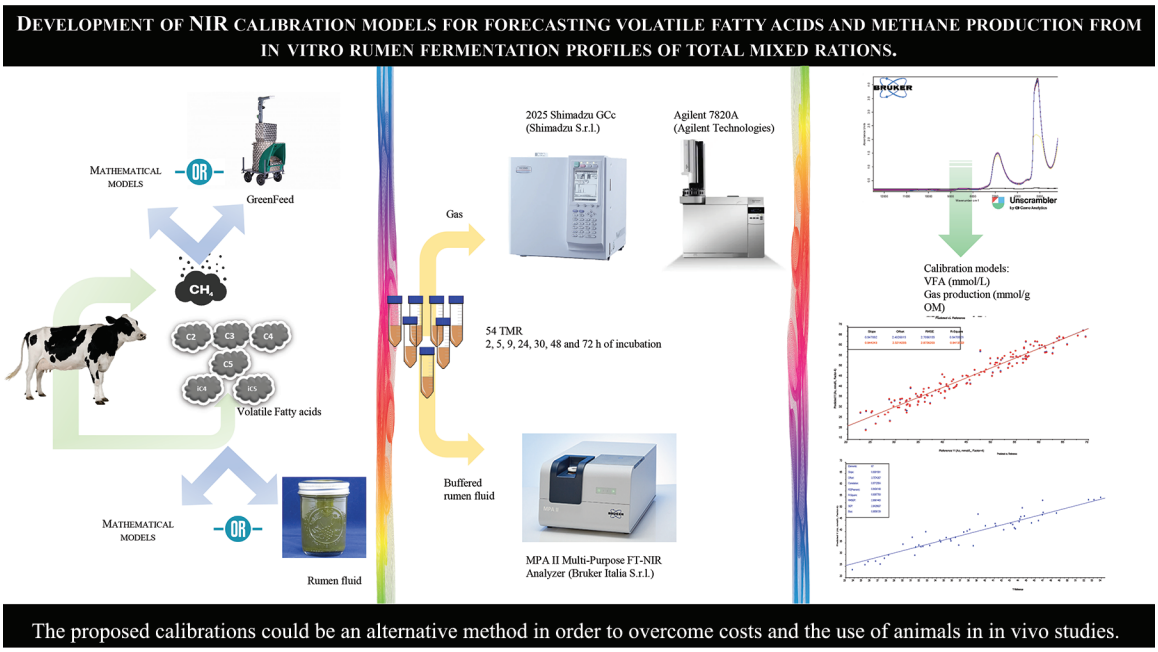
JDS Communications®
2022; 3:19–25

https://doi.org/10.3168/jdsc.2021-0156
Short Communication
Animal Nutrition and Farm Systems

Near-infrared calibration models for estimating volatile fatty acids and methane production from in vitro rumen fermentation of different total mixed rations

F. Ghilardelli, G. Ferronato, and A. Gallo*

Graphical Abstract



Summary

Diverse disciplines of science use near-infrared (NIR) spectroscopy for chemical analysis because it is rapid and inexpensive, and it provides reproducible results. Many animal scientists perform in vitro rumen fermentation tests to measure the production of volatile fatty acids (VFA) and methane (indicative of energy loss) from different feeds or diets. We used NIR spectroscopy to develop calibration models that predict the production of different VFA (acetic, propionic, butyric, valeric, isovaleric, and isobutyric acids), total gas, and methane from in vitro rumen fermentation of different silage-based diets. Our models provided reliable estimates of these rumen fermentation products.

Highlights

- Near-infrared (NIR) prediction models accurately predicted volatile fatty acids, methane, and gas production.
- Outputs of models could provide useful information for calibrating rumen mechanistic models.
- Calibrations of valeric and isovaleric acids need to be improved.

