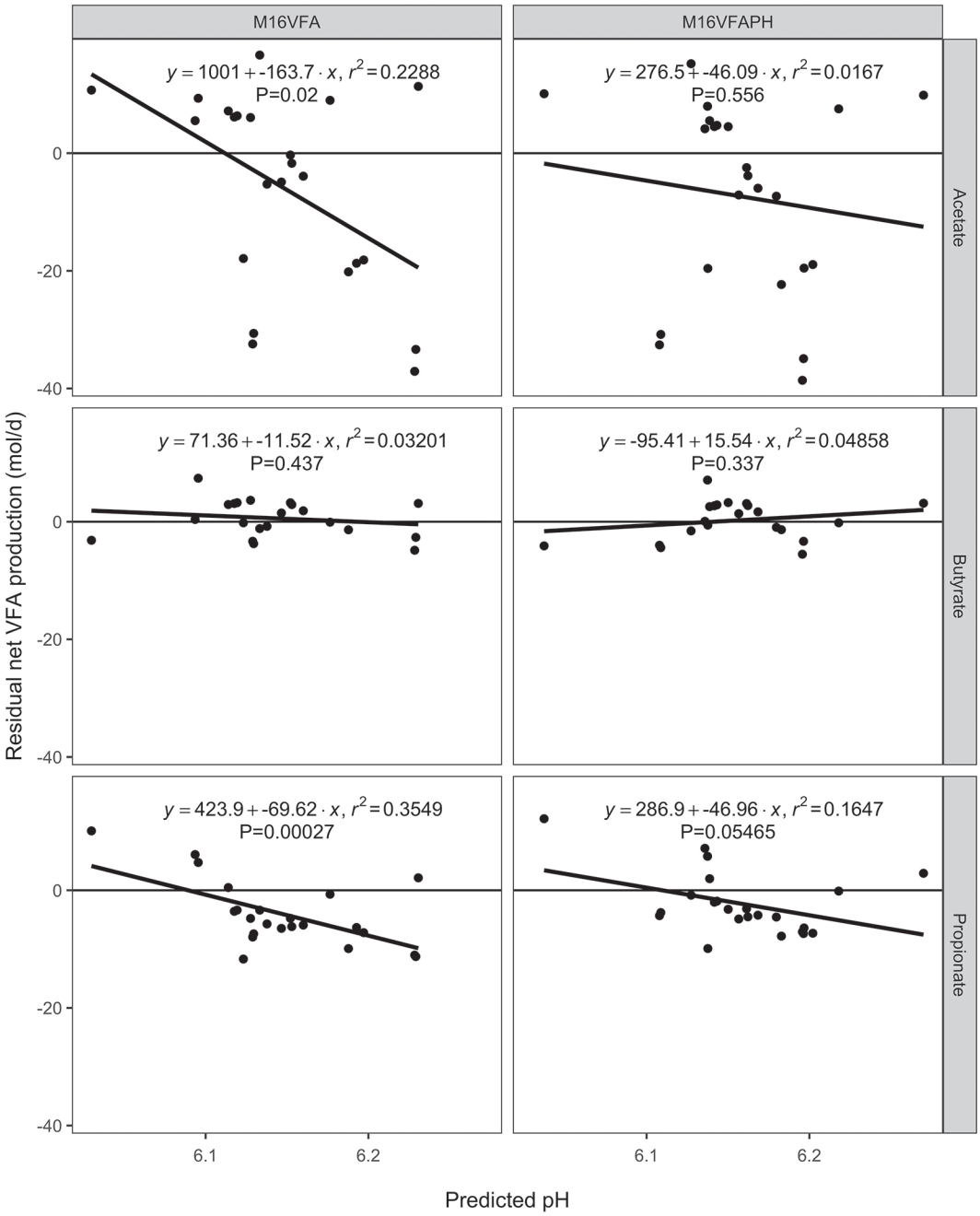

Figure 3. Residual errors of net acetate, propionate, and butyrate production versus predicted pH using the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) and the derived parameters listed in Table 3 with the representation of VFA interconversion without pH (M16VFA) or with (M16VFAPH) pH-regulated VFA absorption.

Table 3. Global sensitivity analyses of net VFA production to stoichiometry and absorption parameters (as described in Table 2) both with (M16VFA) and without (M16) equations to represent VFA interconversion in the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) when VFA absorption was driven by mass action without pH control or with a Michaelis-Menten absorption equation considering pH regulation (M16PH and M16VFAPH, respectively)

