Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows

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ABSTRACT

Volatile fatty acids (VFA), produced in the rumen by microbial fermentation, are the main energy source for ruminants. The VFA profile, particularly the non-glucogenic (acetate, Ac; butyrate, Bu) to glucogenic (propionate, Pr) VFA ratio (NGR), is associated with effects on methane production, milk composition, and energy balance. The aim of this study was to evaluate extant rumen VFA stoichiometry models for their ability to predict in vivo VFA molar proportions. The models were evaluated using an independent data set consisting of 101 treatments from 24 peer-reviewed publications with lactating Holstein cows. All publications contained a full diet description, rumen pH, and rumen VFA molar proportions. Stoichiometric models were evaluated based on root mean squared prediction error (RMSPE) and concordance correlation coefficient (CCC) analysis. Of all models evaluated, the 1998 Friggens model had the lowest RMSPE for Ac and Bu (7.2 and 20.2% of observed mean, respectively). The 2006 Bannink model had the lowest RMSPE and highest CCC for Pr (14.4% and 0.70, respectively). The 2008 Bannink model had comparable predictive performance for Pr to that of the 2006 Bannink model but a larger error due to overall bias (26.2% of MSPE). The 1982 Murphy model provided the poorest prediction of Bu, with the highest RMSPE and lowest CCC (24.6% and 0.15, respectively). The 1988 Argyle and Baldwin model had the highest CCC for Ac with an intermediate RMSPE (0.47 and 8.0%, respectively). The 2006 Sveinbjörnsson model had the highest RMSPE (13.9 and 34.0%, respectively) and lowest CCC (0.31 and 0.40, respectively) for Ac and Pr. The NGR predictions had the lowest RMSPE and highest CCC in the 2 models of Bannink, whereas the lowest predictive performance was in the 2006 Sveinbjörnsson model. It appears that the type of VFA produced is not a simple linear relationship between substrate inputs and pH as currently represented. The analysis demonstrates that most rumen VFA stoichiometric approaches explain a large part of the variation in VFA molar proportions among diets, in particular for Ac, whereas predictive power for Pr and Bu differ largely among approaches. The move toward feed evaluation systems based on animal response might necessitate an improved representation of rumen fermentation, focused on improving our understanding of VFA proportions in diets that vary from the mean.
Key words: volatile fatty acid, rumen, stoichiometry, model evaluation

INTRODUCTION

Volatile fatty acids, produced in the rumen by microbial fermentation, are the main energy source for ruminants (Bergman, 1990). The type of VFA formed in the rumen depends on type of substrate fermented, microbial population, and rumen environment (Bannink et al., 2008). The proportions among individual VFA are of particular interest because different VFA arise from variations in substrate intake and microbial populations, and absorbed VFA have different metabolic pathways in the animal organs. The glucogenic propionate (**Pr**) is a substrate for gluconeogenesis and is the main source of glucose in the animal, whereas the nonglucogenic acetate (**Ac**) and butyrate (**Bu**) are sources for long-chain fatty acid synthesis. Of these 3 VFA, Bu is the most extensively metabolized by the rumen epithelium (McLeod and Baldwin, 2000) and exerts mitotic effects on the rumen epithelium (Mentschel et al., 2001). Changes in the relative supply of individual VFA are related to milk yield and composition (Thomas and Martin, 1988; Seymour et al., 2005) and energy partitioning. For example, Ørskov et al. (1969) reported that isocaloric infusions of Ac, compared with Pr, result in differences in partitioning of energy into milk or body tissues. With Ac infusion, more energy was secreted as milk, whereas with Pr infusion, more

was deposited in body tissue. Moreover, an increase in milk fat percentage occurred with Ac. Acetic acid infusion caused increases in the C12, C14, and C16 fatty acid content of milk fat and decreased the proportion of C18:1 fatty acids. Glucogenic and lipogenic nutrient supply and VFA profile have also been associated with animal energy balance in early lactation (Van Knegsel et al., 2007) and methane production (Ellis et al., 2008). Moreover, increased Pr may promote insulin secretion and may regulate DMI by high-producing dairy cattle (Allen, 2000). However, current energy evaluation systems for cattle are based on ME or net energy (**NE**) and do not explicitly include the effect of type of VFA, but nutrient-based response systems to evaluate feeds for dairy cattle do require a proper representation of type of VFA formed (Hanigan et al., 2006; Dijkstra et al., 2007). For example, Dijkstra et al. (2008b) showed that various energy evaluation systems overestimate energy supply relative to energy requirement on grass-based diets for dairy cattle. A mechanistic model that included prediction of type of VFA, and consequently amount of glucogenic nutrients, proved to be more accurate and precise than the energy evaluation systems.

In the last few decades, several models predicting rumen fermentation stoichiometry have been developed, as reviewed by Dijkstra et al. (2008a). The stoichiometric coefficients developed for various ruminally fermented substrates have been used in several mechanistic whole-rumen models (e.g., Baldwin et al., 1987). Despite repeated efforts to predict rumen VFA proportions correctly, the prediction error of the models remains considerable in the few evaluations published (Bannink et al., 1997a; Hanigan et al., 2006). Bannink et al. (1997a) evaluated the sources of error likely to explain the inability of rumen fermentation models to predict VFA molar proportions correctly, and concluded that the inappropriate representations of VFA coefficients that relate type of VFA formed to type of substrate fermented is among the most probable causes. The aim of the current study was to evaluate rumen VFA stoichiometry coefficients using an independent data set of lactating Holstein cow digestion trials. The stoichiometric coefficients evaluated were Murphy et al. (1982), Argyle and Baldwin (1988), Friggens et al. (1998), Sveinbjörnsson et al. (2006), Bannink et al. (2006), and Bannink et al. (2008).

MATERIALS AND METHODS

VFA Stoichiometry Models

Six VFA stoichiometry prediction models were evaluated using independent data. Murphy et al. (1982) developed a set of stoichiometric coefficients by fitting observed rumen VFA molar proportions to digested soluble carbohydrates, starch, hemicellulose, cellulose, and CP. The approach was similar to that of Koong et al. (1975), but used a larger data set of 108 diets from mainly beef cattle and sheep trials. Separate stoichiometry coefficients, relating type of VFA produced to the various degraded entities, were generated for mainly concentrate and mainly roughage diets. The whole-rumen model of Baldwin et al. (1987) used the average of these stoichiometric coefficients for intermediate diets (45 to 55% concentrate).

Argyle and Baldwin (1988) modified the model of Murphy et al. (1982) by relating the fermentation of soluble carbohydrates and starch to rumen fluid pH. Linear relationships between rumen-digested substrate and rumen pH values below 6.2 were assumed based on in vitro data. These coefficients were used in the whole-cow metabolic model of Baldwin (1995), which includes a rumen model.

Friggens et al. (1998) used an empirical approach to predict rumen VFA stoichiometry, conducting a trial with cannulated sheep fed supplemented grass silage diets. Principal component analysis was used to determine feed fractions significant to the prediction of VFA molar proportions: CP, starch, sugars, and cellulose. Thus, the model of Friggens et al. (1998) uses feed composition, rather than digested feed fractions, to predict VFA molar proportions directly.

Sveinbjörnsson et al. (2006) developed a stoichiometrical submodel of VFA fermentation for the Nordic dairy cow model Karoline. One hundred seven treatments from 29 dairy cattle experiments were used, consisting of mainly grass silage based diets. The model related VFA molar proportions to digested CP, starch, forage NDF (**fNDF**), concentrate NDF (**cNDF**), lactate, and "rest" fraction (DM – ash – NDF – starch – CP – lactate – VFA). Dry matter intake relative to BW and concentrate ether extract were used as inputs in addition to digested feed fractions.

Bannink et al. (2006) used a similar approach to Murphy et al. (1982), but used 182 treatments from 47 digestion trials and only data on lactating Holstein cows to fit the stoichiometric parameters, in contrast to the study of Murphy et al. (1982), which mainly made use of beef cattle and sheep data. Volatile fatty acid molar proportions were related to observed amounts of digested soluble carbohydrates, starch, hemicellulose, cellulose, and CP. In addition to the distinction of mainly concentrate and mainly roughage diets, intermediate diets (40 to 60% roughage) have been recognized in whole-rumen models (e.g., Dijkstra et al., 1992) using coefficient means, similar to Baldwin et al. (1987).

Bannink et al. (2008) fitted VFA stoichiometry coefficients from the same data set of in vivo lactating dairy

cow observations as Bannink et al. (2006), but added the effect of rumen pH on the fermentation pattern of starch and soluble carbohydrates. Sigmoidal relationships between rumen pH and fraction of substrate converted to Ac, Pr, and Bu were assumed. Additionally, nonlinear relationships between VFA concentration and VFA absorption were used. However, because these relationships are described using rumen fluid volume and passage rate, they were not considered in the current evaluation. The profile of VFA was related to digested soluble carbohydrates, starch, hemicellulose, cellulose and CP. Coefficients were determined for mainly concentrate, intermediate, and mainly roughage diets.

Data Set

A data set consisting of 101 treatments from 24 peer-reviewed publications was collected for model evaluation (Broderick et al., 2002; Oba and Allen, 2003a,b; Rigout et al., 2003; DeFrain et al., 2004; Plaizier, 2004; Taweel et al., 2005; Benefield et al., 2006; Gencoglu and Turkmen, 2006; Benchaar et al., 2007; Krizsan et al., 2007; Abrahamse et al., 2008a,b; Boeckaert et al., 2008; Dann et al., 2008; Gehman et al., 2008; Abrahamse et al., 2009; Alamouti et al., 2009; Iqbal et al., 2009; Kelzer et al., 2009; Khafipour et al., 2009; Mahjoubi et al., 2009; Hara and Tanigawa, 2010; Hippen et al., 2010). To guarantee independent evaluation, only treatments that were not used during formation of any of the stoichiometric models mentioned previously were included. To eliminate differences caused by genetic variation only trials using lactating Holstein dairy cattle were used. Treatments that included additives or bST treatments were excluded from the study. All papers reported diet chemical composition (NDF, ADF, starch, crude fat, CP, ash), rumen liquid pH and rumen liquid VFA composition. In experiments where acid detergent lignin (**ADL**) was not reported, the cellulose fraction was determined by correcting ADF using the ratio of ADF to ADL of the relevant feed ingredients according to Dutch feed tables (CVB, 2007). Soluble carbohydrates were calculated as the fraction of DM not accounted for by NDF, starch, CP, crude fat, ash, lactate, and VFA. Monomer equivalents of degraded substrates were calculated assuming molecular weights of 162, 110, and 90.8 g/mol for carbohydrates, protein, and lactate, respectively. Observed VFA not accounted for by Ac, Pr, and Bu were assumed to be valeric and branched-chain fatty acids (**Bc**). Last, to integrate the 4 individual VFA into one characteristic, the nonglucogenic to glucogenic VFA ratio (**NGR**) was calculated as $[Ac + 2 \times Bu + Bc]/[Pr + Bc]$ (Abrahamse et al., 2008b).

