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**Compositional mixed modelling of methane emissions  
and ruminal volatile fatty acids from individual cattle and multiple experiments<sup>1</sup>**

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13 **ABSTRACT:** The aim of the study was to investigate the association of methane (CH<sub>4</sub>)  
14 yields (g/kg DMI) with rumen VFA molar proportions and animal and diet-related covariates  
15 from individual animals and multiple experiments. The dataset available consisted of 284  
16 measurements of CH<sub>4</sub> yields for beef cattle from 6 experiments measured in indirect  
17 respiration chambers. A compositional modelling approach was employed where VFA  
18 measurements were considered as a whole, instead of in isolation, emphasizing their  
19 multivariate relative scale. The analysis revealed expected close groupings of acetate and  
20 butyrate; propionate and valerate; iso-butyrate and iso-valerate. Linear mixed models were  
21 then fitted to examine relationships between CH<sub>4</sub> yield and VFA, represented by meaningful  
22 log-contrasts of components called compositional balances, while accounting for other  
23 animal and diet-related covariates and random variability between experiments. A  
24 compositional balance representing (acetate · butyrate)/propionate best explained the  
25 contribution of VFA to variation in CH<sub>4</sub> yield. The covariates DMI, forage:concentrate  
26 proportion (expressed as a categorical variable diet type: high concentrate, mixed  
27 forage:concentrate or high forage), and diet ME were also statistically significant. These  
28 results provided new insights into the relative inter-relationships amongst VFA  
29 measurements and also between VFA and CH<sub>4</sub> yield. In conclusion, VFA molar proportions  
30 as represented by compositional balances were a significant contributor to explaining  
31 variation in CH<sub>4</sub> yields from individual cattle.

32 **Key words:** methane production, volatile fatty acids, compositional data, mixed models.

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## INTRODUCTION

35 Methane (CH<sub>4</sub>), derived almost entirely (90%) from enteric fermentation, is a major  
36 contributor to greenhouse gas emissions from the livestock sector, and cattle are responsible  
37 for most (77%) of CH<sub>4</sub> emissions (Gerber et al., 2013). While modifying the diet of cattle is  
38 the most effective short-term method for mitigating CH<sub>4</sub> emissions, longer-term the wide  
39 variation in CH<sub>4</sub> yield (g CH<sub>4</sub>/kg DMI) between individual animals (up to 2-fold when fed  
40 the same diet, Rooke et al., 2014) must be exploited. A limitation to this is the relatively slow  
41 output achievable using indirect respiration chambers to measure CH<sub>4</sub> yield and the cost of  
42 such measurements. The amount of CH<sub>4</sub> produced from a specific diet by rumen archaea  
43 depends largely upon the amount of hydrogen (H<sub>2</sub>) produced as an end-product of  
44 fermentation of feed carbohydrates by other organisms in the rumen microbiome. There are  
45 well established stoichiometric relationships between the pattern of VFA and H<sub>2</sub> produced by  
46 rumen fermentation and resulting CH<sub>4</sub> formation (Wolin, 1960; Murphy et al., 1982; Alemu  
47 et al., 2011), but these stoichiometric relationships have usually been modelled using data at  
48 the diet level. **In the current study, we use CH<sub>4</sub> and VFA measurements for individual  
49 animals from 6 experiments to address the hypothesis that VFA pattern in an individual  
50 animal could be used as an explanatory variable in accounting for variation in CH<sub>4</sub> yield in  
51 addition to other diet and animal characteristics.** A key methodological novelty is that we  
52 consider VFA (expressed as molar proportions) as a composition of intrinsically co-  
53 dependent amounts carrying only relative information. This was embedded into a linear  
54 mixed modelling framework to account for the variability originating from the multiple study  
55 structure of the data.

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## MATERIALS AND METHODS

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The experiments which were included in the database were conducted at Scotland's Rural College (SRUC) Beef and Sheep Research Centre in Edinburgh. Each individual experimental protocol was approved by SRUC's Animal Welfare and Ethical Review Body, the Animal Experiments Committee and was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act, 1986.

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### *Description of the Data*

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The data analyzed here were obtained from 6 experiments carried out at SRUC between 2011 and 2014. The cattle used were either steers (Rooke et al., 2014, Troy et al., 2015, 2016, Duthie et al., 2017; exp. 1–4) or beef cows (Duthie et al., 2015; exp. 5 (unpublished) and of varying breed types (Aberdeen Angus x Limousin; Limousin x Aberdeen Angus; Luining; Charolais cross bred; exp. 6, Aberdeen Angus x Limousin and Limousin x Aberdeen Angus). Diets fed were of 3 types which were used as a categorical variable, diet type, in analysis. High concentrate diets (Concentrate; < 100 g forage/kg DM) were based on ground barley and barley straw with either rapeseed meal or distillers dark grains (similar to distillers grains with solubles but low in sulfur). Mixed forage:concentrate diets (Mixed; 400 – 600 g forage/kg DM) were based on grass silage, whole crop barley silage, barley, and either rapeseed meal or distillers dark grains. High forage diets (Forage; > 700 g forage/kg DM) consisted of barley straw and either grass silage or brewers' grains (Duthie et al., 2015) or a mixture of grass silage (696 g/kg DM) and whole crop barley silage (293 g/kg DM) in exp. 5. All diets were offered ad libitum as total mixed rations (fed once daily) and had been fed for at least 3 weeks before measurements of CH<sub>4</sub> yield. Methane output was measured (48 h) using indirect respiration chambers. As it was not possible to take rumen samples whilst animals were in the chambers, a single sample of rumen fluid for VFA analysis was taken by stomach tube within 2 h of cattle leaving the chambers

82 (approximately 25 h after feed bins were last refilled). Full details of methodology can be  
83 found in Rooke et al. (2014); Duthie et al. (2015) and Troy et al. (2015). The constituents of  
84 the diets are summarized in Table 1 and mean cattle BW, DMI, and CH<sub>4</sub> production in Table  
85 2. It should be noted that only animals which had complete records of DMI, CH<sub>4</sub>, and VFA  
86 were used in the current analysis; thus, there may be small discrepancies from the original  
87 references.

88 TABLE 1 (DIET CONSTITUENTS)

89 TABLE 2 (SUMMARY OF VARIABLES)

90 ***VFA Measurements as Compositional Data***

91 Data accounting for relative parts of a whole are known as compositional data (see  
92 e.g. Aitchison, 1986; Pawlowsky-Glahn et al., 2015 for discussion, formal properties and  
93 principles). This is the case for chemical mixtures such as VFA composition when expressed  
94 as portions of the whole in either weight or volume units. Thus, the components of the VFA  
95 mixture are intrinsically co-dependent positive amounts carrying only relative information.  
96 Changes in one or several components affect the remaining ones and, consequently, an  
97 equivalent symmetric overall change should be measured on the latter as well. The relative  
98 and symmetric scale is commonly recognized in practice by re-expressing the data in  
99 proportions adding up to 1 or similar units like mmol/mol used here. It is important to note  
100 that the data do not necessarily have to add up to the same constant total. Given a  $D$ -part  
101 composition  $\mathbf{x} = [x_1, \dots, x_D]$ , the statistical analysis focuses on the log-ratios  $\ln(x_i/x_j)$   
102 between components. Using this type of transformation, the results do not depend on the  
103 units of measurement and the relative scale of the data is considered. It does not matter either  
104 whether the original full mixture or a subset (subcomposition) of components, which may or  
105 may not add to total VFA, are used as here.

106 ***Basic Compositional Statistics for the VFA Data Set***

107           Following Pawlowsky-Glahn and Egozcue (2002), the composition best representing  
108 the center of a data set  $\mathbf{X} = [x_{ij}]_{n \times D}$  consisting of  $n$  compositional samples  $\mathbf{x}_i$  of  $D$   
109 components is given by the so-called compositional geometric mean (**CGM**), or  
110 compositional center, as  $\text{CGM}(\mathbf{X}) = C(g_1, \dots, g_D)$ , where  $g_j = (\prod_{i=1}^n x_{ij})^{1/n}$ , for  $j =$   
111  $1, \dots, D$ , is the geometric mean of the the  $j$ th column of  $\mathbf{X}$ . The closure operator  $C$  normalizes  
112 the resulting vector of geometric means to be expressed in the chosen scale. For example, if  
113 working with proportions,  $C$  would mean multiplying each component by  $1/\sum_{j=1}^D g_j$  so that  
114 the total sum of each composition is 1. Moreover, instead of using ordinary correlations, the  
115 relative variability structure of  $\mathbf{X}$  is given by the matrix of log-ratio variances  $\mathbf{T} = [\tau_{ij}]_{D \times D}$ ,  
116 where  $\tau_{ij} = \text{var}(\ln(x_i/x_j))$ , for  $i, j = 1, \dots, D$ , with  $\text{var}$  referring to the ordinary variance  
117 measure. Relationships between components are then understood in terms of proportionality.  
118 A log-ratio variance which is close to 0 indicates that 2 components  $x_i$  and  $x_j$  are nearly  
119 proportional (highly co-dependent); that is, their log-ratio is nearly constant. A measure of  
120 global dispersion is provided by the total variance  $\text{totvar}(\mathbf{X}) = 1/2D \sum_{i,j=1}^D \tau_{ij}$ . Estimators  
121 of these measures from data are obtained by the standard maximum likelihood procedure.

### 122 ***Log-ratio Coordinate Representation***

123           Recent advances in the area (Egozcue et al., 2003) allow the definition of isometric  
124 (metric-preserving) mappings between the original composition  $\mathbf{x}$  and log-ratio coordinates  
125 living in the ordinary real space, which facilitates the use of standard statistical methods.  
126 These isometric log-ratios (**ilr**) can be constructed in infinitely many different ways, although  
127 they are essentially orthogonal rotations of one to another. This means that results from any  
128 set of ilr coordinates transform back into the same results in terms of the original  
129 composition. Note that compositions of size  $D$  correspond to vectors of isometric log-ratio  
130 coordinates of size  $D - 1$  (the actual degrees of freedom of the composition). A procedure  
131 known as sequential binary partition (**SBP**; Egozcue and Pawlowsky-Glahn, 2005) allows the

132 construction of tailored ilr coordinates, usually called compositional balances ( $b_i$ , where  
 133  $i = 1, \dots, D - 1$ ), representing log-contrasts between subsets of components of  $\mathbf{x}$ . This is of  
 134 great practical relevance because balances can be defined according to insights from  
 135 exploratory data analysis (see Model A1 below) or using biological knowledge.