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Near-infrared calibration models for estimating volatile fatty acids and methane production from in vitro rumen fermentation of different total mixed rations

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Abstract: Volatile fatty acids (VFA) and methane (CH_4) are the major products of rumen fermentation. The VFA are considered an energy source for the animal and rumen microbiota, and CH_4 (which is released by eructation) is considered an energy loss. Quantification of these fermentation products is fundamental for the evaluation of feeds and diets, and provides important information regarding the use of nutrients by ruminants. Near-infrared (NIR) spectroscopy is increasingly used for the evaluation of animal feeds because it is rapid, nondestructive, noninvasive, and inexpensive; does not require reagents; and the results are reproducible. The aim of this study was to develop NIR calibration models for estimating the production of VFA (acetic, propionic, butyric, valeric, isovaleric, and isobutyric acids), total gas, and CH_4 using in vitro gas production tests with buffered rumen inoculum throughout fermentation. Fifty-four total mixed rations (TMRs) were examined, and rumen fluid was manually collected from 2 dry Holstein dairy cows that had ruminal fistulas and were fed at maintenance energy levels. Then, 30 mL of buffered rumen fluid was incubated in bottles with ~220 mg of TMR. The total gas, VFA, and CH_4 were measured after 2, 5, 9, 24, 30, 48, and 72 h of rumen incubation for each TMR. The VFA were measured on 32 randomly selected TMR. In particular, 7 bottles were used for each TMR, one for each incubation time. Methane was measured in the headspace and VFA were measured in the buffered rumen fluid. The bottles were considered experimental units for calibration purposes. The production of CH_4 was quantified from the bottle headspaces by gas chromatography, and total gas production was measured using a pressure transducer at each incubation time. Two aliquots of the fermented liquids were sampled by opening the bottles at each incubation time, and (1) the concentrations of VFA were determined by gas chromatography or (2) spectra were obtained from Fourier-transform NIR spectroscopy. The data were randomly divided into calibration and validation data sets. The average concentrations of acetic acid (45.30 ± 11.92 and 43.86 ± 11.93 mmol/L), propionic acid (14.97 ± 6.08 and 14.38 ± 6.56 mmol/L), butyric acid (8.47 ± 3.47 and 8.65 ± 3.79 mmol/L), total gas (111.34 ± 81.90 and 116.46 ± 82.44 mL/g of organic matter), and CH_4 (9.65 ± 9.45 and 10.35 ± 9.33 mmol/L) were similar in the 2 data sets. The best calibration models were retained based on the coefficient of determination (R^2) and the ratio of prediction to deviation (RPD). The R^2 values for prediction of VFA ranged from 0.69 (RPD = 3.28) for valeric acid to 0.94 (RPD = 4.20) for acetic acid. The models also provided good predictions of CH_4 (R^2 = 0.89, RPD = 3.05) and cumulative gas production (R^2 = 0.91, RPD = 3.30). The models described here precisely and accurately estimated the production of CH_4 and VFA during in vitro rumen fermentation tests. Validations at additional laboratories may provide more robust calibrations.

Volatile fatty acids are the main products of rumen fermentation, and represent about 40 to 70% of digestible energy intake (Dijkstra et al., 2005). The proportions of acetic, butyric, and propionic acids from rumen fermentation determine the amount of hydrogen (H_2) available for methanogenic bacteria (Alemu et al., 2011). In contrast, methane (CH_4) production results from microbial digestion, and ruminants expel CH_4 by belching (eructation). The production of CH_4 indicates an energy loss for ruminants (Ellis et al., 2008) because it decreases the efficiency of the feed use, and it also contributes to global warming (Rossi et al., 2001). Several approaches that use mathematical models or direct laboratory methods can quantify rumen CH_4 and VFA productions. The molar proportions of VFA in the rumen are the consequence of differences in the rate of production, interconversion, and absorption (Morvay et al., 2011). Thus, researchers use stoichiometric coefficients (Bannink et al., 2006) in mechanistic models of the rumen to estimate VFA production. In addition, individual VFA in rumen can be directly determined using GC of samples (Cottyn and Boucque, 1968). The CH_4 can also be quantified using mathematical models or direct measurements (Negussie et al., 2017), and indirect estimates can be from empirical or process-based mechanistic models.

The development and use of these equations requires measurements of many parameters in different conditions (e.g., physiological stage of the animal, feeding strategy, DMI) to achieve high prediction accuracy (Patra, 2016). The most commonly used techniques for direct analysis are respiration chambers, the sulfur hexafluoride (SF_6) tracer method, and the automated head-chamber system (i.e., GreenFeed). These all provide reliable measurements but are not suitable for large-scale applications (Patra, 2016).

