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Evaluation of Models to Predict the Stoichiometry of Volatile Fatty Acid Profiles in Rumen Fluid of Dairy Cattle

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To dad, who would have loved this

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Interpretive summary

Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of dairy cattle. Morvay et al. An accurate prediction of the proportions between individual volatile fatty acids (VFA) in the rumen, the main energy source of ruminants, is of interest because they are associated with methane production and milk composition. The aim of this study was to evaluate six rumen VFA models for their ability to predict in vivo VFA molar proportions. The results indicate that different models vary in predictive performance. Nevertheless, the move towards feed evaluation systems based on animal response might require an improved representation of rumen fermentation than is provided by current VFA stoichiometry models.

Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of dairy cattle

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ABSTRACT

Volatile fatty acids (VFA), produced in the rumen by microbial fermentation, are the main energy source for ruminants. The proportions of individual ruminal VFA are of particular interest because the VFA profile, particularly the ratio between nonglucogenic (acetate, Ac; butyrate, Bu) to glucogenic (propionate, Pr) VFA (NGR), is associated with effects on methane production, milk composition and energy balance. In the last few decades, several models have been developed to predict rumen fermentation stoichiometry. The aim of the current study was to evaluate rumen VFA stoichiometry models for their ability to predict in vivo VFA molar proportions. The models were evaluated using an independent dataset consisting of 101 treatments from 24 peer-reviewed publications with lactating dairy cows. All publications contained a full diet description, rumen pH and rumen VFA molar proportions. Ruminal digestibility was estimated using the rumen fermentation model of Dijkstra et al. (1992). Stoichiometric models were evaluated based on mean squared prediction error (MSPE) and concordance correlation coefficient (CCC) analysis. Of all models evaluated, Friggens et al. (1998) had the lowest root MSPE for Ac and Bu (7.2 and 20.2% of observed mean, respectively). Bannink et al. (2006) had the lowest RMSPE and highest CCC for Pr (14.4% and 0.70, respectively). The predictions of Bannink et al. (2008) had comparable predictive performance for Pr to that of Bannink et al. (2006) but a larger error due to overall bias (26.2% of MSPE). Murphy et al. (1982) provided the poorest prediction of Bu, with the highest RMSPE and lowest CCC (24.6% and 0.15, respectively). Argyle and Baldwin (1988) had the highest CCC for Ac with an intermediate RMSPE (0.47 and 8.0%, respectively). Sveinbjörnsson et al. (2006) had the highest RMSPE (13.9 and 34.0%, respectively) and lowest CCC (0.31 and 0.40, respectively) for Ac and Pr. NGR predictions had the lowest RMSPE and highest CCC in the models of Bannink et al. (2006) and Bannink et al. (2008), whereas the lowest predictive performance was by Sveinbjörnsson et al. (2006). It appears that type of VFA produced is not a simple linear relationship of substrate inputs and pH as currently represented. The move towards feed evaluation systems based on animal response might require an improved representation of rumen fermentation than is provided by current VFA models if VFA production patterns are to be predicted with sufficient degree of accuracy.

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 of 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 undergo separate metabolic pathways. 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. Consequently, the VFA profile has been associated with animal energy balance in early lactation (Van Knegsel et al., 2007), milk yield and composition (Seymour et al., 2005), and methane production (Ellis et al., 2008). Current energy evaluation systems for cattle are based on metabolizable energy (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).

In the last few decades, a number of models predicting rumen fermentation stoichiometry have been developed, as reviewed by Dijkstra et al. (2008). 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 correctly predict rumen VFA proportions, the prediction error of the models remains considerably high 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 correctly predict VFA molar proportions, and concluded that the inappropriate representations of VFA coefficients is among the most probable causes. The aim of the current study was to evaluate rumen VFA stoichiometry models using an independent dataset of dairy cattle digestion trials. The models 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

Volatile Fatty Acid Stoichiometry Models

Six VFA prediction models were evaluated using independent data. Murphy et al. (1982) developed a set of stoichiometric coefficients by fitting 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 dataset of 108 diets in mainly beef

cattle and sheep trials. Separate stoichiometry coefficients were generated for mainly-concentrates 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% concentrates).

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 digested substrate and rumen pH values below 6.2 were assumed, based on in vitro data. These coefficients were used in the whole-rumen model of Baldwin (1995).