### 136 ***Building Balances for the VFA Composition***

137 Balances are obtained using SBP by successive splits of the components of the VFA  
 138 composition  $\mathbf{x}$  into 2 mutually exclusive groups until only groups of 1 component are left (see  
 139 left-hand side of Table 4). These two groups are coded by the signs + and – respectively. The  
 140 collection of  $D - 1$  balances  $b_i$ , for  $i = 1, \dots, D - 1$ , is obtained as

$$b_i = \frac{\sqrt{r_i s_i}}{\sqrt{r_i + s_i}} \ln \frac{(\prod_{k=1}^{r_i} x_{ik}^+)^{1/r_i}}{(\prod_{k=1}^{s_i} x_{ik}^-)^{1/s_i}}, \quad [1]$$

141 where  $x_{ik}^+$  and  $x_{ik}^-$  refer to the subsets of  $r_i$  and  $s_i$  components going, respectively, into the +  
 142 (numerator) and – (denominator) groups. The  $D - 1$  balances fully represent the information  
 143 in the composition  $\mathbf{x}$  and, as previously said, they are appropriate to be used in standard  
 144 statistical modelling. Note that the log-ratio term in Eq. [1] is computed as the ratio  
 145 between the geometric means of the corresponding + and – components. It is multiplied by a  
 146 normalizing constant to give the  $b_i$  that allow balances to be compared and confers on them  
 147 desirable geometric properties. A balance then measures the relative importance, in geometric  
 148 mean, of one group against the other by means of a log-contrast between them. Table 4 (left)  
 149 depicts two alternative but equivalent balance representations of the VFA composition used  
 150 in this work to produce a linear mixed model for CH<sub>4</sub> emissions. For example, the balance  $b_1$   
 151 in Model A1 was computed as

$$b_1 = \sqrt{\frac{8}{6}} \ln \frac{(\text{iso-butyrate} \cdot \text{iso-valerate} \cdot \text{butyrate} \cdot \text{acetate})^{1/4}}{(\text{valerate} \cdot \text{propionate})^{1/2}}. \quad [2]$$

152 In Model A2, the SBP is modified to isolate a balance of particular biological interest



153 (balance  $b_2$  in Model A2) so that its significance can be statistically tested. Note that,  
 154 regardless of the balance representation chosen, it holds that  $\text{totvar}(\mathbf{X}) = \sum_{i=1}^{D-1} \text{var}(b_i)$  for a  
 155 given compositional data set  $\mathbf{X}$ . Thus, the balances can be ranked according to their  
 156 contribution to explaining the total variability within the data set.

### 157 *A Compositional Linear Mixed Model for CH<sub>4</sub> Emissions*

158 A linear mixed model (LMM) approach was adopted to integrate quantitative  
 159 findings from the 6 different studies. The linear association between CH<sub>4</sub> emissions  
 160 expressed as g/d or g/kg DMI (see Table 2) was moderately high and positive (Pearson's  
 161  $r = 0.75$ ). Accordingly, similar estimates of the effects of the given explanatory variables  
 162 would be obtained. For the purpose of this work we used CH<sub>4</sub> yields expressed in g/kg DMI  
 163 as the response variable. Methane yield was log transformed to more closely satisfy the  
 164 normality assumption of the model residuals. Formally, the response vector  $\mathbf{y}_i$  from the  $i$ th  
 165 experiment was modelled as

$$\begin{aligned} \mathbf{y}_i &= \mathbf{B}_i \cdot \beta_1 + \mathbf{C}_i \cdot \beta_2 + \mathbf{Z}_i \cdot f_i + \varepsilon_i, \quad i = 1, \dots, 6, \\ f_i &\sim N(0, \sigma_f^2), \\ \varepsilon_i &\sim N(\mathbf{0}, \sigma^2 \mathbf{I}), \end{aligned} \quad [3]$$

166 where  $\beta_1$  and  $\beta_2$  were the coefficients of the fixed effects associated with, respectively, (a)  
 167 the VFA balances ( $\mathbf{B}_i$  matrix) as obtained from Eq. [1] given a SBP, and (b) the other  
 168 explanatory covariates ( $\mathbf{C}_i$  matrix). The BW and DMI summarized the information about  
 169 animal covariates in the model. For diet-related covariates, diet type (Concentrate, Mixed or  
 170 Forage) was included as an explanatory factor representing the diet contents (forage, starch,  
 171 and neutral detergent fiber) across experiments, along with metabolizable energy (ME). All  
 172 the covariates were log transformed to be introduced in the model. The term  $\mathbf{Z}_i \cdot f_i$  was the  
 173 experiment random effects term, with  $f_i$  assumed to be normally distributed with mean 0 and  
 174 variance  $\sigma_f^2$ . The within-group random errors  $\varepsilon_i$  were assumed to be normally distributed

175 with means 0 and variances  $\sigma^2$ . Random variability of model intercepts between experiments  
176 was assumed, which implied that the design matrix of the random effects  $\mathbf{Z}_i$  equaled a unit  
177 vector. The random effects  $f_i$  and the random errors  $\varepsilon_i$  were assumed to be independent for  
178 different experiments and independent of each other for the same experiment.

179 Model fitting was conducted by restricted maximum likelihood estimation. The  
180 marginal statistical significance of the fixed effect coefficients was assessed by conditional  $t$ -  
181 tests (Pinheiro and Bates, 2000). Conditional  $F$ -tests were applied to jointly test for the  
182 significance of the VFA balance coefficients and, hence, of the VFA composition. Statistical  
183 significance was concluded when associated  $P$ -values were  $< 0.05$ . An approximate model  
184 goodness-of-fit measure for mixed models was provided by using the marginal and  
185 conditional  $R^2$  coefficients for mixed models ( $R_m^2$ , % variance explained by fixed terms; and  
186  $R_c^2$ , % variance explained by both fixed and random terms) proposed in Nakagawa and  
187 Schielzeth (2013). Note however that, due to the complications added by the random effects  
188 structure in mixed models, these are pseudo- $R^2$  coefficients. Hence, interpretation and  
189 comparison of model explained variances based on these coefficients must be conducted with  
190 extreme caution. Comparison of models with nested fixed effect structures and the same  
191 random effect structure was conducted using the Akaike information criterion (AIC) and  
192 likelihood ratio test (LRT) as obtained from maximum likelihood estimation of the models.  
193 Statistical analyses, including compositional analyses, and modelling were conducted in the  
194 R system for statistical computing v3.2 (R Core Team, 2016).

195

196

## RESULTS

### 197 *Exploratory Analysis of the VFA Composition*

198 Table 3 shows compositional summary statistics for the VFA composition across all  
199 the experiments (other ordinary statistics for individual diets are supplied in Appendix 1).

200 The overall CGM reveals that, as expected, acetate was the most abundant VFA (mean, 656  
201 mmol/mol), whereas iso-butyrate, iso-valerate, and valerate were all present at < 20  
202 mmol/mol. The results per diet type illustrate the differences in mean VFA profiles between  
203 them. The variation matrix indicates that acetate and butyrate held the strongest  
204 proportionality association ( $\tau = 0.10$ ). Contrarily, propionate and butyrate or iso-valerate  
205 were the least proportionally associated components ( $\tau = 0.29$ ).

206

## TABLE 3 (VFA SUMMARY)

207 The relative variation structure of the data and the relationships between samples  
208 (points) and VFA components (rays) were approximately represented in Fig. 1 using a  
209 compositional biplot (Aitchison and Greenacre, 2002) explaining 68% of the data variability.  
210 The lengths of the links between arrowheads approximate the log-ratio variances (Table 3) of  
211 the corresponding components. Thus, propionate showed in general the greatest log-ratio  
212 variances (lowest proportionality) with all the others, particularly with iso-valerate and  
213 butyrate (relationship highlighted using dashed lines). The samples were distinguished by  
214 diet type. Concentrate diet type mostly associated with greater relative amounts of valerate  
215 and propionate, Forage diet type mostly linked to acetate and the Mixed diet type  
216 intermediate between Concentrate and Forage diet types but also linked to greater relative  
217 amounts of butyrate and iso-valerate.

218

## FIGURE 1 (BIPLOT)

219 The variation matrix in Table 3 was used to arrange the VFA components into  
220 homogenous groups by the Ward's clustering method (Ward, 1963). This allowed for a  
221 hierarchical representation of the structure of proportionality relationships between VFA  
222 components in a dendrogram (Fig. 2).

223

## FIGURE 2 (DENDROGRAM)

224 Three groups of VFA components can be clearly distinguished: propionate and  
225 valerate, acetate and butyrate, and iso-butyrate and iso-valerate. This configuration is  
226 coherent with the biplot analysis above and can be used to define a set of compositional  
227 balances between VFA components based only on the proportionality structure inferred from  
228 the data. In particular, the balance contrasting propionate and valerate against the remaining  
229 VFA components (balance  $b_1$  in Eq. [2]), corresponding to the top split in Fig. 2, explained  
230 most of the total variability (totvar) in the data set (34.42%) and was used as starting point to  
231 generate a collection of balances through SBP (Table 4, SBP for Model A1). Note that the  
232 subsequent balances  $b_2, b_3, b_4$ , and  $b_5$  corresponded to log-contrasts between the components  
233 located at each of the two branches of the nodes of the dendrogram as indicated in Fig 2.