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Alternatively, in vitro methods that measure gas production by buffered rumen inoculum can be used to estimate ruminant feedstuff value, characterize rumen fermentation dynamics, and measure CH_4 production over time (Serment et al., 2016). Under controlled conditions, these in vitro methods quantify VFA and CH_4 at different times, and these measurements are inexpensive to acquire and provide reproducible results. Getachew et al. (2005) reported that in vitro and in vivo techniques yielded similar measurements of CH_4 production, although the scientific literature has contradictory results regarding the agreement of data from these different methods (Maccarana et al., 2016). In addition, Bhatta et al. (2006) compared results from the in vivo SF_6 method with those from the in vitro rumen simulation technique (i.e., RUSITEC) and the in vitro gas production technique. They concluded that methane estimation using the gas production technique was very close to that measured by the in vivo technique, and the average coefficients of determination ranged from 0.92 to 0.99, depending on the diet. Recently, Danielsson et al. (2017) compared CH_4 production measured by the GreenFeed system and gas production for 49 different diets. These authors reported a coefficient of determination of 0.96 when in vitro data were used to predict in vivo CH_4 emission.

To avoid the need for an in vivo trial, some mechanistic models simulating the rumen ecosystem have provided results that successfully reproduced rumen fermentation pathways. Recently, Muñoz-Tamayo et al. (2016) proposed a mechanistic rumen model that considered microbial metabolisms, acid-base reactions, and liquid-gas transfers based on in vitro data from Serment et al. (2016). These researchers subsequently validated this mechanistic model using an external data set, with in vitro data on VFA and CH_4 production obtained by rumen fermentation of high-silage diets (Muñoz-Tamayo et al., 2019). A critical consideration for the accurate representation of the rumen ecosystem when using these models is the availability of data for model calibration and validation. Near-infrared (**NIR**) spectroscopy is an inexpensive and simple technique that can potentially provide these data.

Animal scientists who study livestock and feed evaluation are increasingly using NIR spectroscopy. The advantages of this method are that it is rapid, it does not require reagents, it is nondestructive and noninvasive, and it provides reproducible results at very low cost. Some researchers have used infrared calibration in the near- or mid-infrared (**MIR**) regions to measure many different biologic substrates (Yakubu et al., 2020). In the NIR region, the peaks are overtones and combination peaks of molecular vibrations. These nonfundamental excitations are weaker than the fundamental bands and arise from the O–H, C–H, S–H, and N–H stretching modes (Zhang et al., 2009). The main constraints for obtaining accurate calibration models are the accuracy, variability, and distribution of the primary data, as well as the concentration and physical structure of the chemical compounds and types of samples. Despite these limitations, Zhang et al. (2021) successfully used NIR spectroscopy to evaluate single feed or TMR chemical composition and in situ disappearance, and other researchers successfully used NIR spectroscopy to measure in vitro digestibility (Andrés et al., 2005; Mentink et al., 2006). However, little information is available on the accuracy of the NIR method for predicting the production of total gas, VFA, and CH_4 from a rumen fluid sample.

The aim of this study was to assess the use of NIR calibration models to determine the major rumen fermentation parameters by direct analysis of fermented buffered rumen fluids. The specific fermentation products produced by incubating TMR in vitro with a buffered rumen fluid collected from fistulated animals were VFA (acetic, propionic, butyric, valeric, isovaleric and isobutyric acids), total gas, and CH_4.

A total of 54 TMR were collected from dairy farms in the Po valley of northern Italy during 2018. Details regarding locations of dairy farms and TMR sampling technique were previously reported (Atzori et al., 2021). These TMR were dried in a 65°C forced-air oven to constant weight, and then ground to a particle size of 1 mm in a rotor speed laboratory mill (Pulverisette 19). For in vitro gas production tests, a sample (~220 mg) was added into a 100-mL glass bottle (Gallo et al., 2016). Each experimental run consisted of 18 TMR that were incubated together. There were 7 replicates of each TMR sample, 1 for each incubation time. In addition, 4 bottles with only buffered rumen fluid (blanks) and 4 bottles with a starch sample (internal standard, Gelose 80 maize starch; Penford Food Ingredients Co.) were incubated in each experimental run. Blanks and internal standards were used to verify gas production dynamics of each run. Indeed, the cumulative blanks or internal standard gas production of all fermentation runs performed in the experiment had to be within the average value ± 1 standard deviation to be considered acceptable. If this condition was not met, the run was repeated.

