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The impact of divergent breed types and diets on methane emissions, rumen characteristics and performance of finishing beef cattle

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Short title: Methane emissions and performance of beef cattle

26 **Abstract**

27 This study was undertaken to further develop our understanding of the links between
28 breed, diet and the rumen microbial community and determine their effect on
29 production characteristics and methane (CH₄) emissions from beef cattle. The
30 experiment was of a two × two factorial design, comprising two breeds (CHX,
31 crossbred Charolais; LU, purebred Luing) and two diets (concentrate-straw or silage-
32 based). In total, 80 steers were used and balanced for sire within each breed, farm of
33 origin, and BW across diets. The diets (fed as total mixed rations) consisted of (g/kg
34 dry matter (DM)) forage to concentrate ratios of either 500:500 (Mixed) or 79:921
35 (Concentrate). Steers were adapted to the diets over a four week period and
36 performance and feed efficiency were then measured over a 56 day test period.
37 Directly after the 56 day test, CH₄ and carbon dioxide (CO₂) emissions were
38 measured (six steers / week) over a 13 week period. Compared to LU steers, CHX
39 steers had greater average daily gain (ADG; $P<0.05$) and significantly ($P<0.001$)
40 lower residual feed intake. CHX steers had superior conformation and fatness scores
41 ($P<0.001$) than LU steers. Although steers consumed, on a DM basis, more
42 Concentrate than Mixed diet ($P<0.01$), there were no differences between diets in
43 either ADG or feed efficiency during the 56 day test. At slaughter, however,
44 Concentrate-fed steers were heavier ($P<0.05$) and had greater carcass weights than
45 Mixed-fed steers ($P<0.001$). Breed of steer did not influence CH₄ production, but it
46 was substantially lower when the Concentrate rather than Mixed diet was fed
47 ($P<0.001$). Rumen fluid from Concentrate-fed steers contained greater proportions of
48 propionic acid ($P<0.001$) and lower proportions of acetic acid ($P<0.001$), fewer
49 archaea ($P<0.01$) and protozoa ($P=0.09$) but more *Clostridium* Cluster XIVa ($P<0.01$)
50 and *Bacteroides* plus *Prevotella* ($P<0.001$) than Mixed-fed steers. When the CH₄ to

51 CO₂ molar ratio was considered as a proxy method for CH₄ production (g/kg DM
52 intake), only weak relationships were found within diets. In conclusion, while feeding
53 Concentrate and Mixed diets produced substantial differences in CH₄ emissions and
54 rumen characteristics, differences in performance were influenced more markedly by
55 breed.

56

57 **Keywords:** beef cattle, concentrate, forage, methane, performance

58

59 **Implications**

60 The effects of diet and breed on steer performance and methane (CH₄) emissions
61 were measured. Methane emissions on a high concentrate (920 g/kg DM) diet were
62 less (0.68) when compared to a mixed forage / concentrate (500 g/kg DM) diet.
63 Although energy lost as CH₄ was reduced on the high concentrate diet, animal
64 performance and carcass quality did not differ between diets. The CH₄ to CO₂ ratio in
65 expired air did not relate well to daily CH₄ production and may therefore have limited
66 use as a proxy for daily CH₄ production.

67

68 **Introduction**

69 Ruminant livestock systems are under continued political pressure to reduce their
70 greenhouse gas (GHG) outputs. Worldwide, beef production systems generate 2.9
71 Mt of CO₂-Equivalent emissions per year and CH₄ emissions accounted for 44% of
72 total GHG emissions (Gerber *et al.*, 2013b). The global human population is
73 expected to exceed 9 billion by 2050, with meat consumption projected to increase
74 by more than 75% compared to 2005 (Alexandratos and Bruinsma, 2012). Achieving

75 this level of production, whilst reducing the environmental impact of ruminant
76 livestock production, represents a considerable challenge.