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 fermented 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. 107 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**), concentrates NDF (**cNDF**), lactate and "rest" fraction (DM – ash – NDF – starch – CP – lactate – VFA). Dry matter intake relative to body weight and concentrates ether extract were used as input in addition to digested feed fractions.

Bannink et al. (2006) employed a similar approach to Murphy et al. (1982), but used 182 treatments from 47 digestion trials with data of lactating cows only to fit the stoichiometric parameters, in contrast to the study of Murphy et al. (1982) which made us of mainly 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 distinction of mainly-concentrates and mainly-roughage diets, intermediate diets (40 to 60% roughages) have been recognized by whole-rumen models (e.g. Dijkstra et al., 1992) using coefficient means, similarly to Baldwin et al. (1987).

Bannink et al. (2008) fit VFA stoichiometry coefficients from the same dataset 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, but these require information on rumen fluid volume and passage rate and thus 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-concentrates, intermediate and mainly-roughage diets.

Dataset

A dataset consisting of 101 treatments from 24 peer-reviewed publications was collected for model evaluation (Abrahamse et al., 2008a, b, 2009; Alamouti et al., 2009; Benchaar et al., 2007; Benefield et al., 2006; Boeckaert et al., 2008; Broderick et al., 2002; Dann et al., 2008; DeFrain et al., 2004; Gehman et al., 2008; Gencoglu and Turkmen, 2006; Hara and Tanigawa, 2010; Hippen et al., 2010; Iqbal et al., 2009; Kelzer et al., 2009; Khafipour et al., 2009; Krizsan et al., 2007; Mahjoubi et al., 2009; Oba and Allen, 2003a, b; Plaizier, 2004; Rigout et al., 2003; Taweel et al., 2005). To guarantee an independent evaluation, only treatments which were not used during the formation of any of the models were included. Only trials using lactating Holstein dairy cattle were used, to eliminate differences caused by genetic variation. Treatments which 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 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 feed tables. 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 branched-chain fatty acids (**Bc**). Lastly, to integrate the four individual VFA into one characteristic, the non-glucogenic to glucogenic VFA ratio (**NGR**) was calculated as: [Ac + 2×Bu + Bc] / [Pr + Bc].

The model of Sveinbjörnsson et al. (2006) requires cNDF, fNDF and lactate as input parameters. Lactate content was determined using feed table values (CVB, 2007) and considered to be entirely digested in the rumen, as is assumed by Sveinbjörnsson et al. (2006). When experiments did not report cNDF and fNDF, these were calculated from NDF content of the separate feed ingredients according to feed tables. Digested fractions of fNDF and cNDF were estimated using table degradation kinetics data of the separate feedstuffs. The Argyle and Baldwin (1988) model estimates the production 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), of 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 feeding tables (CVB, 2007), which are based on in situ rumen digestion trials. Predicted rumen digested feed fractions were then used as input for the models 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 ones predicted by the models.

Observed rumen VFA originate either in VFA entering the rumen or as result of substrate fermentation. In experiments where VFA were reported in the diet or injected intra-ruminally, observed amounts entering the rumen were added to predicted VFA produced from substrate degradation. Predicted VFA molar proportions were then re-calculated 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 % of the observed mean), which was calculated according to Bibby and Toutenburg (1977). The RMSPE comprises of three sources of error, expressed as % 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 of two terms, viz. Cb and ρ. The Cb is a bias correction factor and provides a measure of accuracy, i.e. 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's correlation coefficient which provides a measure of precision. The term 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 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 (St-Pierre, 2003).

RESULTS

The collected dataset included a wide range of diet chemical composition and feed intake (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. The 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 of all models are presented in Tables 4 to 9 and visualized in Figures 1 to 4. Predictive performance varied with type of VFA. RMSPE and CCC values were in agreement in most models, i.e. models with lower RMSPE had higher CCC and vice-versa, with the exception of the model of Argyle & Baldwin (1988). RMSPE tended to increase in the order Ac < Pr < Bu < Bc. In general, Ac tended to be underpredicted, 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, 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 model provided the poorest prediction of Bu of compared to 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 accuracy value for Ac was the highest of all models (0.95, Fig. 1), whereas Pr and Bu tended to be underpredicted (Fig. 2 and 3, respectively).