A buffer-mineral solution (Menke and Steingass, 1988) was prepared on the same day as inoculation, and it was added to a water bath at 39°C under continuous CO_2 flushing to create anaerobic conditions. The pH was adjusted to 6.5 to 6.6. Rumen fluids were manually collected from 2 Holstein dry dairy cows that had ruminal fistulas (BW 625 ± 10 kg) and were maintained at the CERZOO experimental station (San Bonico, Piacenza, Italy). These cows received maintenance diets, and rumen samples were collected after the morning feeding (NRC, 2001). The TMR had 12% CP and 55% NDF, determined by using amylase and sodium sulfite, on a DM basis, and consisted of grass hay (75% DM), corn silage (15% DM), and a protein vitamin mineral supplement (10% DM). On the day of rumen collection, the rumen fluid inoculum was transferred into 2 warmed thermos flasks, combined, filtered through 2 layers of cheesecloth, added to the buffered mineral solution, and then maintained at 39°C in a water bath and flushed with CO_2. The rumen inoculum was maintained in a warm insulated flask and used within 20 min of sampling. The rumen inoculum was then diluted (1:2 vol/vol rumen fluid:buffer-mineral solution) and the buffered rumen mixture was maintained in continuous agitation, at constant temperature, and under anaerobic conditions. As described by Pirondini et al. (2012) and Serment et al. (2016) with minor modifications, about 30 mL of diluted rumen fluid was transferred into each bottle containing the TMR. First, the bottle headspace (70-mL volume) was flushed with CO_2 and the bottle was then hermetically closed with rubber caps and degassed. The headspace pressure was recorded after 2, 5, 9, 24, 30, 48, and 72 h using a gas pressure transducer (digital test gauge XP2i, Crystal Engineering Corp.). The remaining gas was then released by puncturing the cap with a needle, and the pressure was brought back to atmospheric level. To preserve normal microbial activity, the headspace pressure never exceeded 48 kPa, as recommended by Theodorou et al. (1994). The gas pressure results were converted to moles of gas using the ideal gas law:

$$n = p \times V/(R \times T), \quad [1]$$

where n is moles of gas, p is pressure (kPa), V is headspace volume (L), R is the gas constant (8.314 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$), and T is temperature (K).

One bottle that contained substrate and fermented liquid was used at each incubation time to measure CH_4 in the headspace and VFA concentrations in the buffered rumen inoculum. For this procedure, the bottle was put on ice to stop fermentation, and 1 mL of headspace gas was then sampled using a 2-mL gas-tight glass syringe with a pressure lock (VICI, Precision Sampling Inc.) to collect a sample. This 1-mL sample of gas was analyzed using a GC system (Agilent 7820A, Agilent Technologies) using N_2 as a carrier. An external standard mixture of CO_2 and CH_4 prepared by SIAD S.p.A. (Bergamo, Italy) was used for instrument calibration. Peak areas were calculated by automatic integration. The amount of CH_4 (mmol) produced between adjacent time points and the final amount were calculated as described by Tavendale et al. (2005).

Then, 2 aliquots of fermented rumen fluid were stored at −20°C and used for measurements by 2 different methods. In the first