234 *Associations between CH<sub>4</sub> Emissions, VFA Composition, and Diet and Animal Covariates*  
235 *Model A1.*

236 Table 4 (top, Model A1) shows parameter estimates and statistical significances from  
237 the model for CH<sub>4</sub> yield based on VFA balances according to the relative variation structure  
238 of the data as detailed above. All the balances in Model A1 but  $b_5$  ( $P = 0.832$ ) and  $b_4$   
239 ( $P = 0.338$ ) were statistically significant. A joint test for the coefficients of the balances  
240 confirmed a statistically significant effect of the VFA composition as a whole ( $F =$   
241  $9.931$ ;  $P < 0.001$ ). The Ln BW was the only covariate not statistically significant ( $P =$   
242  $0.129$ ). The marginal and conditional  $R^2$  coefficients (64.65% and 68.60% respectively)  
243 reflected an acceptable model fit. Ordinary checks for normality and homogeneity of  
244 variances of the model residuals were satisfactory (not shown).

245 TABLE 4 (CLMM results)

246 *Model A2.*

247 The fact that some balances were not statistically significant in Model A1 raised the  
248 question of whether some VFA components were not relevant to explain CH<sub>4</sub> yield. Some

249 further exploratory analysis suggested that the SBP used for Model A1 could be refined by  
 250 using a balance representing the main VFA responsible for H<sub>2</sub> production (acetate and  
 251 butyrate) and consumption (propionate) during carbohydrate fermentation. This was achieved  
 252 by defining the SBP shown in Table 4 (bottom) for Model A2, with a new balance  $b_2$  given  
 253 by

$$b_2 = \sqrt{\frac{2}{3}} \ln \frac{(\text{acetate} \cdot \text{butyrate})^{1/2}}{\text{propionate}}. \quad [4]$$

254 Under this arrangement, which is simply a re-parametrization of Model A1, only the  
 255 new balance  $b_2$  was statistically significant. This result clearly picks out the log-contrast  
 256 acetate-butyrate versus propionate (Eq. [4]) as the main driver of the relationship between  
 257 CH<sub>4</sub> yield and VFA composition. It is important to stress at this point that Model A2 is  
 258 entirely equivalent to Model A1, except for the use of a different set of ilr coordinates which  
 259 allowed to test for the significance of a relationship of biological interest and enhanced  
 260 interpretability. Overall measures,  $R^2$  coefficients, AIC, model intercepts, estimates for the  
 261 remaining covariates and random effects estimates were all identical. The technical reason for  
 262 this is that different ilr transformations are orthogonal rotations of each other, and those  
 263 estimates are invariant under such rotations. The AIC measure was used to rank the  
 264 explanatory variables according to their relative importance by the sum of Akaike weights  
 265 (Burnham and Anderson, 2002) over all possible models from Model A2 in which the  
 266 variable was included. Note that all the statistically significant variables (diet type, Ln DMI,  
 267 Ln ME, and  $b_2$ ; see Table 4) were given the same Akaike weight of 1 (values range between  
 268 0 and 1) and, hence, they were all considered of analogous importance in the model.

### 269 **Model B.**

270 Instead of considering the entire VFA composition, we fitted here a simplified LMM  
 271 for CH<sub>4</sub> yield based only on balance  $b_2$  from Model A2 (Eq. [4]; summary statistics in

272 Appendix 1) and the statistically significant covariates (see parameter estimates in Table 5).  
273 A LRT to compare Model A2 with Model B provided a statistically non-significant result  
274 (LR statistic = 6.36;  $P = 0.273$ ) and, hence, supported the use of the simplified model  
275 following the principle of parsimony. The estimated  $\beta$  coefficients for the covariates were  
276 essentially the same as obtained before. Note that a model not including  $b_2$  as explanatory  
277 variable but including the other covariates produced overall estimates  $R_m^2 = 57.11\%$ ,  
278  $R_c^2 = 62.33\%$  and AIC = -170.28. The corresponding LRT (LR statistic = 42.87;  $P < 0.001$ )  
279 supported the inclusion of the VFA compositional balance  $b_2$  in the model.

#### 280 TABLE 5 (MODEL B RESULTS)

281 The relationships between CH<sub>4</sub> yield and the subset of VFA components involved in  
282 Eq. [4] are depicted in Fig. 3. Propionate was entered independently whereas acetate and  
283 butyrate concentrations, on the one hand, and the remaining VFA components, on the other  
284 hand, were entered together by geometric mean to obtain the [propionate, acetate · butyrate,  
285 others] subcomposition. The observed values were displayed on a ternary diagram, with the  
286 diet types distinguished by color and shape. Model B was used to produce expected Ln CH<sub>4</sub>  
287 yields from randomly generated samples of this subcomposition. An interpolated surface was  
288 fitted to them and used to fill the ternary diagram with colors according to the values.

#### 289 FIGURE 3 (TERNARY DIAGRAM)

290 Each vertex of the ternary diagram corresponds with corner VFA subcompositions  
291 consisting of 100% of the component represented in there and 0% of any other. Maximum  
292 data variability occurred along the propionate to (acetate · butyrate) direction, with little  
293 variability observed in the direction pointing to the other vertex. The lowest expected CH<sub>4</sub>  
294 yields were associated with the greatest propionate concentrations, whereas the greatest  
295 expected CH<sub>4</sub> yields were associated with the greatest (acetate · butyrate) concentrations.

296 Using Model B as basis, separate LMMs were fitted to check whether the relationship  
297 between the disclosed VFA balance (Eq. [4]) and CH<sub>4</sub> yield was reproduced within a diet  
298 type. For both Concentrate and Mixed diet types we obtained a similar statistically highly  
299 significant positive estimated  $\beta$  coefficient for the balance representing the (acetate  
300 butyrate) / propionate ratio ( $P < 0.001$ ), thus supporting the association once the effects of  
301 DMI and ME were accounted for within these diet types (see summary tables in Appendix 2).  
302 As expected the amount of unexplained random variability increased in relation to the overall  
303 Model B. The effect of the VFA balance was however negligible within the Forage diet type  
304 ( $\hat{\beta} = 0.073$ ;  $P = 0.704$ ; summary table not shown). The number of animals on the Forage  
305 diet type was lower (45 animals versus 88 and 151 on the Concentrate and Mixed diet types  
306 respectively) and the forage constituents of the 2 trials which contributed to the Forage diet  
307 type were very different, which resulted in a sparse data set for this case.

308

309

## DISCUSSION

310 The use of compositional methods within the natural sciences is rapidly increasing,  
311 with applications found in the study of environmental pollution (Howel, 2007), aroma  
312 volatile compounds (Korhonová et al., 2009), meat fatty acid composition (Ros-Freixedes  
313 and Estany, 2014) and free-ranging animal diets (Stewart et al., 2014), among others. It has  
314 been shown that treating the components in isolation, without relating them to each other,  
315 may lead to misleading or paradoxical conclusions. For example, the standard linear  
316 correlation measured between same 2 components can dramatically vary depending on the  
317 other components considered in the composition (Palarea-Albaladejo and Martín-Fernández,  
318 2013). As an illustration of this, using our own VFA data, the correlation between iso-  
319 butyrate and iso-valerate is 0.62 when the entire 6-component VFA composition is  
320 considered, whereas it is -0.87 when working with only the [iso-butyrate, iso-valerate,

321 valerate] subcomposition. Other common inconsistencies include statistical confidence  
322 intervals covering nonsensical negative values or singularity and collinearity problems in  
323 linear models (Fox, 1997; Hron et al., 2012). In this work, we introduced compositional  
324 linear mixed models to investigate the association between rumen VFA and CH<sub>4</sub> yield from  
325 individual cattle, while accounting for the effects of other animal and diet-related covariates  
326 and the random variation originating from multiple experiments. Meaningful normalized log-  
327 contrasts (compositional balances) were defined between subsets of VFA components and  
328 their contribution to variability in CH<sub>4</sub> yields investigated.

329         Exploratory analysis revealed interesting proportionality associations between the  
330 VFA components and their connections with different diet types (Fig. 1). The VFA  
331 composition as a whole had a statistically significant association with CH<sub>4</sub> yield. The links  
332 between VFA components depicted in Fig. 2, and used in Model A1 (Table 4), described well  
333 the underlying stoichiometry of carbohydrate fermentation (Wolin, 1960). Thus, VFA  
334 associated with H<sub>2</sub> production, acetate and butyrate, were closely related and well separated  
335 from propionate and valerate which are associated with H<sub>2</sub> consumption. The branched chain  
336 VFA, iso-butyrate and iso-valerate were also closely related as might be expected given that  
337 these VFA are products of the catabolism of branched-chain AA. The closer alignment of the  
338 branched chain VFA with acetate and butyrate than with propionate and valerate is consistent  
339 with the requirement for these iso-acids by structural carbohydrate fermenting bacteria such  
340 as *Ruminococcus albus* (Allison and Bryant, 1963; Liu et al., 2014; Wang et al., 2015).  
341 Acetate, propionate, and butyrate are quantitatively the most important VFA in the rumen  
342 environment. When a log-contrast representing these 3 VFA (Eq. [4]) was used in Model A2  
343 (Table 4), it was the only compositional balance having a statistically significant effect on  
344 CH<sub>4</sub> yield. Note that it is analogous to the so-called glucogenic ratio [(acetate + butyrate) /  
345 propionate]; the  $\beta$  coefficient was positive as would be expected because H<sub>2</sub> available for



346 CH<sub>4</sub> formation would be expected to be positively correlated with this ratio. Indeed, this is  
347 the best empirical representation of the mechanistic relationships proposed by Wolin (1960)  
348 relating CH<sub>4</sub> production to VFA. The relationship was consistent across **concentrate and**  
349 **mixed** diet types. Thus, compositional analysis produced relationships between VFA which  
350 described accurately the underlying biology. Note that using an alternative SBP to isolate a  
351 balance between butyrate only and propionate (in the numerator and denominator  
352 respectively of Eq. [1]) provided results very similar to those using the balance in Eq. [4]. We  
353 also implemented a SBP to test the compositional balance representing the exchange between  
354 acetate and propionate only, which has been advocated in Janssen (2010) and Sauvant et al.  
355 (2011). Its effect on CH<sub>4</sub> yield was highly statistically significant as well ( $P = 0.005$ ),  
356 however in this case it was not the only statistically significant balance in the model and,  
357 hence, the results were not so neat. This would then confer butyrate concentrations relative to  
358 propionate a leading role in the balance given by Eq. [4].