The model of Sveinbjörnsson et al. (2006) requires cNDF, fNDF, and lactate as input parameters. Lactate content was determined using Dutch feed table values (CVB, 2007) and considered to be entirely digested in the rumen, as assumed by Sveinbjörnsson et al. (2006). When experiments did not report cNDF and fNDF, these were calculated from the NDF content of the separate feed ingredients using feed tables. Digested fractions of fNDF and cNDF were estimated using tabulated degradation kinetics data of the separate feedstuffs. The Argyle and Baldwin (1988) model estimates the molar proportions of Ac, Pr, and Bu only. Therefore, Bc predicted by Argyle and Baldwin (1988) was assumed equal to Bc predicted by Murphy et al. (1982).

The amount of independent literature reporting full diet descriptions, rumen VFA proportions, as well as duodenal nutrient flows not yet used in any of the stoichiometric model derivations was insufficient for the purpose of the current study. Therefore, digested feed fractions were determined using the dynamic, mechanistic rumen model of Dijkstra et al. (1992), for which duodenal flows were evaluated by Neal et al. (1992). The model consists of 17 state variables and includes partitioning of rumen microflora into amylolytic and fibrolytic bacteria and protozoa. Nutrient fluxes are described by enzymatic and mass action kinetics. Degradation characteristics required as input for the rumen model were obtained from Dutch feed tables (CVB, 2007), which are based on in situ rumen digestion trials. Predicted rumen truly digested substrates were then used as input with the stoichiometric coefficients of Murphy et al. (1982), Argyle and Baldwin (1988), Sveinbjörnsson et al. (2006), Bannink et al. (2006), and Bannink et al. (2008) to regress observed VFA molar proportions against those predicted by the models. The derivation of substrate degradation in the Dijkstra et al. (1992) model is independent from any of the stoichiometric approaches evaluated in the present paper, as none of the data used to develop the model of Dijkstra et al. (1992) were used in the various stoichiometric coefficients evaluated.

Volatile fatty acids present in the rumen are of 2 origins: either they have entered the rumen (through the diet or an infusion) or they have been produced in the rumen as result of substrate fermentation. In experiments where VFA were reported in the diet or infused intraruminally, observed amounts entering the rumen were added to predicted VFA produced from substrate degradation. Predicted VFA molar proportions were then recalculated based on total rumen VFA. For the models of Friggens et al. (1998) and Sveinbjörnsson et al. (2006), which contain predictions of rumen VFA

molar proportions directly rather than VFA production per unit of fermented substrate, the production of each VFA was estimated based on stoichiometric principles.

Statistical Analysis

Prediction errors of VFA stoichiometry models were determined by root mean squared prediction error (**RMSPE**, expressed as a percentage of the observed mean), which was calculated according to Bibby and Toutenburg (1977). The RMSPE comprises 3 sources of error, expressed as percentage of RMSPE: error due to bias (**ECT**), error due to deviation of the regression slope from 1 (**ER**), and random error (**ED**). In addition, the accuracy and precision of the models were evaluated using concordance correlation coefficient (**CCC**) analysis, according to Lin (1989). Concordance correlation coefficients range from –1 to +1, where values closer to +1 indicate a more precise and accurate model. This coefficient comprises 2 components; namely, Cb and ρ. The Cb is a bias correction factor and provides a measure of accuracy; that is, how close the line of regression of observed against predicted values is to the line of unity. The Cb value ranges from 0 to 1, where a higher value indicates a more accurate model. The ρ is the Pearson correlation coefficient, which provides a measure of precision. The measure μ (location shift) is used to calculate Cb and represents an underestimation and overestimation of predictions at positive and negative values, respectively.

For a graphical representation of the VFA molar proportion predictions of each model, observed values were regressed against predicted values (Piñeiro et al., 2008). Similarly, residuals calculated as observed minus predicted values were regressed against predicted values, as the slope is expected to be zero under the assumption of an unbiased model (St-Pierre, 2003).

RESULTS

The collected data set included a wide range of diet chemical compositions and feed intakes (Table 1) and of digested substrates (Table 2). Despite that, several correlations between input parameters were found. Sugars and starch were negatively correlated, whereas positive correlations were found between sugars and the "rest" fraction and between cellulose and fNDF (Table 3). Additionally, observed molar proportions of Ac and Pr were negatively correlated. Observed NGR was positively correlated with Ac and Bu and negatively with Pr, resulting from the definition of this ratio (Table 3).

Statistics for VFA stoichiometry predictions by all models are presented in Tables 4, 5, 6, 7, 8, and 9 and visualized in Figures 1, 2, 3, and 4. Predictive performance varied with type of VFA. In general, with the exception of Sveinbjörnsson et al. (2006), a large proportion of variation in VFA molar proportion was predicted well, and a large proportion of the error was random, particularly for Ac. The RMSPE and CCC values generally were in agreement in most models; that

Table 1. Summary of the independent data set used to evaluate the VFA stoichiometric approaches[1]

Item	Mean	Median	SD	Minimum	Maximum
DMI (kg/d)	21.5	21.9	3.4	15.2	27.6
BW (kg)	627	625	48	533	715
Concentrates (% of DMI)	45	48	12	14	63
Chemical composition (g/kg of DM)					
NDF	344	342	56	231	482
ADF	210	205	31	152	299
CP	170	168	17	128	227
Starch	204	234	82	31	324
Crude fat	38	35	10	21	64
Lactate	19	22	11	0	42
Concentrate ether extract	19	18	12	0	57
Concentrate NDF	85	72	36	16	171
Forage NDF	259	249	59	165	399
Observed molar proportions[2]					
(mol of VFA/100 mol of total VFA)					
Acetate	62.5	62.7	5.2	44.7	75.4
Propionate	22.4	21.1	4.9	13.8	44.5
Butyrate	11.3	11.6	2.5	5.8	18.0
Bc	3.9	4.0	1.4	0.2	10.5
NGR	3.5	3.6	0.8	1.3	5.9
Rumen pH	6.2	6.2	0.3	5.7	6.9

[1]Mean, median, SD, minimum, and maximum of input values (n = 101).

[2]Ac = acetate, Pr = propionate, Bu = butyrate, Bc = valeric acid and branched-chain fatty acids, NGR = nonglucogenic to glucogenic VFA ratio, calculated as [Ac + 2 × Bu + Bc]/[Pr + Bc].

Table 2. Mean, median, SD, minimum, and maximum of feed fraction digestion rates in the rumen (kg/d) as estimated by the model of Dijkstra et al. (1992) in the independent data set used to evaluate the VFA stoichiometric approaches (n = 101)

Item	Mean	Median	SD	Minimum	Maximum
Cellulose	2.5	2.4	0.7	0.8	4.2
Hemicellulose	1.8	1.7	0.6	0.5	2.9
CP	2.3	2.4	0.4	1.5	3.3
Starch	4.0	4.6	1.8	0.5	7.1
Sugars	2.7	2.5	1.3	0.2	6.0
Concentrate NDF	1.3	0.9	0.9	0.2	4.1
Forage NDF	3.0	2.9	1.0	0.7	5.9

is, models with lower RMSPE had higher CCC and vice versa, with the exception of the model of Argyle and Baldwin (1988). The RMSPE tended to increase in the order Ac < Pr < Bu < Bc. In general, Ac tended to be underpredicted and Bc tended to be overpredicted, whereas no clear pattern was observed with Pr and Bu. Coefficient of determination values (R^2), representing the fraction of explained variation in observed data, ranged from 0.00 to 0.57 across models and tended to increase in the order Bc < Bu < Ac < Pr.

The model of Murphy et al. (1982) had errors mainly due to random variation for Ac and Pr (89.1 and 80.5% of MSPE, respectively; Table 4). The accuracy value for Ac was the highest of all models (0.95, Figure 1), whereas Pr and Bu tended to be underpredicted (Figures 2 and 3, respectively). The model provided the poorest prediction of Bu compared with the other models, with the highest RMSPE and lowest CCC (24.6% of observed mean and 0.15, respectively), and a relatively large error due to overall bias (33.4%).

The Argyle and Baldwin (1988) model had intermediate RMSPE for Ac, Pr, and Bu (8.0, 16.2, and 22.7%, respectively; Table 5). Concordance correlation coefficients were variable, with high values for Ac and Pr (0.47 and 0.67, respectively) and an intermediate value for Bu (0.22). Accuracy values for Ac and Pr were very high (0.93 and 0.95, respectively), but Bu tended to be underpredicted (Figure 3). Model errors for Ac and Pr were mainly due to random variation (80.3 and 90.5%, respectively); however, large error due to bias was observed for Bu (20.7%).

The empirical model of Friggens et al. (1998) provided an improved prediction of molar proportions of Bu compared with the other models, with the lowest RMSPE and highest CCC (20.2% and 0.37, respectively; Table 6). Acetate predictions had the lowest RMSPE and a relatively high CCC (7.2% and 0.43, respectively). Model errors for Ac and Bu were mainly due to random variation (89.2 and 85.8%). In contrast, a large bias error was observed for Pr (34.2%), with Pr being underpredicted (Figure 2).

The Sveinbjörnsson et al. (2006) model performed relatively poorly in predicting Ac and Pr, with the highest RMSPE (13.9 and 34.0% of observed mean) and lowest CCC (0.31 and 0.40, respectively; Table 7) of all models. Markedly large errors due to overall bias were observed for Ac and Pr (78.3 and 69.8% of MSPE, respectively). The model had the highest accuracy value for Bu of all models (0.86) but tended to underpredict Ac and overpredict Pr (Figures 1 and 2, respectively).