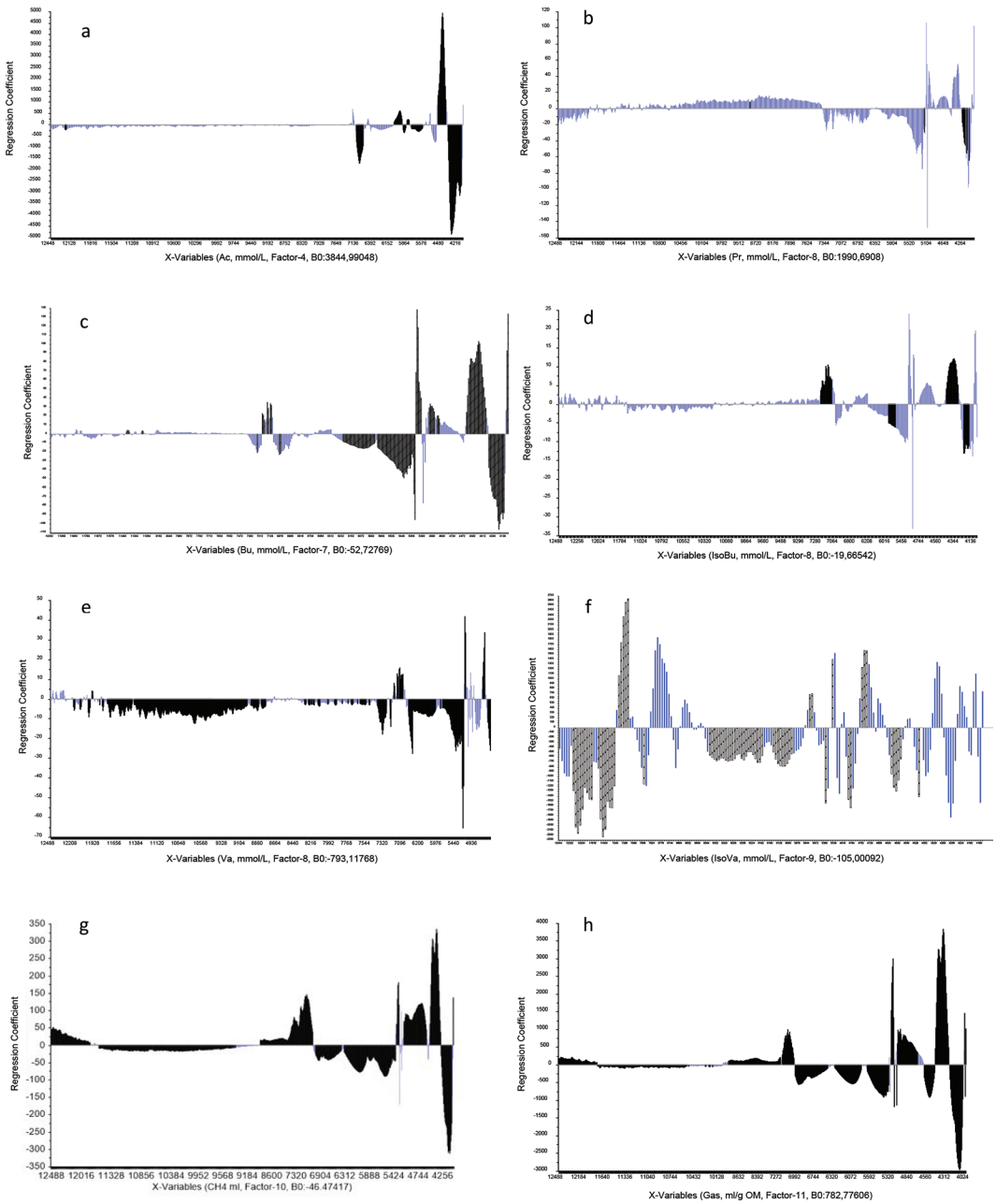

Figure 1. Selection of latent variables for each partial least squares model: (a) acetic acid; (b) propionic acid; (c) butyric acid; (d) isobutyric acid; (e) valeric acid; (f) isovaleric acid; (g) methane; and (h) total gas.

Table 1. Levels of in vitro rumen fermentation products in the calibration and validation data sets