The Baldwin and Argyle (1988) model had intermediate RMSPE for Ac, Pr and Bu (8.0, 16.2 and 22.7%, respectively; Table 5). CCC were variable, with high value 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 (Fig. 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 to the other models, with the lowest RMSPE and highest CCC (20.2% and 0.37, respectively; Table 6). Ac 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 (Fig. 2).

The model of Sveinbjörnsson et al. (2006) 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 (Fig. 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, there was a tendency to overpredict Bu (Fig. 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 of Bannink et al. (2006) for Pr, with comparable low RMSPE and high CCC values (Table 9). However, a large error due to bias was observed for Ac, Pr and Bu (25.0, 26.2 and 37.7% of MSPE). RMSPE and CCC for Ac an Bu were intermediate. Accuracy terms for Ac and Pr were relatively high (0.78 and 0.88, respectively), whereas Bu tended to be overpredicted (Fig. 3).

Predictive performance of NGR varied between the models (Fig. 4). The model of Bannink et al. (2006) showed rather 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 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

Volatile Fatty Acid Stoichiometry Model Predictions

Predictive performance of six models of VFA rumen stoichiometry were compared using a dataset of lactating dairy cattle from recent publications. Although a large fraction of observed variation remained unexplained, the results of the present study showed an improved performance compared to previous evaluations of VFA stoichiometry models (e.g. Bannink et al., 1997b; Neal et al., 1992). 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, similarly to the dataset that was used to evaluate the models in the current study. This is likely to have largely influenced the observed relatively good performance of these models. The Murphy et al. (1982) and Sveinbjörnsson et al. (2006) models, which showed a relatively reduced performance compared to the other models, used large datasets 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 the 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 based on few mainly in vitro studies, but their modification of sugars and starch fermentation coefficients to pH-dependent ones resulted in improved predictions compared to those of Murphy et al. (1982).

The relatively good predictive performance of the empirical model of Friggens et al. (1998) is in spite of both inter-species difference and the attribution of VFA molar

proportions solely to feed composition, without considering animal and rumen environment factors (e.g. DMI, digestibility). On theoretical grounds, ruminal substrate degradation has to be expected to explain 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 and effects of ingredient-specific degradation characteristics of these nutrients. For example, exchanging barley and maize in high-concentrate diets hardly changed dietary chemical composition, but did significantly affect 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, whilst other stoichiometric models based on rumen degraded substrates do predict alterations in molar proportions related to the higher starch degradation of the barley diet compared with the maize 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 to that of Bannink et al. (2006; Tables 8 and 9), despite both fitting stoichiometric coefficients from the same dataset, with the former including a direct effect of pH and assuming 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 predictions. 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 within the coefficient fitting process of Bannink et al. (2008). This process favored a more accurate prediction in the lower pH range, which are associated with an alteration of the rumen fermentation pattern. The current dataset, with an average rumen pH of 6.2 (SD = 0.3), thus 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 $\leq$ 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 with a high intake of rapidly fermentable substrates in dairy cattle. This is supported by the improved predictive performance of Argyle and Baldwin (1988) compared to 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 nonglucogenic to glucogenic VFA ratio is related to the efficiency with which VFA are used for productive purposes, because it provides an indication of the partitioning of energy between milk and body mass (Van Knegsel et al., 2007). The models of Bannink et al. (2006) and Bannink et al. (2008) showed improved predictive performance of NGR compared to 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 overpedicted NGR (Table 6). In the aggregation of nonglucogenic and glucogenic VFA, opposing 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 the distinct hydrogen production or uptake associated with each acid.

To ensure a fully independent evaluation, data on substrate duodenal flows in experiments used in the development of the stoichiometry models could not be used in the present study. All models apart from 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 satisfactorily predict N, NDF, starch and sugars duodenal flows. Moreover, Benchaar et al. (1998) showed that the Dijkstra et al. (1992) model had the lowest prediction error of methane production of four extant models. A drawback of the 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.