The model of Bannink et al. (2006) showed an improved predictive performance for Pr compared with the other models, with the lowest RMSPE and highest CCC (14.4% and 0.70, respectively; Table 8). Model performance for Ac and Bu was comparable to that of Friggens et al. (1998). However, a tendency to overpredict Bu was observed (Figure 3). Model errors for Ac and Pr were mainly due to random variation (83.2 and 99.5%, respectively), whereas a large bias error was observed for Bu (22.5%; Table 8).

The Bannink et al. (2008) model performed similarly to that Bannink et al. (2006) for Pr, with comparable low RMSPE and high CCC values (Table 9). However, a significant error due to bias was observed for Ac, Pr, and Bu (25.0, 26.2, and 37.7% of MSPE). Root mean squared prediction error and CCC for Ac and Bu were intermediate. Accuracy terms for Ac and Pr were relatively high (0.78 and 0.88, respectively), whereas Bu tended to be overpredicted (Figure 3).

Predictive performance of NGR varied between the models (Figure 4). The model of Bannink et al. (2006) showed good predictive performance, with the lowest RMSPE and highest CCC of all models (16.8% and 0.59, respectively; Table 8), and model error was mainly due to random variation (94.3%). The Bannink et al. (2008) model performed similarly to that of Bannink et al. (2006), with comparable low RMSPE and high CCC values and decomposition, but slightly lower precision (Table 9). Friggens et al. (1998) had a large bias error for NGR (28.7%), and NGR was overpredicted (Table 6). In contrast, the error of the model of Murphy et al. (1982) was largely due to random

Table 3. Pearson correlation among digested feed fractions (g/kg of DM), observed VFA molar proportions (mol of VFA/100 mol of total VFA), and feeding level (kg of DM/kg of BW per day) in the independent data set used to evaluate the VFA stoichiometric approaches (n = 101)[1]

	Su	St	CP	Ce	Hc	fNDF	cNDF	Re	FL	Ac	Pr	Bu	Bc	NGR
Su	1.000													
St	-0.788*	1.000												
CP	-0.241*	0.273*	1.000											
Ce	0.275*	-0.625*	-0.293*	1.000										
Hc	0.026	-0.400*	-0.218*	0.421*	1.000									
fNDF	0.307*	-0.639*	-0.245*	0.788*	0.619*	1.000								
cNDF	-0.332*	0.162	-0.002	-0.041	0.320*	-0.385*	1.000							
Re	0.990*	-0.781*	-0.400*	0.261*	-0.028	0.278*	-0.342*	1.000						
FL	-0.120	0.373*	0.245*	-0.234*	-0.623*	-0.438*	-0.156	-0.085	1.000					
Ac	0.121	-0.140	-0.089	0.110	0.220*	0.097	0.137	0.062	0.044	1.000				
Pr	-0.221*	0.192	0.038	-0.121	-0.278*	-0.173	-0.081	-0.158	0.218*	-0.823*	1.000			
Bu	0.342*	-0.307*	0.000	0.114	0.153	0.197*	-0.035	0.347*	-0.536*	-0.239*	-0.284*	1.000		
Bc	-0.241*	0.373*	0.133	-0.239*	-0.071*	-0.150	-0.071	-0.254*	-0.044	-0.376*	0.036	0.139	1.000	
NGR	0.280*	-0.286*	-0.118	0.159	0.255*	0.174	0.105	0.223*	-0.223*	0.858*	-0.925*	0.218*	-0.301*	1.000

[1]Su = soluble carbohydrates, St = starch, Ce = cellulose, Hc = hemicellulose, fNDF = forage NDF, cNDF = concentrate NDF, Re = "rest" fraction (DM – ash – NDF – starch – CP – lactate – VFA), FL = feeding level, Ac = acetate, Pr = propionate, Bu = butyrate, Bc = valerate and branched-chain fatty acids, NGR = nonglucogenic to glucogenic VFA ratio calculated as [Ac + 2 × Bu + Bc]/[Pr + Bc].

*Statistical significance of the linear correlation at $\alpha = 0.05$.

variation (79.3%, Table 4). The model of Argyle and Baldwin (1988) had intermediate CCC and RMSPE for NGR (0.39 and 22.3%, respectively), with a relatively large error due to overall bias (24.0% of MSPE; Table 5). Finally, the model of Sveinbjörnsson et al. (2006) performed relatively poorly in predicting NGR with the highest RMSPE and lowest CCC of all models (35.7% of observed mean and 0.25, respectively), and model error was largely due to overall bias (72.7% of MSPE; Table 7).

DISCUSSION

VFA Stoichiometry Model Predictions

In the present analysis, observed and predicted VFA molar proportions were compared. The molar proportions in the rumen are the result of several processes that may differ between individual VFA, including differences in the rate of production, rate of interconversion, and rate of absorption (Bannink et al., 1997a); ideally, rate of production of individual VFA should have been evaluated. However, as already indicated by Murphy et al. (1982), the amount of data and range of diet composition available are too narrow and limited to evaluate the stoichiometric approaches for dairy cattle. Hence, data on VFA molar proportions based on rumen fluid concentrations were used. The results of a rare experiment examining VFA production rates in lactating dairy cattle using radioactive isotope-labeled VFA implied that the readily measured and widely published values for VFA proportions in the rumen provide a close estimate of the molar proportions on acetic and propionic acid produced, although that of butyric acid may be overestimated (Sutton et al., 2003). The VFA pattern is also known to be less sensitive than production rate to site and time of sampling (Murphy et al., 1982).

The ability of 6 models to describe VFA molar proportions in the rumen was compared using an independent data set for lactating Holstein cows from recent publications. Although a considerable fraction of observed variation remained unexplained, in particular for Pr, Bu, and Bc, the results of the present study showed an improved performance compared with previous evaluations of VFA stoichiometry models (e.g., Neal et al., 1992; Bannink et al., 1997b). When comparing model predictive performance, the scope and goals of each model must be considered. The models of Bannink et al. (2006) and Bannink et al. (2008) used a relatively wide range of high-lactating Holstein dairy cow diets for coefficient determination, similar to the data set that was used to evaluate the models in the current study. This is likely to have affected the observed relatively good

Table 4. Evaluation of the predictive performance of the VFA stoichiometric approach of Murphy et al. (1982) for the independent data set[1]

Item	Ac	Pr	Bu	Bc	NGR
Mean observed[2]	62.5	22.4	11.3	3.8	3.5
Mean predicted[2]	62.2	20.6	9.7	7.5	3.2
MSPE[3]	24.3	16.7	7.7	16.5	0.6
RMSPE[4]	7.9	18.2	24.6	105.9	21.7
ECT	0.4	18.6	33.4	82.1	15.2
ER	10.5	0.8	0.6	4.9	5.5
ED	89.1	80.5	66.0	13.0	79.3
CCC[5]	0.40	0.57	0.15	0.02	0.38
Cb	0.95	0.88	0.39	0.19	0.85
ρ	0.42	0.65	0.38	0.11	0.45
μ	0.07	0.42	1.21	−2.93	0.47
R^2	0.18	0.43	0.14	0.01	0.20

[1]Ac = acetate, Pr = propionate, Bu = butyrate, Bc = valerate and branched-chain fatty acids, NGR = non-glucogenic to glucogenic VFA ratio, calculated as [Ac + 2 × Bu + Bc]/[Pr + Bc].
[2]Ac, Pr, Bu, and Bc in mol of VFA/100 mol of total VFA.
[3]Mean squared prediction error (mol of VFA/100 mol of total VFA), according to Bibby and Toutenburg (1977).
[4]Root mean squared prediction error (% of mean observed); ECT = error due to bias (% of MSPE); ER = error due to deviation of the regression slope from 1 (% of MSPE); ED = random error (% of MSPE).
[5]Concordance correlation coefficient, according to Lin (1989): Cb = bias correction factor, ρ = Pearson correlation coefficient, μ = location shift.

performance of these models. The Murphy et al. (1982) and Sveinbjörnsson et al. (2006) models, which showed a poorer performance compared with the other models, used large data sets as well, but these contained observations with mainly sheep and beef cattle in the former and Nordic lactating cows fed grass silage based diets in the latter. Sveinbjörnsson et al. (2006) recognized the possible limitation in applicability of their model to a broader range of diets. In contrast, Friggens et al. (1998) based their model on one study with sheep fed supplemented grass silage, but produced surprisingly good predictions of VFA profiles in the rumen of lactating cattle. Argyle and Baldwin (1988) determined VFA coefficients of sugars and starch affected by rumen pH based on a few, mainly in vitro, studies but their modification of sugars and starch fermentation coefficients to pH-dependent ones resulted in improved predictions compared with those of Murphy et al. (1982).

Table 5. Evaluation of the predictive performance of the VFA stoichiometric approach of Argyle and Baldwin (1988) for the independent data set[1]

Item	Ac	Pr	Bu	Bc	NGR
Mean observed[2]	62.5	22.4	11.3	3.8	3.5
Mean predicted[2]	61.0	21.4	10.1	7.5	3.1
MSPE[3]	24.7	13.1	6.6	16.5	0.6
RMSPE[4]	8.0	16.2	22.7	105.8	22.3
ECT	9.1	7.8	20.7	82.3	24.0
ER	10.6	1.7	0.6	4.6	4.3
ED	80.3	90.5	78.7	13.0	71.6
CCC[5]	0.47	0.67	0.22	0.02	0.39
Cb	0.93	0.95	0.62	0.18	0.80
ρ	0.50	0.70	0.36	0.10	0.49
μ	0.32	0.23	0.72	−2.98	0.60
R^2	0.25	0.49	0.13	0.01	0.24

[1]Ac = acetate, Pr = propionate, Bu = butyrate, Bc = valerate and branched-chain fatty acids, NGR = non-glucogenic to glucogenic VFA ratio, calculated as [Ac + 2 × Bu + Bc]/[Pr + Bc].
[2]Ac, Pr, Bu, and Bc in mol of VFA/100 mol of total VFA.
[3]Mean squared prediction error (mol of VFA/100 mol of total VFA), according to Bibby and Toutenburg (1977).
[4]Root mean squared prediction error (% of mean observed); ECT = error due to bias (% of MSPE); ER = error due to deviation of the regression slope from 1 (% of MSPE); ED = random error (% of MSPE).
[5]Concordance correlation coefficient, according to Lin (1989): Cb = bias correction factor, ρ = Pearson correlation coefficient, μ = location shift.