	Calibration data					Validation data				
Fermentation product	Mean	Maximum	Minimum	SD	CV	Mean	Maximum	Minimum	SD	CV
Acetic acid, mmol/L	45.30	69.41	22.69	11.92	0.26	43.86	64.56	23.81	11.93	0.27
Propionic acid, mmol/L	14.97	33.15	3.77	6.08	0.41	14.38	32.92	5.47	6.56	0.46
Butyric acid, mmol/L	8.47	16.79	2.22	3.47	0.41	8.65	19.70	1.75	3.79	0.44
Valeric acid, mmol/L	1.00	3.07	0.15	0.62	0.62	1.01	3.15	0.16	0.69	0.69
Isobutyric acid, mmol/L	0.60	2.10	0.15	0.35	0.59	0.67	2.11	0.22	0.44	0.66
Isovaleric acid, mmol/L	0.93	3.51	0.22	0.63	0.67	0.90	1.83	0.30	0.49	0.54
Methane, mmol/L	9.65	33.94	0.02	9.45	0.98	10.35	32.30	0.01	9.33	0.90
Total gas, mL/g of OM	111.34	276.89	0.01	81.90	0.74	116.46	280.63	0.01	82.44	0.71

method, VFA concentrations were determined using a GC system (2025 Shimadzu GC, Shimadzu S.r.l.). This device had an autosampler (AOC-20i Shimadzu S.r.l.), flame-ionization detector, and capillary column (DB-FFAP; 30 m, 0.250 mm, 0.25 µm; Agilent Technologies S.p.A.). The injector operated at 200°C and the detector at 220°C. The injection volume was 1 µL and the split ratio was 100:1. In the second method, Fourier-transform (**FT**)-NIR spectroscopy of samples was performed using an MPA II Multi-Purpose FT-NIR Analyzer (Bruker Italia S.r.l. Unipersonale). Before this analysis, the buffered rumen fluid was thawed at 37°C in a water bath for 15 min, cooled in a water and ice solution, centrifuged at 3,800 × *g* for 16 min at 6°C, and placed in a thermostatically controlled water bath at 30°C for 10 min. All spectra were acquired in duplicate. The flow cell thermostat was set to 30°C and the resolution was 8 cm^{-1} in the range of 12,488 to 4,000 cm^{-1}. For methane, a primary database was created and measurements were recorded from each bottle of each fermented TMR. There were 378 observations (7 bottles for each of the 54 TMR). For measurements of gas production, results were obtained on all remaining unopened bottles at each incubation time. For VFA, 32 randomly selected TMR were sampled and used to generate measurements for NIR calibrations. The databases were analyzed to determine fermentation parameters and NIR spectra using a statistical filtering procedure, based on univariate and multivariate approaches. The detection of outliers in the primary data was determined using the threshold technique of calculating an acceptability range:

$$Threshold_{min} = \text{mean} - a \times \text{SD}; \; Threshold_{max} = \text{mean} + a \times \text{SD}, \quad [2]$$

where the control parameter (*a*) was set to 2 (Yang et al., 2019). Then, principal component analysis (**PCA**) was used to analyze spectra and detect outliers according the influence plot, based on Hoteling's T^2 statistic with a 5% limit. The acquired spectra were processed by Unscrambler X software (version 10.5.1, CAMO Software) for development of calibration curves using partial least squares (**PLS**) fitting. In particular, spectra were randomly assigned to a calibration set or a validation set in a 70:30 ratio. Different preprocessing methods were used to remove physical variations in the spectra (Rinnan et al., 2009). These methods corrected for scatter (standard normal variate method) and spectral derivation (Savitzky-Golay polynomial derivative method). Using the calibration set, a PLS regression model with a cross-validation leave-one-out criterion and a nonlinear iterative PLS (NIPALS) algorithm were performed using NIR wavelengths as the predictor terms. To avoid redundancy and collinearity and improve the robustness of the calibration models, specific NIR spectral regions were selected according to an optimal number of latent variables (Gowen et al., 2011). In this procedure, the software set the residual variance to quantify the effect of adding an additional factor to the model on the increase of R^2 (Figure 1). An R^2 value close to 1 indicates good linear dependence between the observed and predicted values; that is, good predictability of the model (Sileoni et al., 2013). If the variance between 2 consecutive factors was less than 6%, the latent factor extraction process was stopped and the model was developed using the optimal number of factors (Tian et al., 2021). All regression models were then applied to the validation set to determine the accuracy of predictions. In particular, the accuracy of the models was evaluated by calculating the coefficient of determination (R^2), the root mean square error, the standard error of calibration, the standard error of cross-validation, the standard error of prediction (**SEP**), and the ratio of prediction to deviation (**RPD**). The models that provided the best predictions were retained (Aptula et al., 2005).