Aspects to be Improved

Several aspects of rumen fermentation not included in the models are likely to have contributed to the error in VFA predictions 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 the fractional absorption rates of all VFA being 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, large 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 in order to determine whether such a detailed representation of VFA absorption improves the prediction of VFA profiles compared to 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 rate affects the redox balance and thus were suggested to 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 degradation rates, because e.g. NDF breakdown is reduced in low pH values, associated with mainly-concentrates diets (Argyle and Baldwin, 1988). However, this distinction does not represent the variation within concentrate and roughage feed types. Bannink et al. (2008) took a step further 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, 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 is also not incorporated into 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 drop in pH associated with bacterial fermentation (Williams and Coleman, 1997). Nagorcka et al. (2000) developed a VFA stoichiometry model using coefficients derived from 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 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 in part due to the limited in vivo data available on protozoal activity and VFA proportions compared to bacteria (Dijkstra et al., 2008). Thus, further research in this domain would be essential in order to be able to incorporate the in vivo contribution of protozoa into VFA models. Additionally, the need to distinguish the three microbial groups in such VFA stoichiometry models (Nagorcka et al., 2000) renders this approach less practical and such a model more difficult to be evaluated with independent in vivo observations.

The models assume that all fermented substrates are equally partitioned between microbial growth and VFA production. However, 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 the fractional passage rate in most mechanistic rumen models (e.g. Baldwin et al., 1987; Dijkstra et al., 1992). Nevertheless, Bannink et al. (2000) conducted simulations that showed that this assumption only slightly affects their coefficient estimates, and therefore the error in estimated stoichiometry of VFA production and predicted VFA molar proportions might not be substantial.

The inadequate prediction of rumen VFA production rates remains a weakness of current whole-rumen models, which aim to predict nutrient absorption and duodenal flows. Despite the unexpected, good performance of the dietary-level model of Friggens et al.

(1998), these type of models are not able to respond to physical or chemical feed treatments or to variable degradation rates of specific nutrients, and thus mechanistic approaches are to be preferred. Adequate representations of additional rumen factors in VFA stoichiometry models may result in better predictive performance, although the risk of over complexity and unidentifiable parameters should not be overlooked.

CONCLUSIONS

The six evaluated VFA stoichiometry models varied considerably in their ability to predict rumen VFA molar proportions in lactating cows. The model of Bannink et al. (2006) and to a lesser extent the models of Friggens et al (1998) and Bannink et al. (2008) and showed an improved predictive performance over the models of Argyle and Baldwin (1988), Murphy et al. (1982) and Sveinbjörnsson et al. (2006). The move towards feed evaluation systems based on animal response might require an improved representation of rumen fermentation than is provided by current VFA models if VFA production patterns are to be predicted with a sufficient degree of accuracy.

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

	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
Chemical composition (g/ kg 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
cEE	19	18	12	0	57
cNDF	85	72	36	16	171
fNDF	259	249	59	165	399
Observed molar proportions (mol VFA/ 100 mol total VFA)					
Ac	62.5	62.7	5.2	44.7	75.4
Pr	22.4	21.1	4.9	13.8	44.5
Bu	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]cEE = concentrates ether extract, cNDF = concentrates neutral detergent fiber, fNDF = roughage neutral detergent fiber, 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 standard deviation (SD), minimum and maximum of feed fraction[1] digestion rates in the rumen (kg/ d) as estimated by the model of Dijkstra et al. (1992) (n = 101)

	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
cNDF	1.3	0.9	0.9	0.2	4.1
fNDF	3.0	2.9	1.0	0.7	5.9

[1] cNDF = concentrates neutral detergent fiber, fNDF = roughage neutral detergent fiber.

Table 3. Pearson's correlation among digested feed fractions (g/ kg DM), observed VFA molar proportions (mol VFA/ 100 mol total VFA) and feeding level (kg DM/ kg BW/ d) (n = 101)

	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

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

Su = soluble carbohydrates, St = starch, Ce = cellulose, Hc = hemicellulose, fNDF = forages neutral detergent fiber, cNDF = concentrates neutral detergent fiber, 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].

Table 4. Evaluation of the predictive performance[1] of Murphy et al. (1982)

	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 = nonglucogenic to glucogenic VFA ratio, calculated as: [Ac + 2×Bu + Bc] / [Pr + Bc].

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

[3] Mean squared prediction error (mol VFA/ 100 mol 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's correlation coefficient, μ = location shift.

Table 5. Evaluation of the predictive performance[1] of Argyle and Baldwin (1988)

	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

See Table 4 for footnotes.

Table 6. Evaluation of the predictive performance[1] of Friggens et al. (1998)

	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

See Table 4 for footnotes.