Table 6. Evaluation of the predictive performance of the VFA stoichiometric approach of Friggens et al. (1998) for the independent data set[1]

Item	Ac	Pr	Bu	Bc	NGR
Mean observed[2]	62.5	22.4	11.3	3.8	3.5
Mean predicted[2]	63.9	19.9	12.2	4.0	3.9
MSPE[3]	20.4	18.0	5.2	1.9	0.6
RMSPE[4]	7.2	18.9	20.2	36.4	21.6
ECT	10.6	34.2	14.1	1.1	28.7
ER	0.1	0.1	0.1	0.5	4.3
ED	89.2	65.7	85.8	98.4	67.1
CCC[5]	0.43	0.55	0.37	0.24	0.48
Cb	0.77	0.79	0.75	0.70	0.83
ρ	0.56	0.70	0.50	0.34	0.58
μ	−0.39	0.62	−0.49	−0.15	−0.60
R^2	0.31	0.49	0.25	0.12	0.33

[1]Ac = acetate, Pr = propionate, Bu = butyrate, Bc = valerate and branched-chain fatty acids, NGR = non-glucogenic to glucogenic VFA ratio, calculated as [Ac + 2 × Bu + Bc]/[Pr + Bc].

[2]Ac, Pr, Bu, and Bc in mol of VFA/100 mol of total VFA.

[3]Mean squared prediction error (mol of VFA/100 mol of total VFA), according to Bibby and Toutenburg (1977).

[4]Root mean squared prediction error (% of mean observed); ECT = error due to bias (% of MSPE); ER = error due to deviation of the regression slope from 1 (% of MSPE); ED = random error (% of MSPE).

[5]Concordance correlation coefficient, according to Lin (1989): Cb = bias correction factor, ρ = Pearson correlation coefficient, μ = location shift.

The relatively good predictive performance of the empirical model of Friggens et al. (1998) is in spite of both interspecies difference and the attribution of VFA molar proportions solely to feed composition, without considering animal and rumen environment factors (e.g., DMI, digestibility). Ruminal substrate degradation explains a significant part of the variation in rumen VFA molar proportions next to feed composition. Even though empirical models are capable of providing accurate predictions, their applicability to predict rumen VFA molar proportions in combination with specific aspects of rumen function is limited. The model of Friggens et al. (1998) does not account for the origin of the sugars, starch, cellulose, or CP or the effects of ingredient-specific degradation characteristics of these nutrients. For example, exchanging barley and corn in high-concentrate diets hardly changed dietary chemical composition, but did significantly affect starch

Table 7. Evaluation of the predictive performance of the VFA stoichiometric approach of Sveinbjörnsson et al. (2006) for the independent data set[1]

Item	Ac	Pr	Bu	Bc	NGR
Mean observed[2]	62.5	22.4	11.3	3.8	3.5
Mean predicted[2]	54.8	28.7	10.5	6.0	2.5
MSPE[3]	75.9	57.9	6.7	7.5	1.6
RMSPE[4]	13.9	34.0	22.9	71.4	35.7
ECT	78.3	69.8	9.0	60.4	72.7
ER	2.6	9.6	10.5	10.8	2.1
ED	19.1	20.6	80.5	28.8	25.1
CCC[5]	0.31	0.40	0.27	0.02	0.25
Cb	0.46	0.58	0.86	0.38	0.44
ρ	0.67	0.70	0.31	0.05	0.56
μ	1.53	−1.20	0.39	−1.77	1.57
R^2	0.45	0.49	0.10	0.00	0.31

[1]Ac = acetate, Pr = propionate, Bu = butyrate, Bc = valerate and branched-chain fatty acids, NGR = non-glucogenic to glucogenic VFA ratio, calculated as [Ac + 2 × Bu + Bc]/[Pr + Bc].

[2]Ac, Pr, Bu, and Bc in mol of VFA/100 mol of total VFA.

[3]Mean squared prediction error (mol of VFA/100 mol of total VFA), according to Bibby and Toutenburg (1977).

[4]Root mean squared prediction error (% of mean observed); ECT = error due to bias (% of MSPE); ER = error due to deviation of the regression slope from 1 (% of MSPE); ED = random error (% of MSPE).

[5]Concordance correlation coefficient, according to Lin (1989): Cb = bias correction factor, ρ = Pearson correlation coefficient, μ = location shift.

Table 8. Evaluation of the predictive performance of the VFA stoichiometric approach of Bannink et al. (2006) for the independent data set[1]

Item	Ac	Pr	Bu	Bc	NGR
Mean observed[2]	62.5	22.4	11.3	3.8	3.5
Mean predicted[2]	60.5	22.4	12.4	4.6	3.4
MSPE[3]	22.4	10.4	5.5	2.9	0.4
RMSPE[4]	7.6	14.4	20.7	44.3	16.8
ECT	16.5	0.0	22.5	20.5	5.7
ER	0.2	0.5	3.1	4.9	0.1
ED	83.2	99.5	74.4	74.5	94.3
CCC[5]	0.43	0.70	0.33	0.04	0.59
Cb	0.79	0.94	0.58	0.50	0.91
ρ	0.54	0.75	0.56	0.09	0.66
μ	0.49	−0.01	−0.72	−0.88	0.23
R^2	0.29	0.56	0.32	0.01	0.43

[1]Ac = acetate, Pr = propionate, Bu = butyrate, Bc = valerate and branched-chain fatty acids, NGR = non-glucogenic to glucogenic VFA ratio, calculated as [Ac + 2 × Bu + Bc]/[Pr + Bc].

[2]Ac, Pr, Bu, and Bc in mol of VFA/100 mol of total VFA.

[3]Mean squared prediction error (mol of VFA/100 mol of total VFA), according to Bibby and Toutenburg (1977).

[4]Root mean squared prediction error (% of mean observed); ECT = error due to bias (% of MSPE); ER = error due to deviation of the regression slope from 1 (% of MSPE); ED = random error (% of MSPE).

[5]Concordance correlation coefficient, according to Lin (1989): Cb = bias correction factor, ρ = Pearson correlation coefficient, μ = location shift.

degradation in the rumen and VFA molar proportions, with increased Pr levels in the barley diet (Sutton et al., 1980). Such changes in VFA molar proportions will not be reflected in the Friggens et al. (1998) estimates, whereas the other stoichiometric models are based on rumen-degraded substrates and do predict alterations in molar proportions related to the higher starch degradation of the barley diet compared with the corn diet. Similarly, chemical and physical processing may affect the fermentation pattern without changing feed composition (e.g., Joy et al., 1997; Krause et al., 2002), which would also not be reflected in the estimates of Friggens et al. (1998).

The predictive performance of Bannink et al. (2008) did not show improvement compared with that of Bannink et al. (2006; Tables 8 and 9), despite both fitting stoichiometric coefficients from the same data set, with the former including a direct effect of pH and assuming

Table 9. Evaluation of the predictive performance of the VFA stoichiometric approach of Bannink et al. (2008) for the independent data set[1]

Item	Ac	Pr	Bu	Bc	NGR
Mean observed[2]	62.5	22.4	11.3	3.8	3.5
Mean predicted[2]	59.9	24.3	13.0	2.9	3.3
MSPE[3]	27.4	13.6	7.2	2.9	0.4
RMSPE[4]	8.4	16.5	23.7	44.1	18.1
ECT	25.0	26.2	37.7	29.2	9.8
ER	2.6	0.0	1.1	0.3	1.6
ED	72.4	73.8	61.2	70.6	88.6
CCC[5]	0.39	0.67	0.25	0.08	0.56
Cb	0.78	0.88	0.49	0.29	0.91
ρ	0.50	0.76	0.51	0.27	0.62
μ	0.62	−0.44	−1.07	1.36	0.31
R^2	0.25	0.57	0.26	0.07	0.38

[1]Ac = acetate, Pr = propionate, Bu = butyrate, Bc = valerate and branched-chain fatty acids, NGR = non-glucogenic to glucogenic VFA ratio, calculated as [Ac + 2 × Bu + Bc]/[Pr + Bc].

[2]Ac, Pr, Bu, and Bc in mol of VFA/100 mol of total VFA.

[3]Mean squared prediction error (mol of VFA/100 mol of total VFA), according to Bibby and Toutenburg (1977).

[4]Root mean squared prediction error (% of mean observed); ECT = error due to bias (% of MSPE); ER = error due to deviation of the regression slope from 1 (% of MSPE); ED = random error (% of MSPE).