We first calculated the means, SD, ranges, and coefficients of variation (**CV**) for data on the production of VFA, CH_4, and total gas for the calibration and validation data sets (Table 1). The calibration set had the greatest CV for isobutyric acid (0.59), isovaleric acid (0.67), valeric acid (0.62), CH_4 (0.98), and total gas (0.74). The average concentration between calibration and validation sets of acetic acid was 44.58 ± 1.92 mmol/L, the average of propionic acid was 14.64 ± 6.32 mmol/L, and the average of butyric acid was 8.56 ± 3.63 mmol/L. The wide range in these concentrations was related to the different fermentation times (rumen sampling times).

We assessed the performance of prediction models using NIR data for each parameter (Table 2). The prediction of VFA levels could be considered satisfactory, in that the R^2 ranged from 0.69 (SEP = 0.21 mmol/L) for valeric acid to 0.94 (SEP = 2.84 mmol/L) for acetic acid. The model also provided good predictions for CH_4 (R^2 = 0.89, SEP = 3.06 mmol/L) and total cumulative gas production (R^2 = 0.91, SEP = 24.99 mL/g of OM) as measured from 2 h to 72 h of rumen incubations. Williams (2001) recommends an RPD value >3 to define a predictive model as excellent. Thus, all of our models were excellent except the model for isovaleric acid (RPD = 1.95, Table 2). In particular, the low RPD for isovaleric acid suggests that our method can simply distinguish between high and low values, and that a more accurate NIR spectroscopy calibration must be used for this branched VFA.

Only a few previous studies reported calibration models to predict VFA and CH_4 from direct analysis of rumen fluid from NIR spectroscopy. For example, Turza et al. (2002) found that NIR

spectroscopy had the potential to properly predict rumen fluid composition when a specially designed fiber optic method was used. In particular, their predictive models achieved satisfactory results, with R^2 values ranging from 0.81 (isobutyric acid) to 0.92 (acetic acid). The performance of our predictive models for acetic, butyric, valeric, and isobutyric acids were in line these previous results. Our models had lower R^2 values for propionic acid (0.81) and isovaleric acid (0.73) than did the models reported by Turza et al. (2002). These differences could be attributable to the greater variation of the primary data used in our validation and calibration data sets.

Udén and Sjaunja (2009) developed rumen fermentation models to predict acetate, propionate, and butyrate, and reported satisfactory accuracy. Specifically, they analyzed 308 samples for calibration using FT-MIR spectroscopy of samples of semi-artificial rumen fluids that were spiked with acetate, propionate, and butyrate, with and without bicarbonate and phosphate. Tagliapietra et al. (2015) analyzed in vitro rumen fermentation fluid using FT-MIR spectroscopy with Bayesian models and reported similar accuracy in their predictive models. In particular, these researchers developed different VFA calibration models using 8 diets for lactating cows that differed in the content of fiber, CP, lipids, and starch. These diets were fermented for specific times (24 and 48 h) and there were 4 different in vitro incubations. Their results for acetic acid (R^2 = 0.92) and butyric acid (R^2 = 0.84) were similar to our results, but they reported better results for the other VFA (propionic acid: R^2 = 0.90; isobutyric acid: R^2 = 0.91, isovaleric acid: R^2 = 0.93, valeric acid: R^2 = 0.91). This could be due to their use of different spectral regions in the predictive models. In particular, absorbance in the MIR region is directly related to the concentration of individual compounds, but absorbance in the NIR region results from overtones and their combinations (Udén and Sjaunja, 2009).

Zhang et al. (2009) developed an FT-NIR calibration for VFA and ethanol in the effluent of an anaerobic H_2-producing bioreactor. Generally, the discriminant absorption bands in calibration models are related to the frequencies of the third overtone of C–H bonds and the second overtone of O–H bonds, to the first overtone of C–H combinations (7,200–6,100 cm^{-1} and 6,100–5,400 cm^{-1}) and the first overtone of O–H bonds (7,200–6,100 cm^{-1}), and to C–H bond combinations and C–H and O–H combinations (4,900–4,100 cm^{-1}), consistent with our results (Figure 1). Previous studies used NIR spectroscopy to predict the kinetics of in vitro fermentation of dairy cow feed for hay, but the results were unsatisfactory (Herrero et al., 1997; Andrés et al., 2005). Another study (Lovett et al., 2004) reported moderately successful results for silage. Our predictive models had good performance and low error in the calibration and validation data sets.