359 In developing models, other animal (DMI, BW) and nutritional (diet type, ME)  
360 covariates were tested and DMI, diet type, and ME were included and their influence on the  
361 model was in the direction expected. Thus, DMI was associated with a negative  $\beta$  coefficient  
362 recognizing that increased DMI is associated with increased rumen outflow and decreased  
363 extent of fermentation and therefore CH<sub>4</sub> production. Diet type was included as the diets fed  
364 were grouped into three distinct forage to concentrate ratios. The use of starch and NDF  
365 concentrations and the log-ratio NDF/starch were tested directly as covariates. However, they  
366 were highly related to each other and the information about the variation in chemical  
367 composition of diets was robustly represented by ME and diet type. From a statistical point of  
368 view, using diet type also provided greater consistency and numerical stability of the  
369 estimation process. Diet type was associated with a positive  $\beta$  coefficient as it changed from  
370 the Concentrate to the Mixed and then to the Forage diet type as expected from the mean

371 values for CH<sub>4</sub> yield for the Concentrate (14.7 g/kg DMI) and Mixed (22.2 g/kg DMI) diet  
372 types (derived from Table 2). At first sight the positive  $\beta$  coefficient for the Forage diet type  
373 appears anomalous as mean CH<sub>4</sub> yield (17.6 g/kg DMI) was less than for the Mixed diet type.  
374 High forage diets are normally associated with high CH<sub>4</sub> yields because the high structural  
375 fiber content of these diets produces an acetate-dominated fermentation which was indeed  
376 observed (Table 3). The explanation for this apparent anomaly is that 2 diets in the Forage  
377 diet type had low ME concentrations (Table 1). Thus, the positive  $\beta$  coefficient associated  
378 with ME in the model likely adjusts responses for the digestibilities of these diets.

379 Many empirical models relating animal and diet variables to CH<sub>4</sub> have been produced  
380 (recent examples include Hristov et al., 2013a; Storlien et al., 2014; Ricci et al., 2013; Ramin  
381 and Huhtanen, 2013). Where CH<sub>4</sub> production is scaled either as g/kg DMI (as in the present  
382 study) or as kJ/MJ GE intake (Hristov et al., 2013a; Ramin and Huhtanen, 2013), terms  
383 related to DMI, diet digestibility and diet composition have been included in models. The  
384 inclusion of VFA in empirical models has been less common, largely because of scarcity of  
385 data. In some models diet composition has been used. Alemu et al., (2011) used VFA molar  
386 proportions predicted from the stoichiometry of fermentation to explain CH<sub>4</sub> and found that  
387 goodness of fit was model dependent. Ramin and Huhtanen (2013) compared the goodness of  
388 fit for CH<sub>4</sub> from models which including observed VFA proportions and concluded as here  
389 that models which included terms relating to combinations of VFA (acetate, propionate, and  
390 butyrate) gave superior explanations of CH<sub>4</sub> yield than individual VFA. However, all models  
391 noted above did not consider the compositional aspect of the data, that is, their natural  
392 relative and symmetric scale, and were based on treatment means for diets and did not use  
393 individual animal data in their analysis. Indeed, use of individual animal data in analysis of  
394 CH<sub>4</sub> production is not common. Mills et al. (2001) compared observed CH<sub>4</sub> production with  
395 that predicted from the mechanistic models of Dijkstra et al. (1992) and found relationships

396 were superior at the treatment than individual animal level; however, data for VFA  
397 proportions was not reported. Robinson et al. (2010) generated a range of VFA  
398 concentrations by varying DMI of sheep ( $n = 10$ ) fed a single diet of lucerne chaff and found  
399 that VFA concentrations only accounted for 25 – 30% of the variance. The present study was  
400 not comparable with Robinson et al. (2010) because of the narrow range of VFA molar  
401 proportions (700 – 730 mmol/mol acetate) used in that study. In the current study, a much  
402 greater variation in CH<sub>4</sub> yields and range of VFA proportions was available.

403 In reviewing mitigation options for reducing enteric CH<sub>4</sub> emissions, Hristov et al. (2013b)  
404 classified strategies into manure management practices and animal husbandry (which  
405 included genetics). Hristov et al. (2013b) noted that from genetic variation a reduction in  
406 predicted CH<sub>4</sub> production in the order of 11 to 26 percent was theoretically possible and that  
407 genomic selection tools could further increase the reduction in CH<sub>4</sub> production. However,  
408 effective application of genomic selection required significant international effort and  
409 collaboration to bring together relevant data because of the large datasets required. Because  
410 phenotypic CH<sub>4</sub> measurements are produced mainly using indirect respiration chambers,  
411 genetic progress is limited by slow throughput and cost. **Therefore, there is a need for indirect  
412 proxy measurements for CH<sub>4</sub> emissions which are capable of rapid throughput and lower cost  
413 than chambers and these have been recently reviewed by Negussie et al. (2017) including  
414 critical appraisal of their limitations.** The rumen samples which were used for measurement  
415 of VFA in the current study were of necessity single spot samples and, therefore, would not  
416 have captured changes in response to feed intake and fermentation. However, as cattle had  
417 not been given access to fresh feed for 25 h when sampled, variation due to short term feed  
418 intake would have been minimized and variability in VFA molar proportions between  
419 animals and experiments due to differences in feeding pattern reduced. Further cattle are

420 often fasted before slaughter and therefore results from the current study could potentially  
421 apply to samples taken at slaughter.

422         Although we found the association between VFA composition and CH<sub>4</sub> yield highly  
423 statistically significant ( $P < 0.001$ ), particularly through the (acetate · butyrate) / propionate  
424 compositional balance, there were other terms involved and the dataset included only beef  
425 cattle. If the use of VFA were implemented in practice, standardized protocols, such as those  
426 for determining residual feed intake (Basarub et al., 2003) may be appropriate. Other factors  
427 including rumen pH, protozoal population, and substrate utilization by methanogens, related  
428 to rumen fermentation of individual animals not captured by the VFA balances are probably  
429 responsible for unexplained variation and have been reviewed by Ellis et al. (2008). More  
430 recently, the genetic background of the animal has been shown to be important (King et al.,  
431 2011; Hernandez-Sanabria et al., 2013) as are inter-related phenotypic factors such as rumen  
432 size (Goopy et al., 2013), feed intake pattern (Carberry et al., 2014) and colonization of the  
433 rumen after birth (Yanez-Ruiz et al., 2015) which determine the host-specificity of the rumen  
434 microbiome (Weimer et al., 2010; Wallace et al., 2015). Indeed, in one of the experiments  
435 which contributed to the present data set (Rooke et al., 2016) the VFA pattern present in  
436 individual animals before imposition of experimental treatments was a significant covariate  
437 for subsequent samples. Apart from animal factors, differences in diet characteristics may  
438 influence the fate of H<sub>2</sub> in the rumen and variability in VFA pattern. Where nitrate was used  
439 as a mitigation strategy (e.g. Troy et al., 2015) reduction of nitrate to ammonia diverts H<sub>2</sub>  
440 away from VFA formation and increases the ratio of acetate to propionate. This is  
441 contradictory to balance  $b_2$  (Table 5) and may have contributed to the unexplained variation  
442 in the overall model.

443

444

## CONCLUSIONS

445 This work demonstrated the use and benefits of a novel statistical approach to the  
446 analysis of VFA compositions from individual animals and multiple experiments. The results  
447 were coherent with biological knowledge and emphasized the contribution of rumen VFA to  
448 explain cattle CH<sub>4</sub> yield at an individual animal level. Further research is needed to determine  
449 other possible contributing factors and investigate the scope for setting up more specialized  
450 empirical models within the same compositional framework to improve predictive capacity  
451 based on VFA measurements.

452

453

#### CONFLICT OF INTEREST

454 The authors state that there is no conflict of interest in relation to this work.

455

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#### REFERENCES

457 Aitchison, J. 1986. *The Statistical Analysis of Compositional Data*. Chapman and Hall,  
458 London.

459 Aitchison, J., and M. Greenacre. 2002. Biplots of compositional data. *J. R. Stat. Soc. Ser. C*  
460 (Applied Stat.) 51:375–392. doi: 10.1111/1467-9876.00275.

461 Alemu, A. W., J. Dijkstra, A. Bannink, J. France, and E. Kebreab. 2011. Rumen  
462 stoichiometric models and their contribution and challenges in predicting enteric  
463 methane production. *Anim. Feed Sci. Technol.* 166–167:761–778. doi:  
464 10.1016/j.anifeedsci.2011.04.054.

465 Allison, M. J. and M. P. Bryant. 1963. Biosynthesis of branched-chain amino acids from  
466 branched-chain fatty acids by rumen bacteria. *Arch. Biochem. Biophys.* 101:269–277.  
467 doi: 10.1016/S0003-9861(63)80012-0.