[5]Concordance correlation coefficient, according to Lin (1989): Cb = bias correction factor, ρ = Pearson correlation coefficient, μ = location shift.
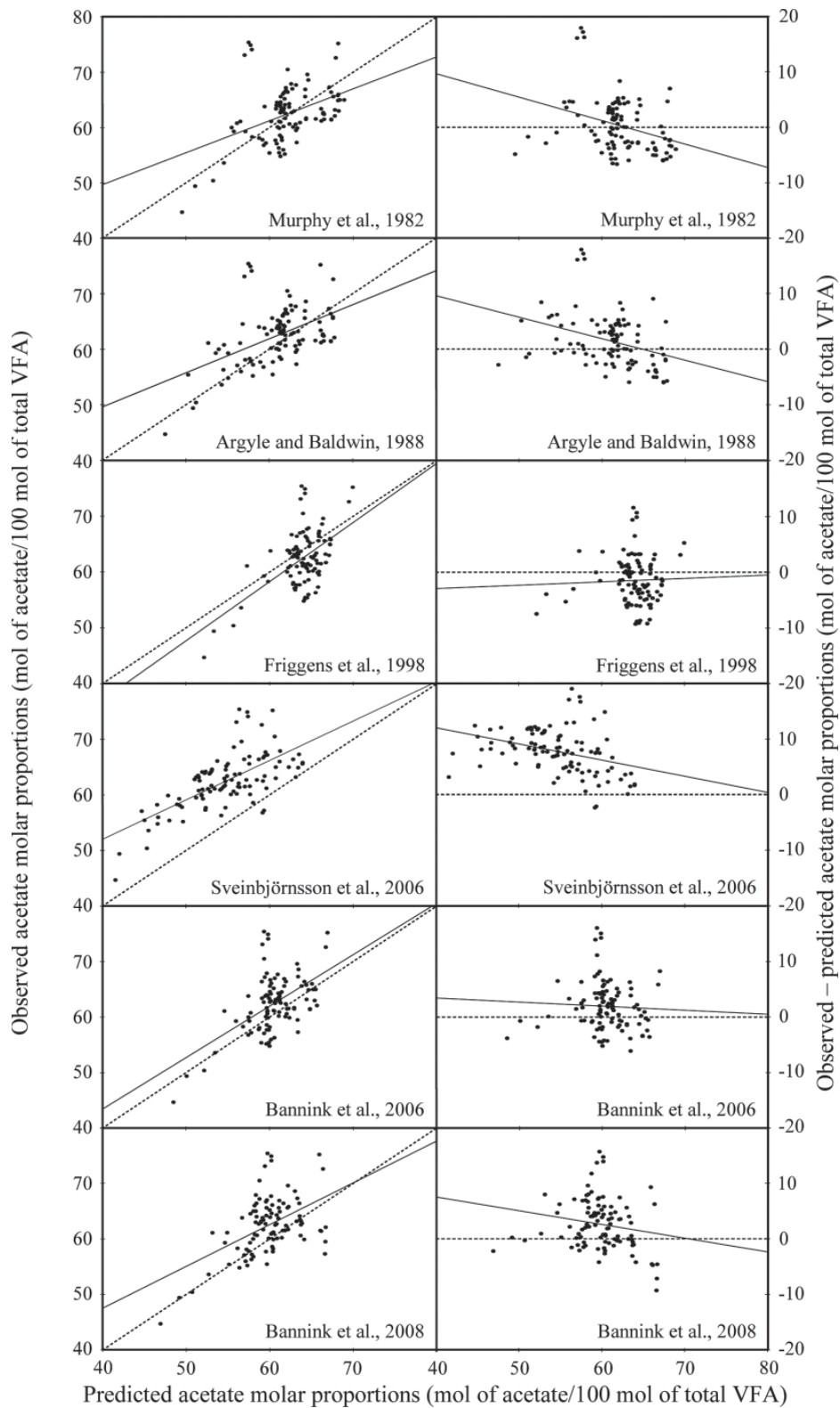
Figure 1. Plots of observed versus predicted (left) and residuals (observed minus predicted) versus predicted (right) acetate molar proportions (mol of acetate/100 mol of total VFA) according to the VFA stoichiometry models of Murphy et al. (1982), Argyle and Baldwin (1988), Friggens et al. (1998), Sveinbjörnsson et al. (2006), Bannink et al. (2006), and Bannink et al. (2008).
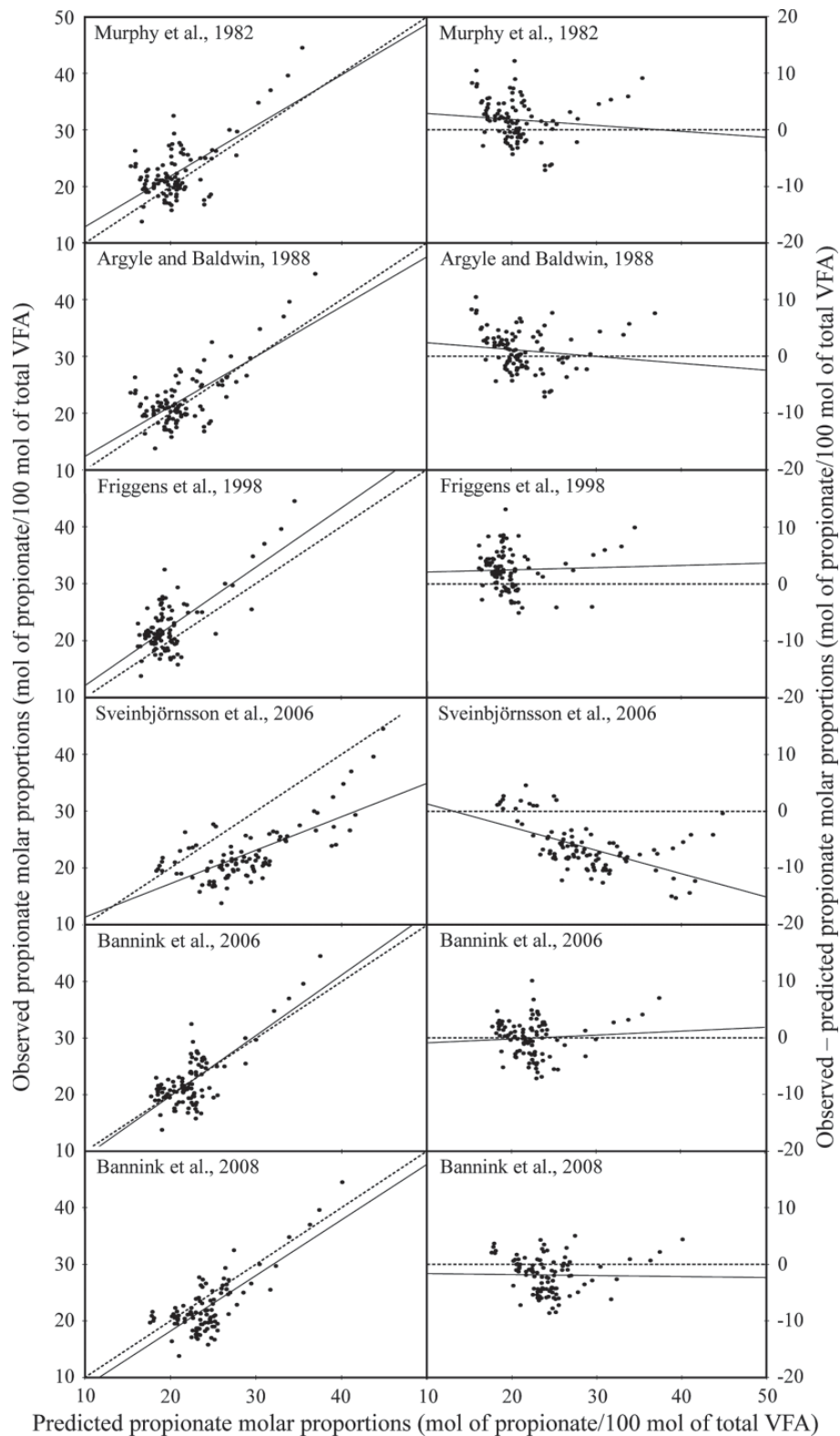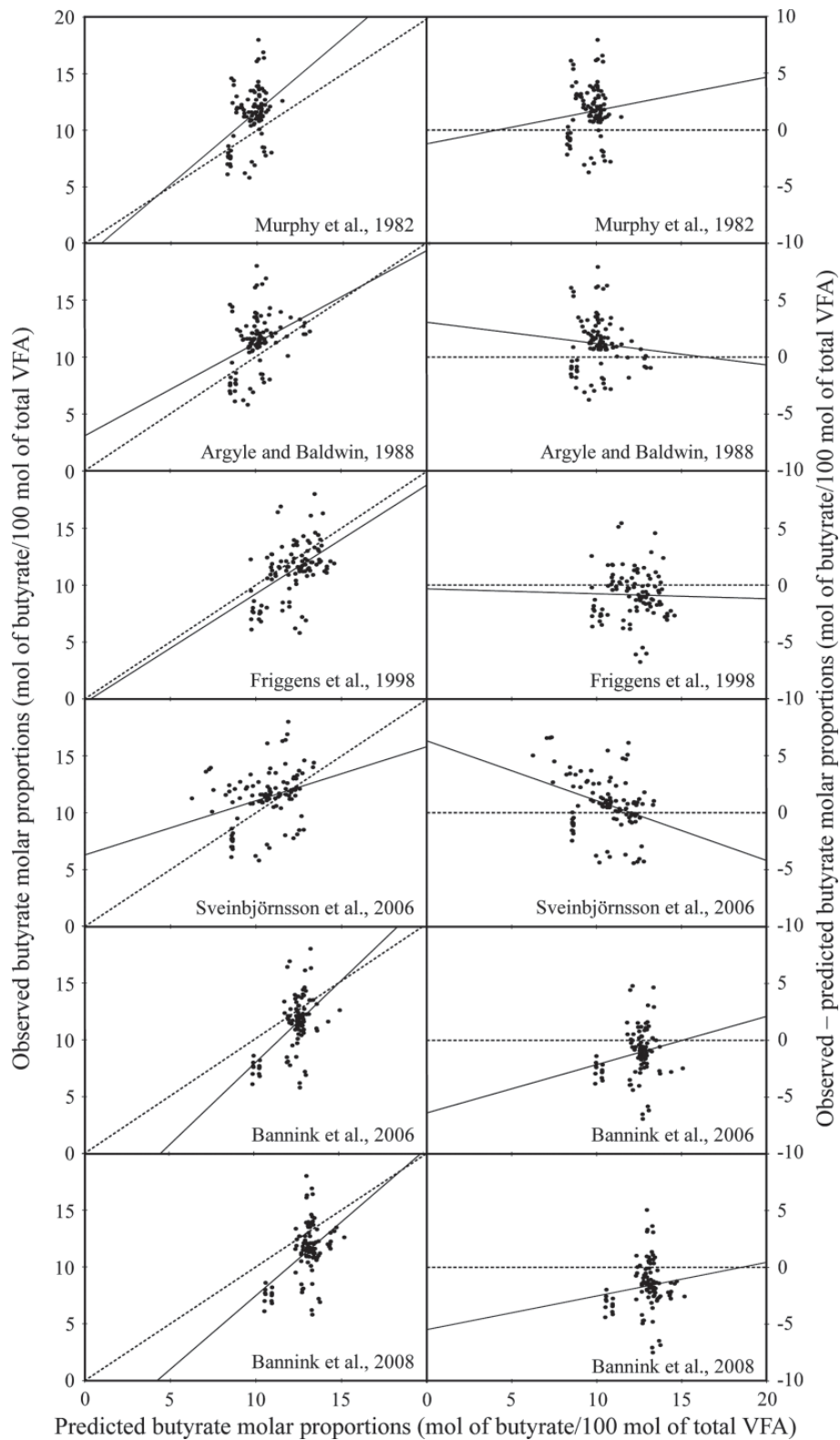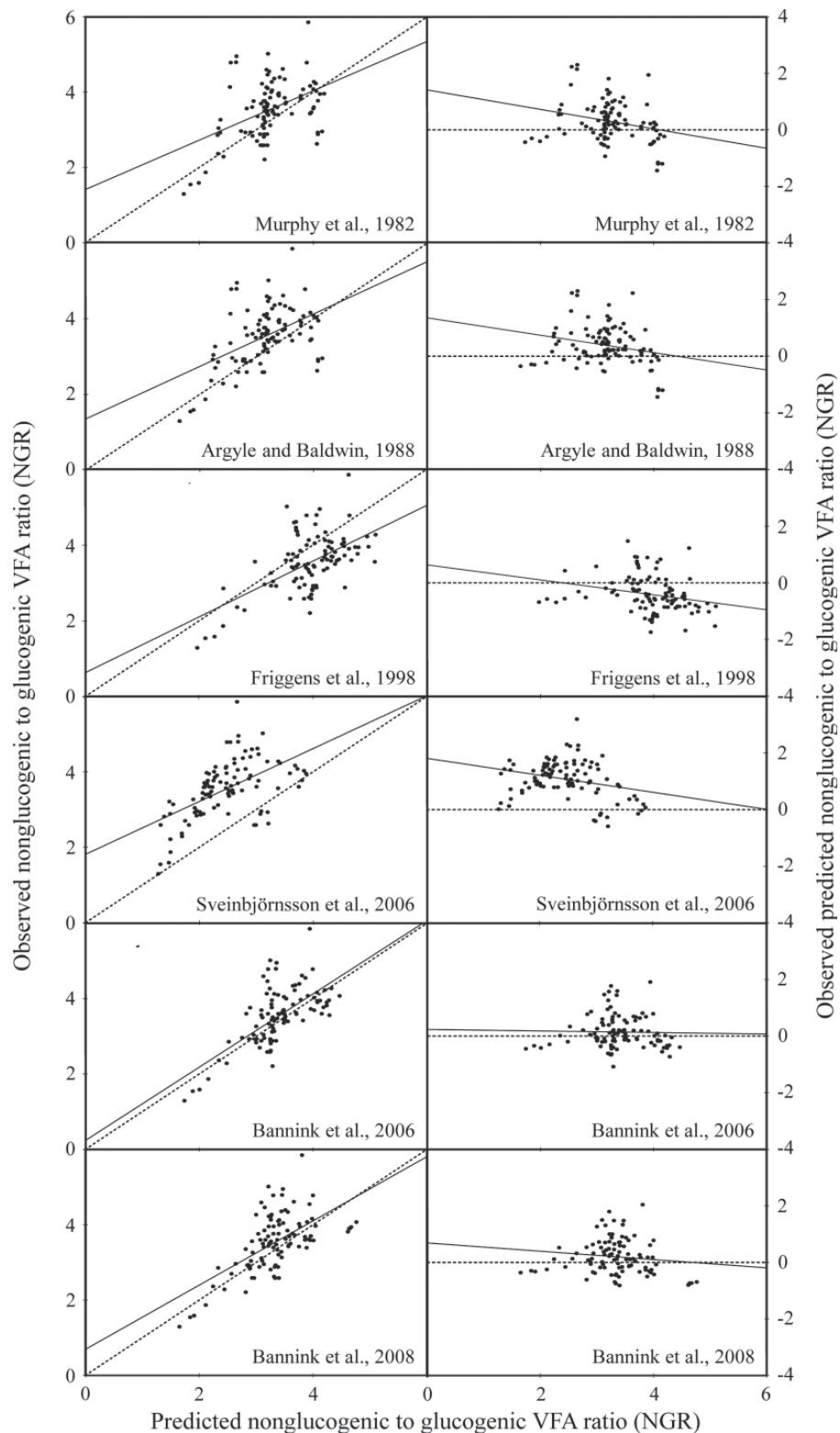
Figure 2. Plots of observed versus predicted (left) and residuals (observed minus predicted) versus predicted (right) propionate molar proportions (mol of propionate/100 mol of total VFA) according to the VFA stoichiometry models of Murphy et al. (1982), Argyle and Baldwin (1988), Friggens et al. (1998), Sveinbjörnsson et al. (2006), Bannink et al. (2006), and Bannink et al. (2008).