Table 7. Evaluation of the predictive performance[1] of Sveinbjörnsson et al. (2006)

	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

See Table 4 for footnotes.

Table 8. Evaluation of the predictive performance[1] of Bannink et al. (2006)

	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

See Table 4 for footnotes.

Table 9. Evaluation of the predictive performance[1] of Bannink et al. (2008)

	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

See Table 4 for footnotes.

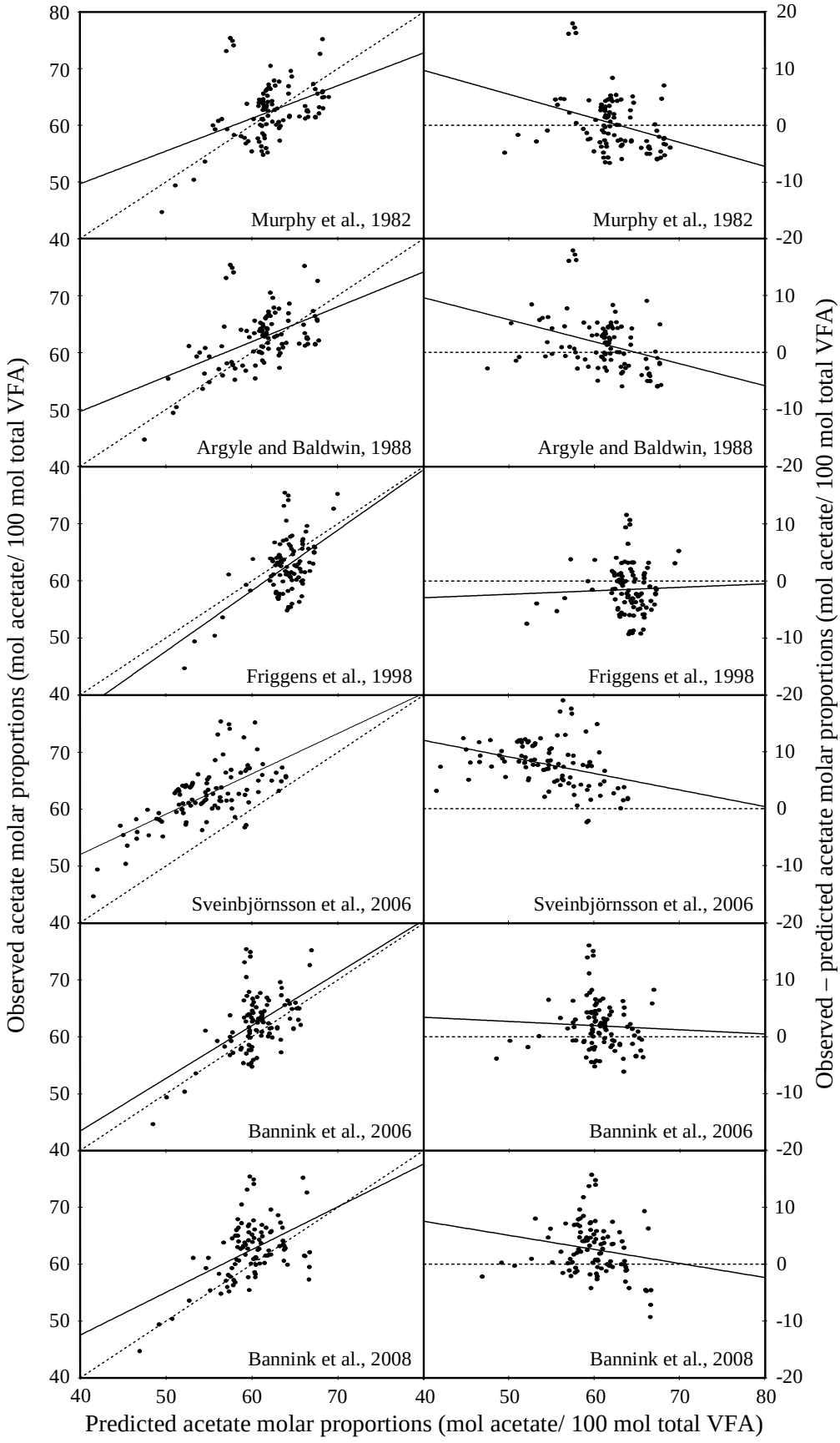
Figure 1. Plots of observed vs. predicted (left) and residuals (observed minus predicted) vs. predicted (right) acetate molar proportions (mol acetate/ 100 mol total VFA) according to the VFA stoichiometry models of Murphy et al. (1982), Argyle and Baldwin (1988), Friggens et al. (1998), Sveinbjörnsson et al. (2006), Bannink et al. (2006), and Bannink et al. (2008).