468 Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003.  
469 Residual feed intake and body composition in young growing cattle. *Can. J. Anim.*

- 470 Sci. 83:189–204. doi: 10.4141/A02-065.
- 471 Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a  
472 practical information-theoretic approach. Springer, New York.
- 473 Carberry, C. A., D. A. Kenny, A. K. Kelly, and S. M. Waters. 2014. Quantitative analysis of  
474 ruminal methanogenic microbial populations in beef cattle divergent in phenotypic  
475 residual feed intake (RFI) offered contrasting diets. *J. Anim. Sci. Biotechnol.* 5(1):41.  
476 doi: 10.1186/2049-1891-5-41.
- 477 Carberry, C. A., S. M. Waters, D. A. Kenny, and C. J. Creevey. 2014. Rumen methanogenic  
478 genotypes differ in abundance according to host residual feed intake phenotype and  
479 diet type. *Appl. Environ. Microbiol.* 80:586–594. doi: 10.1128/AEM.03131-13.
- 480 Casellas, J., and N. Ibáñez-Escriche. 2012. Bayesian recursive mixed linear model for gene  
481 expression analyses with continuous covariates. *J. Anim. Sci.* 90:67–75. doi:  
482 10.2527/jas.2010-3750.
- 483 Dijkstra, J., H. D. S. C. Neal, D. E. Beever, and J. France. 1992. Simulation of nutrient  
484 digestion, absorption and outflow in the rumen - model description. *J. Nutr.*  
485 122:2239–2256.
- 486 Duthie, C. A., J. A. Rooke, J. J. Hyslop, and A. Waterhouse. 2015. Methane emissions from  
487 two breeds of beef cows offered diets containing barley straw with either grass silage  
488 or brewers' grains. *Animal* 9:1680–1687. doi: 10.1017/S1751731115001251.
- 489 Duthie, C. A., J. A. Rooke, S. Troy, J. J. Hyslop, D. W. Ross, A. Waterhouse, and R. Roehe.  
490 2016. Impact of adding nitrate or increasing the lipid content of two contrasting diets  
491 on blood methaemoglobin and performance of two breeds of finishing beef steers.  
492 *Animal* 10:786–795. doi: 10.1017/S1751731115002657.
- 493 Duthie, C. A., M. Haskell, J. J. Hyslop, A. Waterhouse, R. J. Wallace, R. Roehe, and J. A.  
494 Rooke. 2017. The impact of divergent breed types and diets on methane emissions,

- 495 rumen characteristics and performance of finishing beef cattle. *Animal* (in press). doi:  
496 10.1017/S1751731117000301.
- 497 Egozcue, J. J., V. Pawlowsky-Glahn, G. Mateu-Figueras, and C. Barceló-Vidal. 2003.  
498 Isometric logratio transformations for compositional data analysis. *Math. Geol.*  
499 35:279–300. doi: 10.1023/A:1023818214614.
- 500 Egozcue, J. J., and V. Pawlowsky-Glahn. 2005. Groups of parts and their balances in  
501 compositional data analysis. *Math. Geol.* 37:795–828. doi: 10.1007/s11004-005-7381-  
502 9.
- 503 Ellis, J. L., J. Dijkstra, E. Kebreab, A. Bannink, N. E. Odongo, B. W. McBride, and J. France.  
504 2008. Aspects of rumen microbiology central to mechanistic modelling of methane  
505 production in cattle. *J. Agric. Sci.* 146:213–233. doi: 10.1017/S0021859608007752.
- 506 Fox, J. 1997. *Applied regression analysis, linear models, and related methods*. SAGE  
507 Publications, Inc.
- 508 Gerber, P.J., H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, and G.  
509 Tempio 2013. *Tackling climate change through livestock – A global assessment of*  
510 *emissions and mitigation opportunities*. Food and Agriculture Organization of the  
511 United Nations, Rome, Italy.
- 512 Goopy, J. P., A. Donaldson, R. Hegarty, P. E. Vercoe, F. Haynes, M. Barnett, and V. H.  
513 Oddy. 2014. Low-methane yield sheep have smaller rumens and shorter rumen  
514 retention time. *Br. J. Nutr.* 111:578–585. doi: 10.1017/S0007114513002936.
- 515 Hernandez-Sanabria, E., L. A. Goonewardene, Z. Q. Wang, M. Zhou, S. S. Moore, and L. L.  
516 Guan. 2013. Influence of sire breed on the interplay among rumen microbial  
517 populations inhabiting the rumen liquid of the progeny in beef cattle. *PLoS One* 8(3):  
518 e58461. doi: 10.1371/journal.pone.0058461.
- 519 Howel, D. 2007. *Multivariate data analysis of pollutant profiles: PCB levels across Europe*.

- 520 Chemosphere 67:1300–1307. doi: 10.1016/j.chemosphere.2006.11.025.
- 521 Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A.  
522 T. Adesogan, W. Yang, C. Lee, P. J. Gerber, B. Henderson, and J. M. Tricarico.  
523 2013a. SPECIAL TOPICS-Mitigation of methane and nitrous oxide emissions from  
524 animal operations: I. A review of enteric methane mitigation options. *J. Anim. Sci.*  
525 91:5045–5069. doi: 10.2527/jas.2013-6583
- 526 Hristov, A. N., J. Oh, C. Lee, R. Meinen, F. Montes, T. Ott, J. Firkins, A. Rotz, C. Dell, A.  
527 Adesogan, W. Yang, J. Tricarico, E. Kebreab, G. Waghorn, J. Dijkstra, and S.  
528 Oosting. 2013b. Mitigation of greenhouse gas emissions in livestock production – A  
529 review of technical options for non-CO<sub>2</sub> emissions. In: P. Gerber, B. Henderson and  
530 H. P. S. Makkar, editors *FAO Animal Production and Health Paper No. 177*. Food  
531 and Agricultural Organisation, Rome, Italy.
- 532 Hron, K., P. Filzmoser, and K. Thompson. 2012. Linear regression with compositional  
533 explanatory variables. *J. Appl. Stat.* 39:1115–1128. doi:  
534 10.1080/02664763.2011.644268.
- 535 Janssen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation  
536 balances through microbial growth kinetics and fermentation thermodynamics. *Anim.*  
537 *Feed Sci. Technol.* 160, 1–22. doi: <http://dx.doi.org/10.1016/j.anifeedsci.2010.07.002>.
- 538 King, E. E., R. P. Smith, B. St-Pierre, and A. D. G. Wright. 2011. Differences in the rumen  
539 methanogen populations of lactating jersey and holstein dairy cows under the same  
540 diet regimen. *Appl. Environ. Microbiol.* 77:5682–5687. doi: 10.1128/AEM.05130-  
541 11.
- 542 Korhonová, M., K. Hron, D. Klimčíková, L. Müller, P. Bednár, and P. Barták. 2009. Coffee  
543 aroma - statistical analysis of compositional data. *Talanta* 80:710–715. doi:  
544 10.1016/j.talanta.2009.07.054.



- 545 Liu, Q., C. Wang, C. X. Pei, H. Y. Li, Y. X. Wang, S. L. Zhang, Y. L. Zhang, J. P. He, H.  
546 Wang, W. Z. Yang, Y. S. Bai, Z. G. Shi, and X. N. Liu. 2014. Effects of isovalerate  
547 supplementation on microbial status and rumen enzyme profile in steers fed on corn  
548 stover based diet. *Livest. Sci.* 161:60–68. doi: 10.1016/j.livsci.2013.12.034.
- 549 Murphy, M. R., R. L. Baldwin, and L. J. Koong. 1982. Estimation of stoichiometric  
550 parameters for rumen fermentation of roughage and concentrate diets. *J. Anim Sci.*  
551 55:411–421. doi: 10.3168/jds.2012-6042.
- 552 Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining  $R^2$  from  
553 generalized linear mixed-effects models. *Methods Ecol. Evol.* 4:133–142. doi:  
554 10.1111/j.2041-210x.2012.00261.x.
- 555 Negussie, E., de Haas, Y., Dehareng, F., Dewhurst, R. J., Dijkstra, J., Gengier, N., Morgavi,  
556 D. P., Soyeurt, H., van Gastelen, S., Yan, T. and Biscarini, F. 2017. Invited review:  
557 Large-scale indirect measurements for enteric methane emissions in dairy cattle: A  
558 review of proxies and their potential for use in management and breeding strategies. *J.*  
559 *Dairy Sci.*, 100: 2433–2453. doi: 10.3168/jds.2016-12030.
- 560 Palarea-Albaladejo, J., and J. A. Martín-Fernández. 2013. Values below detection limit in  
561 compositional chemical data. *Anal. Chim. Acta* 764:32–43. doi:  
562 10.1016/j.aca.2012.12.029.
- 563 Pawlowsky-Glahn, V., and J. Egozcue. 2002. BLU estimators and compositional data. *Math.*  
564 *Geol.* 34:259–274. doi: 10.1023/A:1014890722372.
- 565 Pawlowsky-Glahn, V., J. J. Egozcue, and R. Tolosana-Delgado. 2015. Modelling and  
566 analysis of compositional data. John Wiley & Sons, Ltd.
- 567 Pinheiro, J. C., and D. M. Bates. 2000. Mixed effects models in S and S-Plus. Springer-  
568 Verlag.
- 569 Poulsen, M., C. Schwab, B. B. Jensen, R. M. Engberg, A. Spang, N. Canibe, O. Hojberg, G.

- 570 Milinovich, L. Fragner, C. Schleper, W. Weckwerth, P. Lund, A. Schramm, and T.  
571 Urich. 2013. Methylophilic methanogenic Thermoplasmata implicated in reduced  
572 methane emissions from bovine rumen. *Nature Commun.* 4:1428. doi:  
573 10.1038/ncomms2432.
- 574 Ramin, M. and P. Huhtanen. 2013. Development of equations for predicting methane  
575 emissions from ruminants. *J. Dairy Sci.* 96:2476–2493. doi: 10.3168/jds.2012-6095.
- 576 R Core Team. 2016. R: A Language and Environment for Statistical Computing. R  
577 Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- 578 Ricci, P., J. A. Rooke, I. Nevison, and A. Waterhouse. 2013. Methane emissions from beef  
579 and dairy cattle: Quantifying the effect of physiological stage and diet characteristics.  
580 *J. Anim Sci.* 91:5379–5389. doi: 10.2527/jas.2013-6544.
- 581 Robinson, D. L., J. Goopy, and R. S. Hegarty 2010. Can rumen methane production be  
582 predicted from volatile fatty acid concentrations? *Anim. Prod. Sci.* 50: 630–636. doi:  
583 10.1071/AN09214.
- 584 Rooke, J. A., R. J. Wallace, C. A. Duthie, N. McKain, S. M. de Souza, J. J. Hyslop, D. W.  
585 Ross, T. Waterhouse, and R. Roehe. 2014. Hydrogen and methane emissions from  
586 beef cattle and their rumen microbial community vary with diet, time after feeding  
587 and genotype. *Br. J. Nutr.* 112:398–407. doi: 10.1017/S0007114514000932.
- 588 Rooke, J. A., S. M. Troy, C.-A. Duthie, J. J. Hyslop, D. W. Ross, T. Waterhouse, R. Roehe,  
589 and R. J. Wallace 2016. Long-term changes in rumen volatile fatty acids (VFA) and  
590 archaea to bacteria (AB) ratios in cattle fed either high concentrate or mixed  
591 forage:concentrate diets with or without the addition of nitrate (NO<sub>3</sub>). *Adv. Anim.*  
592 *Biosci.* 7:53.
- 593 Ros-Freixedes, R., and J. Estany. 2014. On the compositional analysis of fatty acids in pork.  
594 *J. Agric. Biol. Environ. Stat.* 19:136–155. Doi: 10.1007/s13253-013-0162-x.