Figure 3. Plots of observed versus predicted (left) and residuals (observed minus predicted) versus predicted (right) butyrate molar proportions (mol of butyrate/100 mol of total VFA) according to the VFA stoichiometry models of Murphy et al. (1982), Argyle and Baldwin (1988), Friggens et al. (1998), Sveinbjörnsson et al. (2006), Bannink et al. (2006), and Bannink et al. (2008).

Figure 4. Plots of observed versus predicted (left) and residuals (observed minus predicted) versus predicted (right) nonglucogenic to glucogenic VFA ratio (NGR) according to the VFA stoichiometry models of Murphy et al. (1982), Friggens et al. (1998), Argyle and Baldwin (1988), Sveinbjörnsson et al. (2006), Bannink et al. (2006), and Bannink et al. (2008). NGR was calculated as $(Ac + 2 \times Bu + Bc)/(Pr + Bc)$, where Ac = acetate, Pr = proprionate, Bu = butyrate, and Bc = valerate and branched-chain fatty acids.

variable fractional VFA absorption rates. No relationships were found between residuals and various input parameters (e.g., pH, NDF, starch; results not shown) and thus could not provide an explanation for the lack of improved prediction. However, the variable fractional absorption rates assumed by Bannink et al. (2008) in coefficient derivation were not taken into account in the current study. Assuming variable absorption rates would increase the proportions of Ac and reduce Pr and Bu predicted by Bannink et al. (2008), improving its performance and reducing the discrepancy between the Bannink et al. (2006) and Bannink et al. (2008) predictions. Another reason for the lack of improvement despite the inclusion of pH effect could lie in the coefficient fitting process of Bannink et al. (2008). This process favored a more accurate prediction in the lower pH range, lower pH values being associated with an alteration in the rumen fermentation pattern. The current data set, with an average rumen pH of 6.2 (SD = 0.3), may not have been optimal for evaluation of the model of Bannink et al. (2008). To investigate this hypothesis, a subset of observations with lower rumen pH (pH ≤6.0, n = 22) was evaluated against model predictions. Predictive performance improved (results not shown), suggesting that the representation of VFA stoichiometry according to Bannink et al. (2008) has the potential to provide an improved prediction after a refinement of the coefficient fitting process and would be applicable in particular in situations in lactating Holstein cattle with a high intake of rapidly fermentable substrates. However, the amount of data available was rather limited, and a full evaluation requires more data. The findings are supported, however, by the improved predictive performance of Argyle and Baldwin (1988) compared with that of Murphy et al. (1982; Tables 4 and 5). Argyle and Baldwin (1988) included a pH effect while assuming fixed fractional VFA absorption rates, and thus the improved performance suggests that pH alone does explain an additional part of the variation in VFA profiles.

The NGR is related to the efficiency with which VFA are used for productive purposes, as it provides an indication of the partitioning of energy between milk and body mass (Ørskov et al., 1969; Van Knegsel et al., 2007). The models of Bannink et al. (2006) and Bannink et al. (2008) showed improved predictive performance of NGR compared with the other models, which resulted from alternating under- and overpredictions of Ac and Bu, and an accurate prediction of Pr (Tables 8 and 9). In contrast, for example, the model of Friggens et al. (1998) overpredicted Ac and Bu but underpredicted Pr, leading to an overpredicted NGR (Table 6). In the aggregation of nonglucogenic and glucogenic VFA, op-

posing prediction errors within each group are balanced out. Therefore, bearing in mind the common metabolic pathways within each group of VFA, an evaluation of NGR predictions provides a strong overall indication of VFA model performance. However, for the purpose of methane production estimation, an accurate prediction of separate VFA instead of NGR is required, because of distinct hydrogen production or uptake associated with each acid (Benchaar et al., 1998; Ellis et al., 2008).

To ensure a fully independent evaluation, data on substrate duodenal flows in experiments used in the development of the stoichiometry models were not used in the present study. All models apart from that of Friggens et al. (1998) required an input of digested feed fractions, which therefore had to be simulated using the rumen fermentation model of Dijkstra et al. (1992). This rumen fermentation model has been evaluated by Neal et al. (1992), Bannink et al. (1997b), and Mills et al. (2001) and found to predict N, NDF, starch, and sugar duodenal flows satisfactorily. Moreover, Benchaar et al. (1998) showed that the Dijkstra et al. (1992) model had the lowest prediction error for methane production of 4 extant models. None of the stoichiometric approaches evaluated in the present study included experiments or data that were used to develop the Dijkstra et al. (1992) model, and thus this model is independent from the stoichiometric approaches. A drawback of the Dijkstra et al. (1992) model is its limited representation of lipid flows. However, because long-chain fatty acids are not fermented in the rumen, this representation is unlikely to affect fermentable nutrient flows used in the current study as a basis for VFA prediction. The amount of hydrogen consumed in the biohydrogenation of unsaturated fatty acid is also small compared with other sinks (Mills et al., 2001) and unlikely to affect VFA molar profiles, unless diets are supplemented with significant quantities of fat sources rich in unsaturated fatty acids.

Aspects for Improvement

The VFA stoichiometric approaches evaluated in the present paper explained a considerable proportion of error in acetic acid molar proportion among diets, with the exception of Sveinbjörnsson et al. (2006). Several aspects of rumen fermentation not included in the models are likely to have contributed to the somewhat more pronounced error in predictions of Pr, Bu, and Bc found in the current evaluation. Volatile fatty acid molar proportions in the rumen represent a balance between production and disappearance, the latter occurring through absorption and passage. An assumption of all models except Bannink et al. (2008) is that

the fractional absorption rates of all VFA are identical in all diets and pH values, even though they have been shown to depend upon VFA concentrations and rumen pH (Dijkstra et al., 1993). Hanigan et al. (2002) showed that using absorption rates calculated according to Dijkstra et al. (1993) improved Bu and Ac predictions of Baldwin et al. (1987); however, significant bias and slope errors remained with total VFA and Pr predictions, respectively. Additionally, simulation results of Bannink et al. (2006) demonstrated large effects on VFA coefficient estimates when variable absorption rates were introduced into the model. A full evaluation of the VFA stoichiometry model of Bannink et al. (2008), which would necessitate information on fractional absorption rates, rumen fluid passage rate, and rumen fluid volume, would be required to determine whether such a detailed representation of VFA absorption improves the prediction of VFA profiles compared with other models.