Figure 2. Plots of observed vs. predicted (left) and residuals (observed minus predicted) vs. predicted (right) propionate molar proportions (mol propionate/ 100 mol 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).

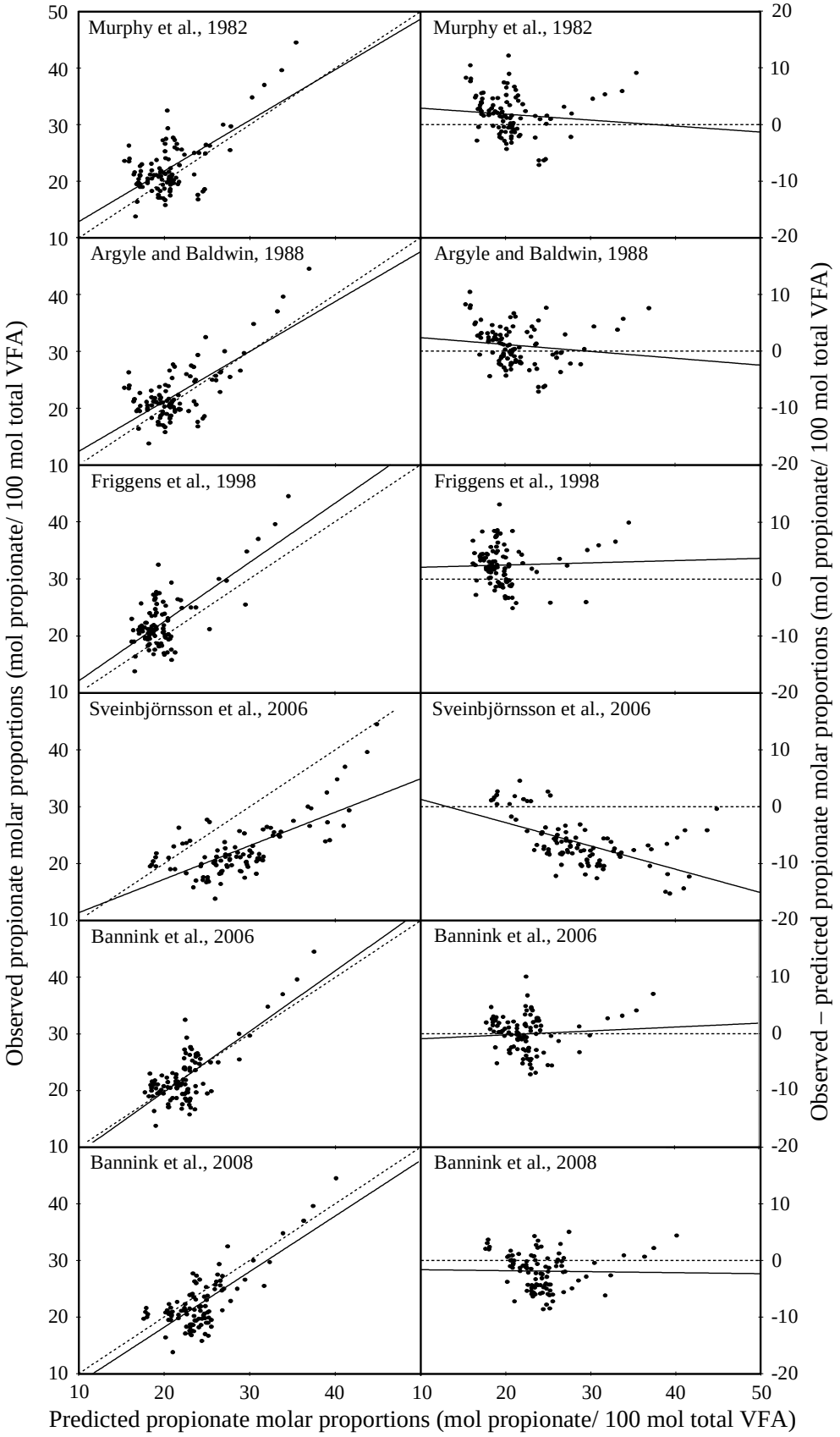
Figure 3. Plots of observed vs. predicted (left) and residuals (observed minus predicted) vs. predicted (right) butyrate molar proportions (mol butyrate/ 100 mol total VFA) according to the VFA stoichiometry models of Murphy et al. (1982), Argyle and Baldwin (1988), Friggens et al. (1998), Sveinbjörnsson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

Figure 4. Plots of observed vs. predicted (left) and residuals (observed minus predicted) vs. 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×Bu + Bc] / [Pr + Bc].