- 595 Rymer, C., and Agnew, R. E. 2004. Summary of characterisation methods. In: C. Thomas,  
596 editor, *Feed into Milk*. Nottingham University Press, Nottingham, UK.
- 597 Sauvant, D., S. Giger-Reverdin, A. Serment and L. Broudicou. 2011. Influences of diet and  
598 rumen fermentation on methane production by ruminants. *INRA Prod. Anim.* 24:433–  
599 446.
- 600 St-Pierre, N. R. 2001. Integrating quantitative findings from multiple studies using mixed  
601 model methodology. *J. Dairy Sci.* 84:741–755. doi: 10.3168/jds.S0022-  
602 0302(01)74530-4.
- 603 Stewart, C., S. Iverson, and C. Field. 2014. Testing for a change in diet using fatty acid  
604 signatures. *Environ. Ecol. Stat.* 21:775–792. doi: 10.1007/s10651-014-0280-9.
- 605 Storlien, T. M., H. Volden, T. Almoy, K. A. Beauchemin, T. A. McAllister, and O. M.  
606 Harstad. 2014. Prediction of enteric methane production from dairy cows. *Acta Agric.*  
607 *Scand. Sect. A-Anim. Sci.* 64:98–109. doi: 10.1080/09064702.2014.959553.
- 608 Troy, S. M., C. A. Duthie, J. J. Hyslop, R. Roehe, D. W. Ross, R. J. Wallace, A. Waterhouse,  
609 and J. A. Rooke. 2015. Effectiveness of nitrate addition and increased oil content as  
610 methane mitigation strategies for beef cattle fed two contrasting basal diets. *J. Anim.*  
611 *Sci.* 93:1815–1823. doi: 10.2527/jas.2014-8688.
- 612 Troy, S. M., C. A. Duthie, J. J. Hyslop, D. W. Ross, R. Roehe, and J. A. Rooke. 2016. The  
613 effects of dietary nitrate addition and increased lipid concentration on methane (CH<sub>4</sub>)  
614 and hydrogen (H<sub>2</sub>) emissions from beef cattle are independent. *Adv. Anim. Biosci.* 7:  
615 50.
- 616 Wallace, R. J., J. A. Rooke, N. McKain, C. A. Duthie, J. J. Hyslop, D. W. Ross, A.  
617 Waterhouse, M. Watson, and R. Roehe. 2015. The rumen microbial metagenome  
618 associated with high methane production in cattle. *BMC Genomics* 16. doi:  
619 10.1186/s12864-015-2032-0.

- 620 Wang, C., Q. Liu, Y. L. Zhang, C. X. Pei, S. L. Zhang, Y. X. Wang, W. Z. Yang, Y. S. Bai,  
621 Z. G. Shi, and X. N. Liu. 2015. Effects of isobutyrate supplementation on ruminal  
622 microflora, rumen enzyme activities and methane emissions in Simmental steers. *J.*  
623 *Anim. Physiol. Anim. Nutr.* 99:123–131. doi: 10.1111/jpn.12191.
- 624 Ward, J. H. 1963. Hierarchical grouping to optimize an objective function. *J. Amer. Statist.*  
625 *Assoc.* 58:236–244. doi: 10.1080/01621459.1963.10500845.
- 626 Weimer, P. J., D. M. Stevenson, H. C. Mantovani, and S. L. C. Man. 2010. Host specificity  
627 of the ruminal bacterial community in the dairy cow following near-total exchange of  
628 ruminal contents. *J. Dairy Sci.* 93:5902–5912. doi: 10.3168/jds.2010-3500.
- 629 Wolin, M. J. 1960. A theoretical rumen fermentation balance. *J. Dairy Sci.* 43:1452–1459.  
630 doi: 10.3168/jds.S0022-0302(60)90348-9.
- 631 Yanez-Ruiz, D. R., L. Abecia, and C. J. Newbold. 2015. Manipulating rumen microbiome  
632 and fermentation through interventions during early life: a review. *Frontiers*  
633 *Microbiol.* 6:1133. doi: 10.3389/fmicb.2015.01133.
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635 APPENDIX 1: SUMMARY STATISTICS OF THE VFA MOLAR PROPORTIONS AND  
 636 THE COMPOSITIONAL BALANCE BASED ON THE (ACETATE · BUTYRATE) /  
 637 PROPIONATE RATIO ( $b_2$  IN EQ. [4], MODELS A2 AND B) PER STUDY AND DIET

Study <sup>1,3</sup>	Diet		Acetate	Propionate	Butyrate	Iso- butyrate	Iso- valerate	Valerate	$b_2$
Concentrate diet-type (forage less than 100 g/kg DM)									
1	1	Minimum	477	163	64	8	6	12	-0.71
		Q1 <sup>2</sup>	554	240	77	10	10	14	-0.38
		Median	560	310	96	11	13	15	-0.24
		Q3 <sup>2</sup>	566	329	122	14	23	17	0.10
		Maximum	645	417	155	17	33	27	0.54
2	3	Minimum	482	152	45	9	4	12	-0.78
		Q1	544	235	80	11	10	14	-0.36
		Median	562	284	95	12	16	16	-0.13
		Q3	590	318	131	16	28	20	0.03
		Maximum	668	411	221	20	72	31	0.47
4	7	Minimum	401	183	46	7	7	8	-0.93
		Q1	500	264	65	8	10	17	-0.57
		Median	535	335	80	14	15	18	-0.46
		Q3	561	402	123	17	21	22	-0.03
		Maximum	635	453	132	21	23	30	0.37
4	8	Minimum	527	126	59	9	10	6	-0.55
		Q1	561	181	66	11	12	10	-0.43
		Median	596	274	83	12	16	16	-0.21
		Q3	629	327	123	13	20	21	0.42
		Maximum	694	359	192	29	28	23	0.84
4	9	Minimum	495	177	50	10	10	10	-0.76
		Q1	527	239	65	15	16	16	-0.62
		Median	539	312	83	23	23	18	-0.30

		Q3	586	364	106	23	26	25	0.05
		Maximum	627	415	176	26	42	28	0.45
Mixed diet-type (400 – 600 g forage / kg DM)									
1	2	Minimum	541	105	107	10	11	10	0.16
		Q1	650	128	111	11	13	12	0.28
		Median	665	170	121	12	14	13	0.46
		Q3	705	190	144	12	15	13	0.64
		Maximum	725	229	152	32	36	17	0.92
2	4	Minimum	596	90	76	11	12	10	-0.18
		Q1	634	158	103	13	16	13	0.17
		Median	648	174	125	15	18	14	0.41
		Q3	665	207	141	16	21	15	0.54
		Maximum	707	269	177	21	27	17	1.07
4	10	Minimum	548	137	75	9	11	8	-0.42
		Q1	614	170	85	13	12	12	0.03
		Median	653	196	110	14	16	16	0.20
		Q3	661	235	119	16	17	20	0.46
		Maximum	686	341	161	17	19	26	0.60
4	11	Minimum	624	118	82	10	6	11	0.03
		Q1	659	144	116	12	8	12	0.33
		Median	668	164	119	13	12	16	0.45
		Q3	682	182	140	14	14	18	0.65
		Maximum	707	229	164	16	15	22	0.79
4	12	Minimum	603	136	84	10	12	6	-0.01
		Q1	623	160	99	12	14	13	0.08
		Median	635	206	107	14	16	16	0.23
		Q3	670	231	113	16	18	18	0.40
		Maximum	717	248	186	19	32	23	0.70
6	14	Minimum	597	120	82	4	8	8	-0.06
		Q1	645	146	115	8	13	11	0.26

		Median	665	180	123	10	15	12	0.38
		Q3	683	200	136	11	16	13	0.63
		Maximum	723	244	154	15	20	24	0.75
6	15	Minimum	650	118	104	7	4	7	0.34
		Q1	664	134	113	8	11	12	0.42
		Median	690	154	122	10	14	12	0.50
		Q3	697	170	138	10	17	14	0.68
		Maximum	720	189	163	11	23	17	0.84
6	16	Minimum	623	140	97	6	10	9	0.23
		Q1	662	150	107	7	12	10	0.38
		Median	678	158	124	9	14	11	0.48
		Q3	695	174	141	10	17	14	0.55
		Maximum	715	197	161	11	21	21	0.65
6	17	Minimum	675	121	93	6	8	1	0.36
		Q1	684	144	113	8	13	9	0.48
		Median	690	147	123	8	13	11	0.56
		Q3	708	154	134	9	15	12	0.59
		Maximum	736	177	143	12	17	14	0.79
Forage diet-type (>700 g forage / kg DM)									
3	5	Minimum	726	106	53	4	5	4	0.04
		Q1	751	129	60	6	6	5	0.23
		Median	767	144	65	6	7	6	0.35
		Q3	792	165	70	8	8	6	0.46
		Maximum	804	189	78	11	14	8	0.69
3	6	Minimum	694	125	63	2	5	4	0.19
		Q1	718	155	69	6	7	5	0.29
		Median	734	163	82	7	8	6	0.31
		Q3	748	170	86	8	9	7	0.37
		Maximum	790	196	99	10	11	11	0.51
5	13	Minimum	662	114	72	0	9	6	0.13

Q1	669	186	84	10	10	10	0.18
Median	676	202	91	10	11	10	0.19
Q3	698	203	98	12	12	11	0.22
Maximum	750	209	110	17	15	12	0.72

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<sup>1</sup>1, Rooke et al., 2014; 2, Duthie et al., 2017; 3, Duthie et al., 2015; 4, Troy et al., 2015; 5, unpublished observations; 6, Troy et al., 2016.

<sup>2</sup>Q1, Q3; respectively first and third quartiles.

<sup>3</sup>To obtain individual data please contact richard.dewhurst@sruc.ac.uk.