Dijkstra (1994) recognized the need to maintain a low redox potential in the rumen through reduction and oxidation of pyridine nucleotides (NAD) as the driving force for rumen VFA production. Among other factors, substrate fractional degradation rates affect the redox balance and thus it was suggested that they be incorporated into VFA stoichiometry models. This is supported by the study of Tamminga et al. (1990), in which large variations among feed ingredients in fractional degradation rates of NDF, starch, and CP were found. Furthermore, Krause et al. (2003) and Sutton et al. (1980) reported a significant effect of starch source on the VFA profile. Fractional degradation rates are not directly implemented into any of the VFA models evaluated in the current study. The differentiation between mainly concentrate and mainly forage diets in the models of Murphy et al. (1982), Argyle and Baldwin (1988), Bannink et al. (2006), and Bannink et al. (2008) somewhat represents differences in fractional degradation rates because, for example, NDF breakdown is reduced at low rumen pH values, associated with mainly concentrate diets (Argyle and Baldwin, 1988). However, this distinction does not represent the variation within concentrate and roughage feed types, nor does it account for differences in feeding patterns. For example, Klusmeyer et al. (1990) observed a tendency to lower Ac:Pr ratio when dairy cattle were fed 4 versus 2 times daily. Bannink et al. (2008) took a further step by including rumen pH as an input parameter to their model. Nevertheless, these approaches contain a certain degree of inaccuracy because the variation in degradation rates cannot be fully explained by pH or type of diet. For example, relating fermentation to rumen pH neglects other factors affecting fermentation such as buffering from feed and saliva (Sudweeks, 1977;

Giger-Reverdin et al., 2002), and thus pH might be an inaccurate indicator of substrate degradation rate.

The effects of differences in fermentation pattern among microbial types on the whole-rumen fermentation profile are also not incorporated in any of the models evaluated in the present study. Particularly, an inclusion of fermentation by protozoa could be beneficial for improved VFA predictions. Protozoa are associated with a higher butyrate production rate than bacteria, and are known to have a buffering effect on the rumen, fermenting starch and sugars less rapidly than bacteria and thus preventing the sharp decrease in pH associated with bacterial fermentation (Williams and Coleman, 1997). Nagorcka et al. (2000) developed a VFA stoichiometry model using coefficients derived from the literature. The model differentiates between amylolytic bacteria, fibrolytic bacteria, and protozoa and assumes, for example, that the fermentation of 1 mol of soluble sugars and starch or hemicellulose results in 0.5 mol of Bu and no Pr. These stoichiometry coefficients are markedly different from those established by any of the models in the present study, and thus could affect the predicted rumen fermentation pattern to a large extent. The lack of protozoal representation in most VFA models is due, in part, to the limited in vivo data available on protozoal activity and VFA proportions compared with bacteria (Dijkstra et al., 2008a). Thus, further research in this domain is essential to be able to incorporate the in vivo contribution of protozoa into VFA models. Additionally, the need to distinguish the 3 microbial groups in such VFA stoichiometry models (Nagorcka et al., 2000) renders this approach less practical and such a model more difficult to evaluate with independent in vivo observations.

The stoichiometric models assume that all digested substrates are equally partitioned between microbial growth and VFA production. However, the proportions of rumen-digested substrate that are incorporated into microbial mass may differ between substrates. Microbial efficiency has been shown to vary considerably due to factors such as fractional growth rates and energy requirements for maintenance (Russell and Wallace, 1997; Dijkstra et al., 2007). Additionally, microbial efficiency is assumed to be dependent on fractional passage rate in most mechanistic rumen models (e.g., Baldwin et al., 1987; Dijkstra et al., 1992). For example, high fractional growth rates with sugars, compared with those with cellulose or hemicellulose, may result in a larger proportion of digested sugars being incorporated into microbial biomass rather than fermented to VFA compared with digested cellulose or hemicellulose. Nevertheless, Bannink et al. (2000) conducted simulations that showed that this assumption of equal partitioning only slightly affects their coefficient estimates, and

therefore its contribution to the error in estimated stoichiometry of VFA production and predicted VFA molar proportions might not be substantial.

The present analysis indicates that the majority of variation among diets in Ac molar proportion was explained by the models. The analysis demonstrates that we need to focus on improving our understanding of the type of VFA produced in diets that vary from the normal range. Despite the good performance of the dietary-level model of Friggens et al. (1998), these types of models are unable to respond to physical or chemical feed treatments or to variable degradation rates of specific nutrients, and thus mechanistic approaches are preferred. Adequate representation of additional rumen factors in VFA stoichiometry models may result in better predictive performance of Pr, Bu, and Bc, although the risk of over-complexity and unidentifiable parameters should not be overlooked.

CONCLUSIONS

The 6 VFA stoichiometry models evaluated varied considerably in their ability to predict rumen VFA molar proportions in lactating Holstein cows. With the exception of the model of Sveinbjörnsson et al. (2006), all models predicted the molar proportions of acetic acid well, whereas prediction accuracy of molar proportions of the other VFA was lower than that of acetic acid. The model of Bannink et al. (2006), and to a lesser extent the models of Friggens et al. (1998) and Bannink et al. (2008), showed an improved predictive performance over the models of Argyle and Baldwin (1988), Murphy et al. (1982), and Sveinbjörnsson et al. (2006). The move toward feed evaluation systems based on animal response might necessitate better representation of rumen fermentation than is provided by current VFA models, in particular that of propionic and butyric acid, focused on improving our understanding of VFA proportions in diets that vary from the mean.

REFERENCES

Abrahamse, P. A., J. Dijkstra, B. Vlaeminck, and S. Tamminga. 2008a. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. J. Dairy Sci. 91:2033–2045.

Abrahamse, P. A., S. Tamminga, and J. Dijkstra. 2009. Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. J. Agric. Sci. 147:721–730.

Abrahamse, P. A., B. Vlaeminck, S. Tamminga, and J. Dijkstra. 2008b. The effect of silage and concentrate type on intake behavior, rumen function, and milk production in dairy cows in early and late lactation. J. Dairy Sci. 91:4778–4792.

Alamouti, A. A., M. Alikhani, G. R. Ghorbani, and Q. Zebeli. 2009. Effects of inclusion of neutral detergent soluble fibre sources in diets varying in forage particle size on feed intake, digestive pro-

cesses, and performance of mid-lactation Holstein cows. Anim. Feed Sci. Technol. 154:9–23.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624.

Argyle, J. L., and R. L. Baldwin. 1988. Modeling of the rumen water kinetics and effects on rumen pH changes. J. Dairy Sci. 71:1178–1188.

Baldwin, R. L. 1995. Modeling Ruminant Digestion and Metabolism. Chapman and Hall, London, UK.

Baldwin, R. L., J. H. M. Thornley, and D. E. Beever. 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. J. Dairy Res. 54:107–131.

Bannink, A., H. De Visser, A. Klop, J. Dijkstra, and J. France. 1997a. Causes of inaccurate prediction of volatile fatty acids by simulation models of rumen function in lactating cows. J. Theor. Biol. 189:353–366.

Bannink, A., H. De Visser, and A. M. Van Vuuren. 1997b. Comparison and evaluation of mechanistic rumen models. Br. J. Nutr. 78:563–581.

Bannink, A., J. France, S. López, W. J. J. Gerrits, E. Kebreab, S. Tamminga, and J. Dijkstra. 2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Anim. Feed Sci. Technol. 143:3–26.

Bannink, A., J. Kogut, J. Dijkstra, J. France, E. Kebreab, A. M. Van Vuuren, and S. Tamminga. 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. J. Theor. Biol. 238:36–51.

Bannink, A., J. Kogut, J. Dijkstra, J. France, S. Tamminga, and A. M. Van Vuuren. 2000. Modelling production and portal appearance of volatile fatty acids in dairy cows. Pages 87–102 in Modelling Nutrient Utilization in Farm Animals. J. P. McNamara, J. France, and D. E. Beever, ed. CAB International, Wallingford, UK.

Benchaar, C., H. V. Petit, R. Berthiaume, D. R. Ouellet, J. Chiquette, and P. Y. Chouinard. 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. J. Dairy Sci. 90:886–897.

Benchaar, C., J. Rivest, C. Pomar, and J. Chiquette. 1998. Prediction of methane production from dairy cows using existing mechanistic models and regression equations. J. Anim. Sci. 76:617–627.

Benefield, B. C., M. Liñeiro, I. R. Ipharraguerre, and J. H. Clark. 2006. NutriDense corn grain and corn silage for dairy cows. J. Dairy Sci. 89:1571–1579.

Bergman, E. N. 1990. Energy contribution of VFA from the gastrointestinal tract in various species. Physiol. Rev. 70:567–590.

Bibby, J., and H. Toutenburg. 1977. Prediction and improved estimation in linear models. John Wiley and Sons, London, UK.

Boeckaert, C., B. Vlaeminck, J. Dijkstra, A. Issa-Zacharia, T. Van Nespen, W. Van Straalen, and V. Fievez. 2008. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. J. Dairy Sci. 91:4714–4727.

Broderick, G. A., D. R. Mertens, and R. Simons. 2002. Efficacy of carbohydrate sources for milk production by cows fed diets based on alfalfa silage. J. Dairy Sci. 85:1767–1776.

CVB. 2007. Tabellenboek Veevoeding 2000 (Dutch Feeding Tables). Voedernormen landbouwhuisdieren en voederwaarde veevoeders (in Dutch). Central Bureau for Livestock Feeding, Lelystad, the Netherlands.

Dann, H. M., R. J. Grant, K. W. Cotanch, E. D. Thomas, C. S. Ballard, and R. Rice. 2008. Comparison of brown midrib sorghum-sudangrass with corn silage on lactational performance and nutrient digestibility in Holstein dairy cows. J. Dairy Sci. 91:663–672.

DeFrain, J. M., A. R. Hippen, K. F. Kalscheur, and D. J. Schingoethe. 2004. Feeding lactose increases ruminal butyrate and plasma β-hydroxybutyrate in lactating dairy cows. J. Dairy Sci. 87:2486–2494.