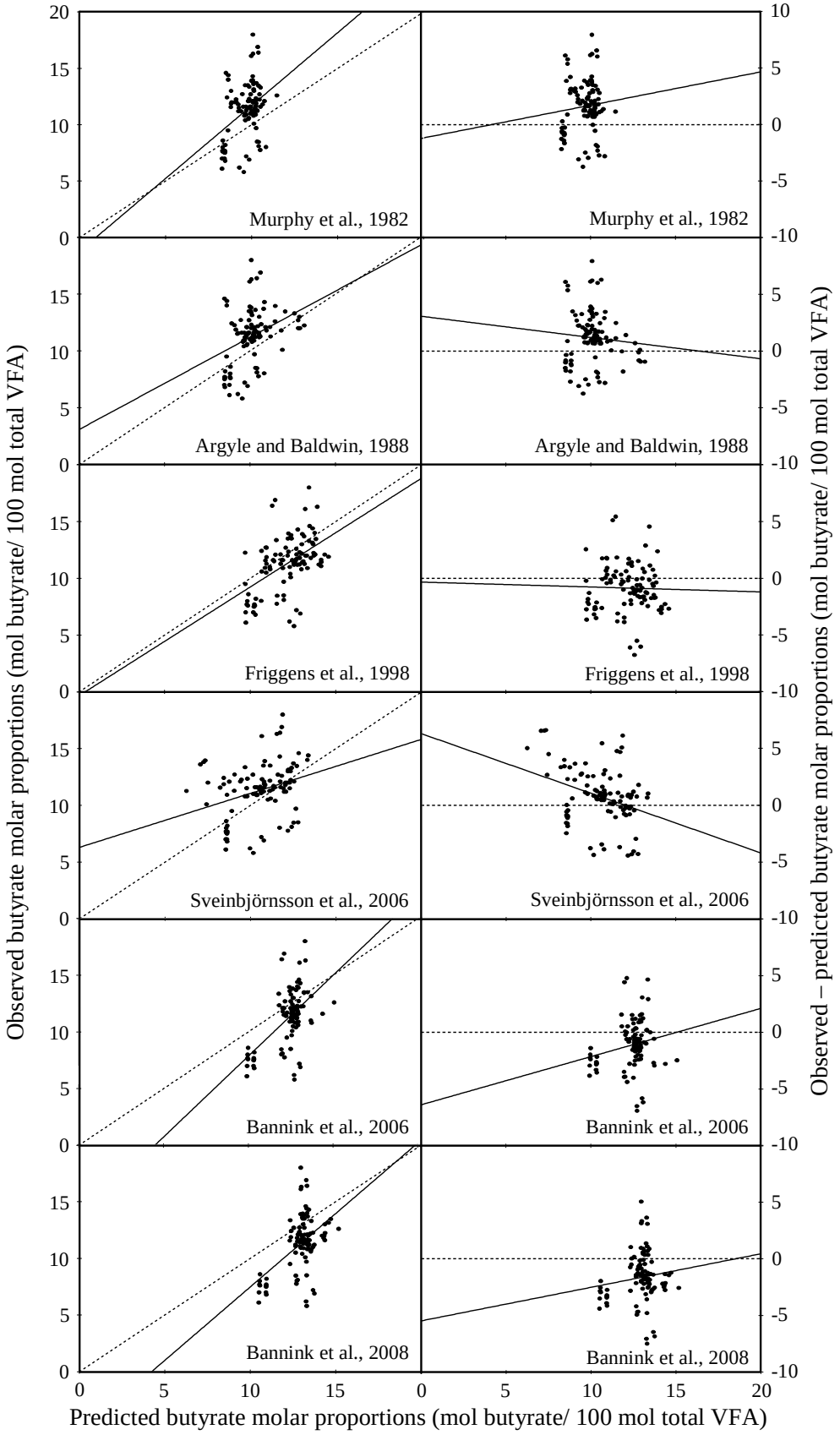
Morvay Figure 1.