	Predicted net VFA production[2]											
	M16			M16PH			M16VFA			M16VFAPH		
Model parameter[1]	Ac	Pr	Bu	Ac	Pr	Bu	Ac	Pr	Bu	Ac	Pr	Bu
VFA stoichiometry coefficient												
fScPrFor	0.010	0.019	0.000	0.008	0.029	0.000	0.001	0.007	0.000	0.001	0.014	0.001
fStPrFor	0.022	0.041	0.000	0.008	0.029	0.000	0.006	0.048	0.001	0.001	0.013	0.001
fHcPrFor	0.002	0.004	0.000	0.004	0.014	0.000	0.000	0.004	0.000	0.001	0.014	0.001
fCePrFor	0.001	0.002	0.000	0.001	0.002	0.000	0.000	0.001	0.000	0.000	0.001	0.000
fScPrCon	0.012	0.023	0.000	0.020	0.069	0.000	0.005	0.043	0.001	0.002	0.022	0.001
fStPrCon	0.183	0.343	0.000	0.201	0.689	0.000	0.049	0.403	0.010	0.045	0.634	0.025
fHcPrCon	0.003	0.006	0.000	0.016	0.054	0.000	0.002	0.017	0.000	0.001	0.021	0.001
fCePrCon	0.001	0.001	0.000	0.001	0.002	0.000	0.000	0.003	0.000	0.000	0.001	0.000
fScBuFor	0.002	0.000	0.010	0.002	0.000	0.005	0.001	0.000	0.004	0.000	0.000	0.004
fStBuFor	0.038	0.000	0.163	0.029	0.000	0.068	0.018	0.002	0.054	0.007	0.001	0.061
fHcBuFor	0.003	0.000	0.014	0.003	0.000	0.008	0.002	0.000	0.005	0.000	0.000	0.004
fCeBuFor	0.003	0.000	0.012	0.005	0.000	0.013	0.002	0.000	0.005	0.001	0.000	0.006
fScBuCon	0.011	0.000	0.048	0.086	0.000	0.202	0.003	0.000	0.009	0.002	0.000	0.019
fStBuCon	0.065	0.000	0.280	0.045	0.000	0.106	0.007	0.001	0.022	0.017	0.002	0.146
fHcBuCon	0.003	0.000	0.014	0.001	0.000	0.002	0.000	0.000	0.001	0.001	0.000	0.005
fCeBuCon	0.002	0.000	0.010	0.001	0.000	0.002	0.000	0.000	0.001	0.000	0.000	0.003
fAaPr	0.295	0.553	0.000	0.024	0.084	0.000	0.042	0.353	0.009	0.017	0.240	0.010
fAaBu	0.073	0.000	0.418	0.187	0.000	0.585	0.013	0.002	0.061	0.046	0.011	0.636
Rate constant for absorption												
KabsAc	0.215	0.006	0.024	—	—	—	0.835	0.015	0.316	—	—	—
KabsPr	0.044	0.001	0.005	—	—	—	0.011	0.061	0.001	—	—	—
KabsBu	0.008	0.000	0.001	—	—	—	0.001	0.038	0.496	—	—	—
Vmax	—	—	—	0.354	0.024	0.01	—	—	—	0.855	0.019	0.069

[1]Definitions for each of the model parameters are given in Table 2.

[2]Ac = acetate, Pr = propionate, Bu = butyrate.

Relevance Relative to Other VFA Prediction Approaches

In experimental settings, VFA are generally measured as concentrations and relative proportions in the rumen. This is mainly because of the complexity and cost associated with measuring actual production rates. Therefore, Murphy et al. (1982) modified the Koong et al. (1995) model to derive VFA stoichiometric parameters by accommodating the relatively larger data reporting VFA molar proportions. Similar efforts for evaluation and rederivation of VFA coefficients were carried out later by Dijkstra et al. (1992), Bannink et al. (1997), Bannink et al. (2006), and Morvay et al. (2011). However, it is important to recognize that VFA concentration does not truly represent the production, as it is the reflection of the balance between production, interconversion, absorption, and outflow from the rumen; thus, it is critical that more studies be conducted with actual production and interconversion rate measurements. This will allow derivation of more accurate predictions of VFA production rates and, in turn, the influence of VFA production on energy availability, methane production, and ruminal health. Achieving better predictions will likely require a more mechanistic representation of VFA production, as little additional progress has been made over the past 30 yr attempting to refine the fairly empirical approach used in most rumen models (Morvay et al., 2011). Although, the current results do not provide any apparent benefit of inclusion of a mechanistic element, such a lack of improvement could largely be due to limited information on factors controlling ruminal environment and the quality of available VFA production data.

CONCLUSIONS

Including VFA interconversion equations based on thermodynamics in the Molly cow model did not improve the prediction of VFA production, as demonstrated by significantly lower LLF for the M16VFA model, when compared with the base M13 model, after rederiving VFA stoichiometric and absorption parameters in both models. We noted some reduction in prediction errors of VFA concentration after rederiving the parameters, but it was also evident in the model without interconversion equations and the overall performance was also similar, indicating that these improvements were also not due to the newly introduced equations. An equation representing the effect of pH on VFA absorption was introduced in both the M16 and M16VFA models, which improved their performance as indicated by χ^2 test of LLF, but the errors of predictions for VFA production were still large. Potential inaccuracy of reported VFA production rates in the literature and the required assumptions used in the thermodynamic equations due to scant data may have contributed to the lack of success in this current effort. This underscores the importance of further experimentation to generate higher-quality data on VFA production and additional observations related to thermodynamic driving variables.

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