Dijkstra, J. 1994. Production and absorption of volatile fatty acids in the rumen. Livest. Prod. Sci. 39:61–69.

Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining, and S. Tamminga. 1993. Absorption of volatile fatty acids from the rumen of lactat-

ing dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. Br. J. Nutr. 69:385–396.

Dijkstra, J., E. Kebreab, A. Bannink, L. A. Crompton, S. Lopez, P. A. Abrahamse, P. Chilibroste, J. A. N. Mills, and J. France. 2008b. Comparison of energy evaluation systems and a mechanistic model for milk production by dairy cattle offered fresh grass-based diets. Anim. Feed Sci. Technol. 143:203–219.

Dijkstra, J., E. Kebreab, J. France, and A. Bannink. 2008a. Modelling protozoal metabolism and volatile fatty acid production in the rumen. Pages 170–188 in Mathematical Modelling in Animal Nutrition. J. France and E. Kebreab, ed. CAB International, Wallingford, UK.

Dijkstra, J., E. Kebreab, J. A. N. Mills, W. F. Pellikaan, S. López, A. Bannink, and J. France. 2007. Predicting the profile of nutrients available for absorption: From nutrient requirement to animal response and environmental impact. Animal 1:99–111.

Dijkstra, J., H. D. St. C. Neal, D. E. Beever, and J. France. 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: Model description. J. Nutr. 122:2239–2256.

Ellis, J. L., J. Dijkstra, E. Kebreab, A. Bannink, N. E. Odongo, B. W. McBride, and J. France. 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agric. Sci. 146:213–233.

Friggens, N. C., J. D. Oldham, R. J. Dewhurst, and G. Horgan. 1998. Proportions of volatile fatty acids in relation to the chemical composition of feeds based on grass silage. J. Dairy Sci. 81:1331–1344.

Gehman, A. M., P. J. Kononoff, C. R. Mullins, and B. N. Janicek. 2008. Evaluation of nitrogen utilization and the effects of monensin in dairy cows fed brown midrib corn silage. J. Dairy Sci. 91:288–300.

Gencoglu, H., and I. I. Turkmen. 2006. Effects of forage source on chewing and rumen fermentation in lactating dairy cows. Rev. Med. Vet. 157:463–470.

Giger-Reverdin, S., C. Duvaux-Ponter, D. Sauvant, O. Martin, I. N. Prado, and R. Müller. 2002. Intrinsic buffering capacity of feedstuffs. Anim. Feed Sci. Technol. 96:83–102.

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.

Hanigan, M. D., D. C. Weakley, F. E. Standaert, and L. R. Reutzel. 2002. Evaluation and refinement of ruminal volatile fatty acid absorption equations in a dynamic, metabolic model of the lactating dairy cow. J. Dairy Sci. 85(Suppl. 1):402. (Abstr.)

Hara, S., and T. Tanigawa. 2010. Effects of length of cut and mechanical processing on utilization of corn silage harvested at the black line stage of maturity by lactating dairy cows. Anim. Sci. J. 81:187–193.

Hippen, A. R., D. J. Schingoethe, K. F. Kalscheur, P. L. Linke, D. R. Rennich, M. M. Abdelqader, and I. Yoon. 2010. *Saccharomyces cerevisiae* fermentation product in dairy cow diets containing dried distillers grains plus solubles. J. Dairy Sci. 93:2661–2669.

Iqbal, S., Q. Zebeli, A. Mazzolari, G. Bertoni, S. M. Dunn, W. Z. Yang, and B. N. Ametaj. 2009. Feeding barley grain steeped in lactic acid modulates rumen fermentation patterns and increases milk fat content in dairy cows. J. Dairy Sci. 92:6023–6032.

Joy, M. T., E. J. DePeters, J. G. Fadel, and R. A. Zinn. 1997. Effects of corn processing on the site and extent of digestion in lactating cows. J. Dairy Sci. 80:2087–2097.

Kelzer, J. M., P. J. Kononoff, A. M. Gehman, L. O. Tedeschi, K. Karges, and M. L. Gibson. 2009. Effects of feeding three types of corn-milling coproducts on milk production and ruminal fermentation of lactating Holstein cattle. J. Dairy Sci. 92:5120–5132.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. J. Dairy Sci. 92:1712–1724.

Klusmeyer, T. H., M. R. Cameron, G. C. McCoy, and J. H. Clark. 1990. Effects of feed processing and frequency of feeding on ruminal fermentation, milk production, and milk composition. J. Dairy Sci. 73:3538–3543.

Koong, L. J., R. L. Baldwin, M. J. Ulyatt, and T. J. Charlesworth. 1975. Iterative computation of metabolic flux and stoichiometric parameters for alternate pathways in rumen fermentation. Comput. Programs Biomed. 4:209–213.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. J. Dairy Sci. 85:1947–1957.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2003. Effects of increasing levels of refined cornstarch in the diet of lactating dairy cows on performance and ruminal pH. J. Dairy Sci. 86:1341–1353.

Krizsan, S. J., G. A. Broderick, R. E. Muck, C. Promkot, S. Colombini, and Å. T. Randby. 2007. Effect of alfalfa silage storage structure and roasting corn on production and ruminal metabolism of lactating dairy cows. J. Dairy Sci. 90:4793–4804.

Lin, L. I. K. 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics 45:255–268.

Mahjoubi, E., H. Amanlou, D. Zahmatkesh, M. Ghelich Khan, and N. Aghaziarati. 2009. Use of beet pulp as a replacement for barley grain to manage body condition score in over-conditioned late lactation cows. Anim. Feed Sci. Technol. 153:60–67.

McLeod, K. R., and R. L. Baldwin VI.. 2000. Effects of diet forage:concentrate ratio and metabolizable energy intake on visceral organ growth and in vitro oxidative capacity of gut tissues in sheep. J. Anim. Sci. 78:760–770.

Mentschel, J., R. Leiser, C. Mulling, C. Pfarrer, and R. Claus. 2001. Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis. Arch. Tierenahr. 55:85–102.

Mills, J. A. N., J. Dijkstra, A. Bannink, S. B. Cammell, E. Kebreab, and J. France. 2001. A mechanistic model of whole tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation and application. J. Anim. Sci. 79:1584–1597.

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.

Nagorcka, B. N., G. L. R. Cordon, and R. A. Dynes. 2000. Towards a more accurate representation of fermentation in mathematical models of the rumen. Pages 37–48 in Modelling Nutrient Utilization in Farm Animals. J. P. McNamara, J. France, and D. E. Beever, ed. CAB International, Wallingford, UK.

Neal, H. D. St. C., J. Dijkstra, and M. Gill. 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: Model evaluation. J. Nutr. 122:2257–2272.

Oba, M., and M. S. Allen. 2003a. Dose-response effects of intrauminal infusion of propionate on feeding behavior of lactating cows in early or midlactation. J. Dairy Sci. 86:2922–2931.

Oba, M., and M. S. Allen. 2003b. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174–183.

Ørskov, E. R., W. P. Flatt, P. W. Moe, A. W. Munson, R. W. Hemken, and I. Katz. 1969. The influence of ruminal infusion of volatile fatty acids on milk yield and composition and on energy utilization by lactating cows. Br. J. Nutr. 23:443–453.

Piñeiro, G., S. Perelman, J. P. Guerschman, and J. M. Paruelo. 2008. How to evaluate models: Observed vs. predicted or predicted vs. observed? Ecol. Modell. 216:316–322.

Plaizier, J. C. 2004. Replacing chopped alfalfa hay with alfalfa silage in barley grain and alfalfa-based total mixed rations for lactating dairy cows. J. Dairy Sci. 87:2495–2505.

Rigout, S., C. Hurtaud, S. Lemosquet, A. Bach, and H. Rulquin. 2003. Lactational effect of propionic acid and duodenal glucose in cows. J. Dairy Sci. 86:243–253.

Russell, J. B., and R. J. Wallace. 1997. Energy yielding and energy consuming reactions. Pages 246–282 in The Rumen Microbial Ecosystem. P. N. Hobson and C. S. Stewart, ed. Blackie Academic and Professional, London, UK.

Seymour, W. M., D. R. Campbell, and Z. B. Johnson. 2005. Relationships between rumen volatile fatty acid concentrations and milk production in dairy cows: A literature study. Anim. Feed Sci. Technol. 119:155–169.

St-Pierre, N. R. 2003. Reassessment of biases in predicted nitrogen flows to the duodenum by NRC 2001. J. Dairy Sci. 86:344–350.

Sudweeks, E. M. 1977. Chewing time, rumen fermentation and their relationship in steers as affected by diet composition. J. Anim. Sci. 44:694–701.

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.

Sutton, J. D., J. D. Oldham, and I. C. Hart. 1980. Products of digestion, hormones and energy utilization in milking cows given concentrates containing varying proportions of barley or maize. Pages 303–306 in Energy Metabolism. L. E. Mount, ed. Butterworths, London, UK.

Sveinbjörnsson, J., P. Huhtanen, and P. Udén. 2006. The Nordic dairy cow model, Karoline — Development of volatile fatty acid submodel. Pages 1–14 in Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches. E. Kebreab, J. Dijkstra, A. Bannink, W. J. J. Gerrits, and J. France, ed. CAB International, Wallingford, UK.

Tamminga, S., A. M. Van Vuuren, C. J. Van Der Koelen, R. S. Ketelaar, and P. L. Van Der Togt. 1990. Ruminal behavior of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredients in dairy cows. Neth. J. Agric. Sci. 38:513–526.

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2005. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. Anim. Feed Sci. Technol. 121:243–256.

Thomas, P. C., and P. A. Martin. 1988. The influence of nutrient balance on milk yield and composition. Pages 97–118 in Nutrition and Lactation in the Dairy Cow. P. C. Garnsworthy, ed. Butterworths, London, UK.

van Knegsel, A. T. M., H. Van Den Brand, J. Dijkstra, W. M. Van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. J. Dairy Sci. 90:3397–3409.

Williams, A. G., and G. S. Coleman. 1997. The rumen protozoa. Pages 73–139 in The Rumen Microbial Ecosystem. P. N. Hobson and C. S. Stewart, ed. Blackie Academic and Professional, London, UK.