Critically, the present study should be considered a preliminary evaluation of the use of NIR spectroscopy to predict the production of CH_4 and VFA from in vitro rumen fermentation. We suggest that future calibrations should be performed by organizing specific collaborative interlaboratory experiments to improve the applicability to research and commercial laboratories. For each diet, we used 7 different bottles for measurements at 7 incubation times to generate profiles of VFA and CH_4, in line with the method previously adopted by Serment et al. (2016). We considered each bottle a separate experimental unit, although some of these bottles con-

Table 2. Near-infrared spectroscopy parameters and levels of in vitro rumen fermentation products from calibration, cross-validation, and prediction[1]

				Calibration					Cross-validation					Prediction				
Fermentation product	Nc	Np	Spectral preprocessing	LV	R^2	RMSE	SEC	Slope	Offset	R^2	RMSE	SECV	Slope	Offset	R^2	RMSE	RPD	SEP	Slope	Offset	Bias
Acetic acid, mmol/L	151	67	SG	4	0.95	2.71	2.71	0.95	2.40	0.94	2.87	2.88	0.94	2.52	0.94	2.91	4.20	2.84	0.93	3.76	0.70
Propionic acid, mmol/L	154	56	—	8	0.80	1.80	1.80	0.80	2.62	0.78	1.91	1.92	0.80	2.62	0.81	1.76	3.69	1.78	0.88	1.62	0.08
Butyric acid, mmol/L	132	54	SNV	7	0.87	1.09	1.10	0.87	1.03	0.84	1.24	1.24	0.86	1.15	0.85	1.15	3.27	1.16	0.94	0.54	0.10
Valeric acid, mmol/L	140	61	SNV	8	0.74	0.17	1.17	0.74	0.22	0.66	0.20	0.20	0.69	0.26	0.69	0.22	3.28	0.21	0.63	0.36	0.07
Isobutyric acid, mmol/L	148	57	—	8	0.81	0.12	0.12	0.81	0.11	0.76	0.13	0.13	0.78	0.12	0.82	0.11	3.94	0.11	0.87	0.07	0.00
Isovaleric acid, mmol/L	145	43	SG	9	0.84	0.23	0.23	0.84	0.14	0.77	0.28	0.28	0.80	0.17	0.73	0.25	1.95	0.25	0.96	0.08	0.04
Methane, mmol/L	260	112	—	9	0.86	3.45	3.45	0.86	1.31	0.85	3.66	3.66	0.86	1.39	0.89	3.05	3.05	3.06	0.89	1.02	−0.14
Total gas, mL/g of OM	877	441	—	11	0.92	23.29	23.30	0.92	10.60	0.91	24.07	24.09	0.91	11.21	0.91	24.97	3.30	24.99	0.09	10.09	0.66

[1]Nc = number of samples of calibration set; Np = number of samples of prediction set; LV = latent variables; SG = Savitzky-Golay polynomial derivative; SNV = standard normal variate; RMSE = root mean square error; SEC = standard error of calibration; SECV = standard error of cross-validation; SEP = standard error of prediction; RPD = ratio of prediction to deviation.

tained the same TMR and were incubated with the same buffered rumen fluid. We know that a further step of intra- or interlaboratory calibration development should be done by analyzing additional TMR to be more representative of different nutritional strategies, environmental conditions, and production systems.

In conclusion, the NIR spectroscopy models presented here provided good predictions of the production of VFA, total gas, and methane from in vitro fermentation of rumen fluid. The outputs of these models could provide useful information for calibrating rumen mechanistic models that simulate the ruminal compartment of dairy cows, with the notable advantages that NIR spectroscopy is rapid and inexpensive. However, it is important to improve the calibrations for valeric acid and isovaleric acid to obtain more accurate NIR models for these VFA.

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Financial support was provided by Fondazione Romeo ed Enrica Invernizzi (Milan, Italy).

The authors have not stated any conflicts of interest.