638 APPENDIX 2: ESTIMATES FOR THE CONCENTRATE AND MIXED DIET TYPES OF  
 639 THE SIMPLIFIED MIXED MODEL FOR METHANE EMISSIONS (G/KG DMI IN LOG  
 640 SCALE) USING THE COMPOSITIONAL BALANCE BASED ON THE (ACETATE ·  
 641 BUTYRATE) / PROPIONATE RATIO ( $b_2$  IN EQ. [4], MODELS A2 AND B) AND THE  
 642 STATISTICALLY SIGNIFICANT COVARIATES FROM THE OVERALL MODEL B

Concentrate diet type				
Fixed effects	$\hat{\beta}$	SE	<i>t</i> -value	<i>P</i> -value
Intercept	0.744	2.106	0.35	0.725
$b_2$	0.297	0.049	6.03	< 0.001
Ln ME, MJ/kg DM	1.026	0.834	1.23	0.222
Ln DMI, g/kg BW <sup>0.75</sup> /d	-0.279	0.098	-2.85	0.006
$R_m^2 = 32.33\%^1, R_c^2 = 38.69\%^1, \hat{\sigma}_f = 0.06^2, \hat{\sigma} = 0.17^2$				

643

Mixed diet type				
Fixed effects	$\hat{\beta}$	SE	<i>t</i> -value	<i>P</i> -value
Intercept	-0.805	1.520	-0.53	0.597
$b_2$	0.206	0.050	4.10	<0.001
Ln ME, MJ/kg DM	1.806	0.614	2.94	0.004
Ln DMI, g/kg BW <sup>0.75</sup> /d	-0.289	0.060	-4.82	< 0.001
$R_m^2 = 21.46\%, R_c^2 = 41.53\%, \hat{\sigma}_f = 0.07, \hat{\sigma} = 0.13$				

<sup>1</sup> $R_m^2, R_c^2$ ; respectively % variance explained by fixed terms (marginal) and by both fixed and random terms (conditional).

<sup>2</sup> $\hat{\sigma}_f, \hat{\sigma}$ ; respectively estimated standard deviations of the random effects and random error terms.

Table 1. Sources of data and nutritional characteristics of the diets used in the study

Study <sup>1</sup>	Diet	Forage, g/kg DM	Starch, g/kg DM	NDF, g/kg DM	ME <sup>2</sup> , MJ/kg DM
Concentrate diet type (forage less than 100 g/kg DM)					
1	1	80	412	254	12.3
2	3	79	415	248	12.8
4	7	84	439	227	12.2
4	8	80	476	204	12.0
4	9	78	416	211	12.9
Mixed diet type (400 – 600 g forage / kg DM)					
1	2	484	234	388	11.9
2	4	505	284	374	12.0
4	10	490	298	289	11.6
4	11	499	318	272	11.4
4	12	497	262	280	12.2
6	14	557	281	308	11.6
6	15	558	308	295	11.4
6	16	555	264	317	11.9
6	17	556	247	313	11.6
Forage diet type (>700 g forage / kg DM)					
3	5	774	65	771	7.4
3	6	1000	0	693	8.1
5	13	1000	36	473	10.7

<sup>1</sup>1, Rooke et al., 2014; 2, Duthie et al., 2017; 3, Duthie et al., 2015; 4, Troy et al., 2015; 5, unpublished observations; 6, Troy et al., 2016a.

<sup>2</sup>ME estimated from feed composition (Rymer and Agnew, 2004).

1 Table 2. Methane emissions, DMI, and BW of cattle included in database

2

Study <sup>1</sup>	Diet	<i>n</i>	BW, kg		DMI, kg/d		Methane, g/d		Methane, g/kg DMI	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Concentrate diet type (forage less than 100 g/kg DM)										
1	1	15	667	45.1	11.2	2.12	153	44.6	13.9	4.07
2	3	35	635	55.4	10.6	1.74	147	31.2	13.9	2.32
4	7	12	675	41.0	10.0	1.55	148	48.1	14.6	3.31
4	8	13	675	54.3	9.1	2.09	136	27.4	15.3	3.39
4	9	13	687	55.7	9.7	2.04	149	32.4	15.8	3.58
Mixed diet type (400 – 600 g forage / kg DM)										
1	2	13	652	37.4	9.4	1.09	218	46.6	23.4	4.99
2	4	33	605	54.5	9.2	1.59	189	40.2	20.5	3.05
4	10	12	703	40.6	9.8	1.55	235	36.8	24.3	5.06
4	11	12	707	30.2	10.4	1.56	212	25.4	20.6	2.60
4	12	12	705	25.7	10.5	1.53	242	35.2	23.2	2.10
6	14	17	673	26.1	10.4	1.80	245	46.9	23.8	3.58
6	15	16	649	52.5	9.8	2.18	214	42.8	22.1	2.68
6	16	18	652	24.1	10.2	1.41	238	38.6	23.4	2.79
6	17	18	652	33.9	10.2	1.86	210	27.5	20.9	2.48
Forage diet type (>700 g forage / kg DM)										
3	5	16	639	78.5	9.4	1.84	126	24.0	13.8	3.36
3	6	17	607	97.1	10.1	1.65	160	33.3	16.5	5.55
5	13	12	704	58.3	13.7	1.77	308	36.9	22.6	2.12

3 <sup>1</sup>1, Rooke et al., 2014; 2, Duthie et al., 2017; 3, Duthie et al., 2015; 4, Troy et al., 2015; 5, unpublished observations; 6, Troy et al., 2016.

4

5 Table 3. Compositional geometric mean (CGM), overall and by diet group, and relative  
6 variation matrix for the VFA composition across experiments

		Acetate	Propionate	Butyrate	Iso- butyrate	Iso- valerate	Valerate
	Overall	656	199	106	11	14	13
CGM <sup>1</sup> , mmol/mol	Concentrate	574	282	96	14	17	17
	Mixed	670	170	121	11	15	13
	Forage	739	162	77	8	8	7
Variation matrix <sup>2</sup>	Acetate		0.19	0.10	0.18	0.25	0.28
	Propionate	0.19		0.29	0.18	0.29	0.16
	Butyrate	0.10	0.29		0.18	0.21	0.23
	Iso-butyrate	0.18	0.18	0.18		0.13	0.15
	Iso-valerate	0.25	0.29	0.21	0.13		0.22
	Valerate	0.28	0.16	0.23	0.15	0.22	

7 <sup>1</sup>Normalized vector of geometric means of the VFA composition.

8 <sup>2</sup>Matrix of log-ratio variances between pairs of VFA components.

9

10 Table 4. Sequential binary partitions (SBP) providing alternative balance coordinate representations ( $b_i, i = 1, \dots, 5$ ) of the VFA composition and estimates of  
 11 the associated compositional mixed model for methane emissions (g/kg DMI) in log scale, Models A1 (top) and A2 (bottom)

	SBP <sup>1</sup>	$b_1$	$b_2$	$b_3$	$b_4$	$b_5$	Fixed effects	$\hat{\beta}$	SE	$t$ -value	$P$ -value	
Model A1	Iso-valerate	+	+		+		Intercept	-2.134	1.041	-2.05	0.041	
	Iso-butyrate	+	+		-		$b_1$	0.163	0.035	4.66	<0.001	
	Butyrate	+	-			+	$b_2$	-0.122	0.043	-2.82	0.005	
	Acetate	+	-			-	$b_3$	0.158	0.047	3.36	0.001	
	Valerate	-		+			$b_4$	0.043	0.044	0.96	0.338	
	Propionate	-		-			$b_5$	-0.017	0.078	-0.21	0.832	
	$r$		4	2	1	1	1	Mixed diet	0.374	0.034	11.13	<0.001
	$s$		2	2	1	1	1	Forage diet	0.732	0.101	7.23	0.002
	$R_m^2 = 64.65\%^2$ AIC = -207.51 <sup>3</sup>							Ln ME, MJ/kg DM	1.753	0.250	7.01	<0.001
	$R_c^2 = 68.60\%^2$ $\hat{\sigma}_f = 0.06^4$ $\hat{\sigma} = 0.16^4$							Ln BW, kg	0.195	0.128	1.52	0.129
Model A2	Acetate	+	+	+			Intercept	-2.134	1.041	-2.05	0.041	
	Butyrate	+	+	-			$b_1$	0.008	0.039	0.22	0.829	
	Propionate	+	-				$b_2$	0.256	0.050	5.11	<0.001	
	Valerate	-			+		$b_3$	0.017	0.078	0.21	0.832	
	Iso-butyrate	-			-	+	$b_4$	0.026	0.036	0.72	0.470	
	Iso-valerate	-			-	-	$b_5$	-0.043	0.045	-0.96	0.338	
	$r$		3	2	1	1	1	Mixed diet	0.374	0.034	11.13	<0.001
	$s$		3	1	1	2	1	Forage diet	0.732	0.101	7.23	0.002
	$R_m^2 = 64.65\%$ AIC = -207.51							Ln ME, MJ/kg DM	1.753	0.250	7.01	<0.001
	$R_c^2 = 68.60\%$ $\hat{\sigma}_f = 0.06$ $\hat{\sigma} = 0.16$							Ln BW, kg	0.195	0.128	1.52	0.129
							Ln DMI, g/kg BW <sup>0.75</sup> /d	-0.400	0.059	-6.84	<0.001	

<sup>1</sup> Symbol + means that a VFA component is allocated to the numerator of the corresponding compositional balance  $b_i$ , whereas - means that it is allocated to the denominator. Letters  $r$  and  $s$  refer to the number of VFA components in numerator and denominator respectively.

<sup>2</sup>  $R_m^2$ ,  $R_c^2$ ; respectively % variance explained by fixed terms (marginal) and by both fixed and random terms (conditional).

<sup>3</sup> Akaike information criterion measure of the relative quality of the model for the full data set.

<sup>4</sup>  $\hat{\sigma}_f$ ,  $\hat{\sigma}$ ; respectively estimated standard deviations of the random effects and random error terms.