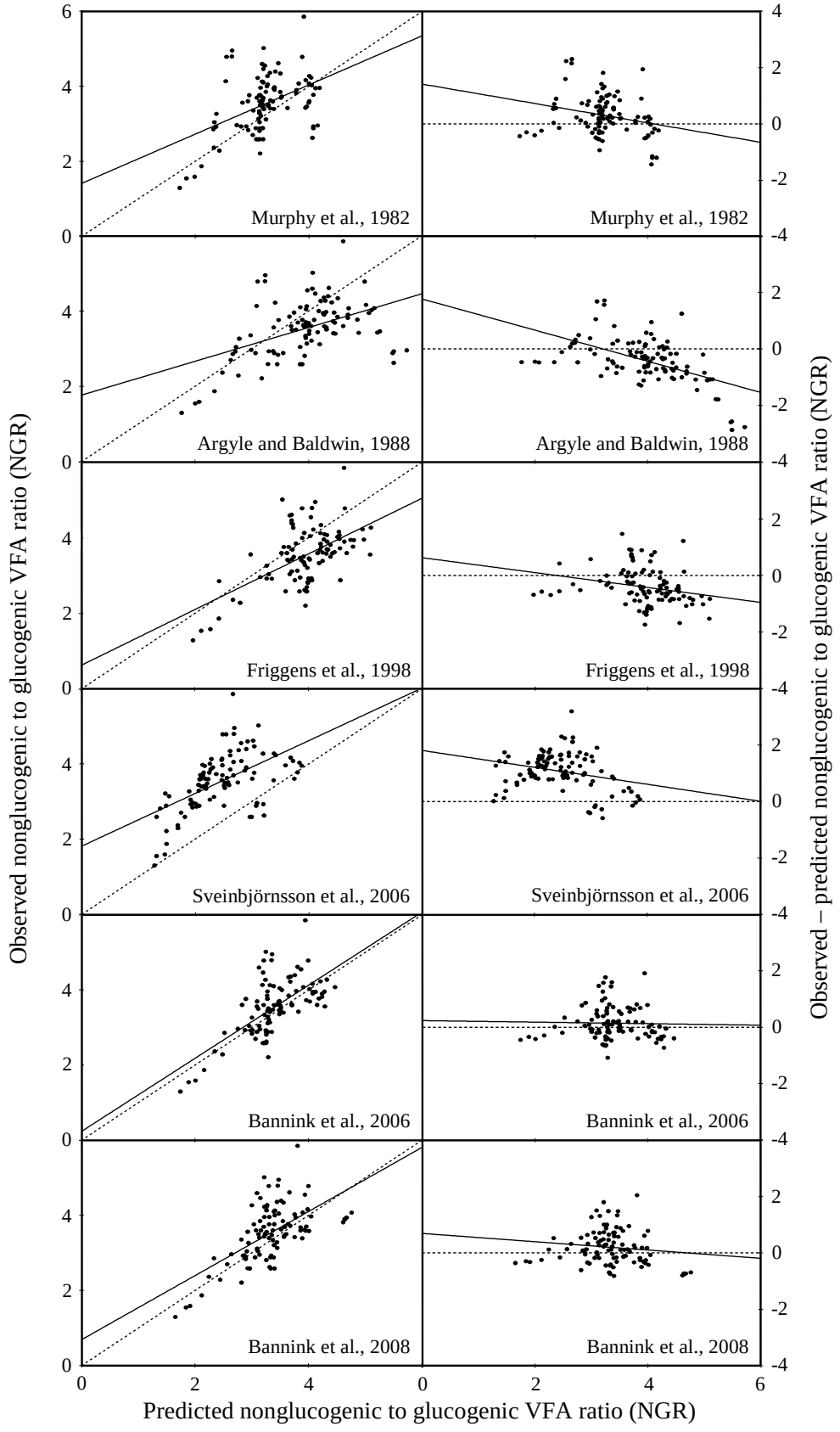
Morvay Figure 2.



Morvay Figure 3.



Morvay Figure 4.



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Yael