- 12 Table 5. Estimates of the simplified mixed model (Model B) for methane emissions (g/kg  
 13 DMI in log scale) using the compositional balance based on the (acetate · butyrate) /  
 14 propionate ratio ( $b_2$  in Eq. [4], Model A2) and the statistically significant covariates

Fixed effects	$\hat{\beta}$	SE	$t$ -value	$P$ -value
Intercept	-0.837	0.613	-1.36	0.174
$b_2$	0.243	0.035	6.97	< 0.001
Mixed diet	0.374	0.033	11.47	< 0.001
Forage diet	0.720	0.105	6.83	0.002
Ln ME, MJ/kg DM	1.725	0.246	6.99	< 0.001
Ln DMI, g/kg BW <sup>0.75</sup> /d	-0.356	0.051	-6.96	< 0.001

$$R_m^2 = 63.51\%{}^1 \quad \text{AIC} = -211.15{}^2$$

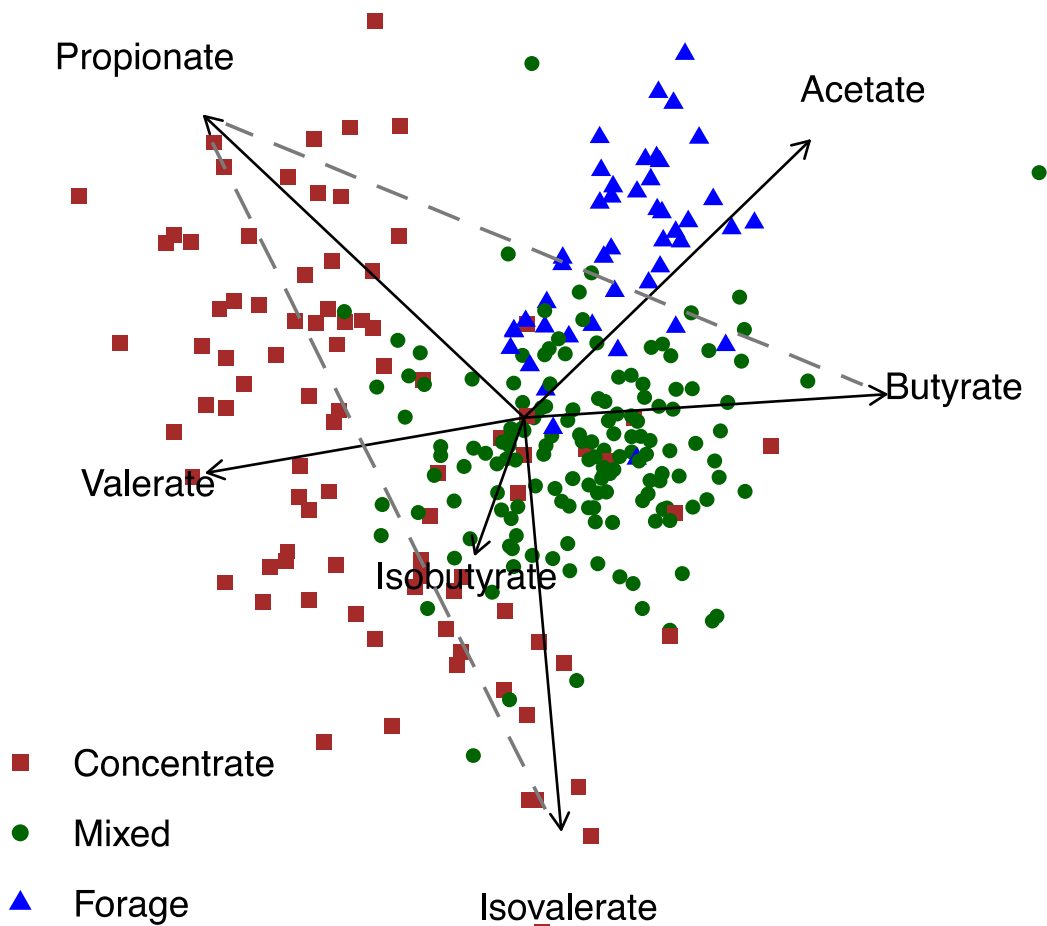
$$R_c^2 = 68.72\%{}^1 \quad \hat{\sigma}_f = 0.06{}^3 \quad \hat{\sigma} = 0.16{}^3$$

<sup>1</sup> $R_m^2$ ,  $R_c^2$ ; respectively % variance explained by fixed terms (marginal) and by both fixed and random terms (conditional).

<sup>2</sup>Akaike information criterion measure of the relative quality of the model for the full data set.

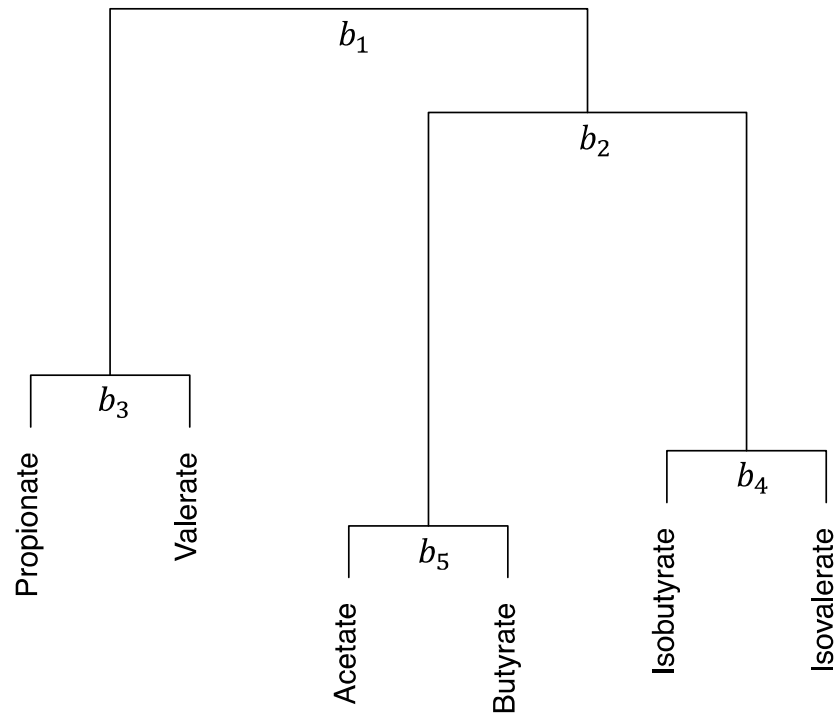
<sup>3</sup> $\hat{\sigma}_f$ ,  $\hat{\sigma}$ ; respectively estimated standard deviations of the random effects and random error terms.

15 Figure 1. Compositional biplot of the VFA (mmol/mol) data set with the components  
 16 represented by rays and the collected samples represented by points according to concentrate,  
 17 mixed, and forage diet types. The links (dashed lines) between arrowheads are proportional to  
 18 the log-ratio variances between the corresponding VFA components.



19  
 20  
 21

- 22 Figure 2. Groupings of VFA (mmol/mol) components according to proportionality  
23 relationships from the variation matrix and associated balances ( $b_i, i = 1, \dots, 5$ ) for Model A1.



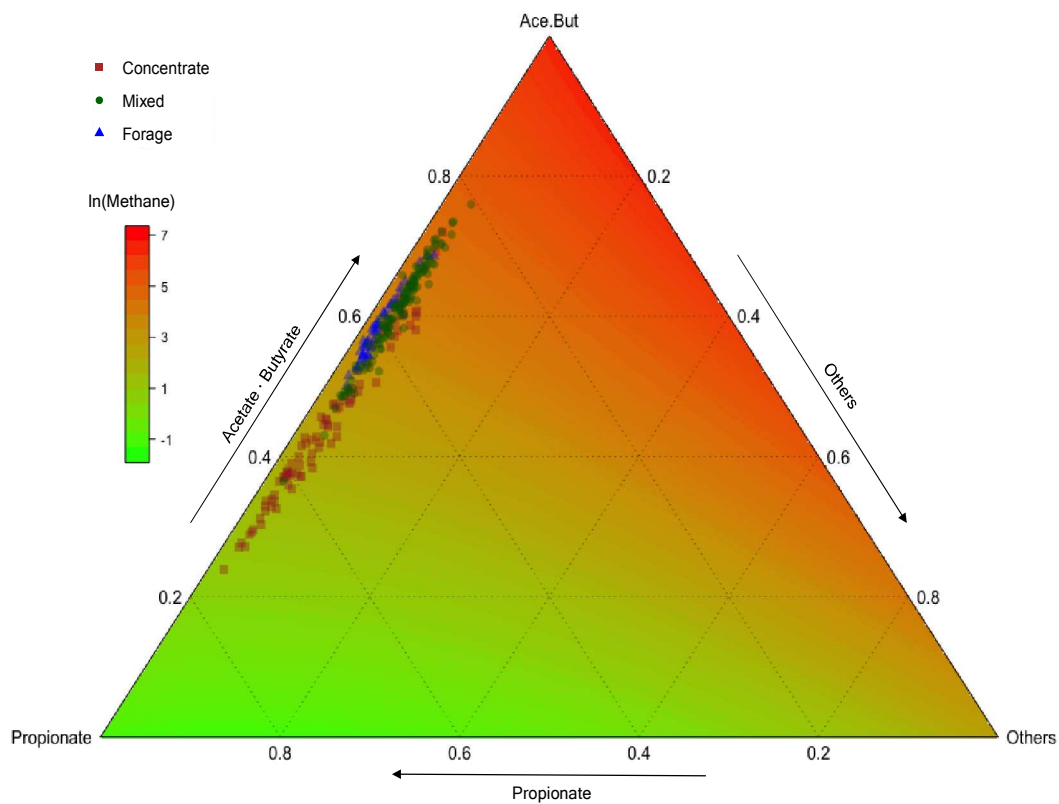
24

25



26 Figure 3. Ternary diagram of the [acetate · butyrate (Ace.But), propionate, others] VFA  
 27 subcomposition for concentrate, mixed, and forage diet types and expected methane  
 28 emissions (g/kg DMI in log scale) from Model B.

29



30

31

Propionate

Acetate

Butyrate

Valerate

Isobutyrate

Isovalerate

Concentrate

Mixed

Forage