77 Ruminants play a crucial role in food security, being able to convert forages
78 and non-human edible food into products for human consumption through enteric
79 fermentation of cellulosic carbohydrates. However, enteric fermentation is the main
80 source of ruminant emissions, as CH₄ is one end product of the microbial digestion
81 process. Methane formation in the rumen depends both on a supply of hydrogen (H₂)
82 from fermentation of feed by bacteria and protozoa and the subsequent conversion of
83 H₂ and carbon dioxide (CO₂) to CH₄ by methanogenic archaea. Enteric CH₄
84 emissions also represent a loss of gross energy to the animal (estimated at 6-10%),
85 which could be used by the animal for production (e.g. deposition of lean meat)
86 (Cottle *et al.*, 2011; Gerber *et al.*, 2013a and 2013b). Understanding the mechanisms
87 of methanogenesis and the microorganisms involved is important for devising
88 sustainable mitigation strategies to lower the environmental impact of ruminant
89 livestock production.

90 Recently Rooke *et al.* (2014) reported that CH₄ emissions were less (0.62 of
91 mixed diet) when a diet containing 900 g concentrates / kg dry matter (DM)
92 (concentrate diet) was fed compared to a diet containing 500 g concentrates /kg DM
93 (mixed diet); further, rumen microbial communities were influenced by the genotype
94 and CH₄ emissions by the sire of cattle (Roehe *et al.*, 2016). In the same study,
95 Wallace *et al.* (2014) demonstrated a positive relationship between the relative
96 abundance of archaea in rumen samples taken at slaughter and the quantities of CH₄
97 produced by individual animals. Furthermore, Wallace *et al.* (2015) has previously
98 demonstrated the influence of microbial communities on CH₄ emissions, and Roehe
99 *et al.* (2016) the impact of the host genetics on CH₄ emissions. Although accurate

100 measurements of CH₄ emissions using respiration chambers are required to develop
101 and test the effectiveness of CH₄ mitigation strategies, for genetic selection of cattle
102 producing lower CH₄ emissions, methods capable of screening large numbers of
103 animals are required such as sampling animals at slaughter (Wallace *et al.*, 2014). In
104 the present study, the same nutritional strategy of Rooke *et al.* (2014) was used. The
105 hypotheses addressed were that CH₄ emissions expressed on a live-weight gain or
106 carcass yield basis would be lower on a high concentrate diet and that differences
107 between breeds in CH₄ emissions would be greater when genetically more diverse
108 breeds of cattle (Charolais and Luings) were tested.

109

110 **Material and methods**

111 This study was conducted at the Beef and Sheep Research Centre, SRUC, UK. The
112 experiment was approved by the Animal Experiment Committee of SRUC and was
113 conducted in accordance with the requirements of the UK Animals (Scientific
114 Procedures) Act 1986.

115

116 *Experimental design, animals and diets*

117 The experiment was of a two × two factorial design, comprising two breeds (CHX,
118 crossbred Charolais; LU, purebred Luings) and two diets (concentrate-based or
119 silage-based). The breed types were selected to represent two commercially relevant
120 breeds where CHX cattle represent a beef breed known for fast growth and excellent
121 carcass conformation, whilst the LU breed is a more extensively managed hardy hill
122 and upland breed. Two diets (as total mixed rations) were generated using a diet
123 mixing wagon and consisted of (g/kg DM) forage to concentrate ratios of either
124 500:500 (Mixed) or 79:921 (Concentrate). The ingredient and chemical composition

125 of the experimental diets are given in Table 1 and the chemical composition of
126 individual components in Table 2. The DM contents of individual components were
127 determined on duplicate samples twice weekly. Bulk feed samples (four per
128 component) were analysed for DM, ash, crude protein, acid detergent fibre, neutral
129 detergent fibre, acid hydrolysed ether extract (AHEE), starch and neutral cellulase
130 and gammanase digestibility (NCGD) (Ministry of Agriculture Fisheries and Food,
131 1992) and gross energy by adiabatic bomb calorimetry. ME values (Thomas, 2004),
132 were either estimated from near infra red spectroscopy (silage and whole crop barley
133 silage), from NCGD and AHEE (barley and wheat distillers dark grains) or from
134 tabulated values for feed composition (straw and molasses).

135 In total, 80 steers were used (n=40 per diet) and each diet was allocated to
136 two pens (four pens in total; 20 steers per pen). Pens were balanced for sire within
137 each breed, farm of origin and BW and were balanced across diets at the start of the
138 experiment. Fresh water was provided *ad libitum* using a water trough, and diets
139 were offered at approximately 1.05 times average daily intake to all steers using 32
140 electronic feeders (HOKO, Insentec, Marknesse, The Netherlands). All steers were
141 bedded on wood fibre and sawdust to ensure that consumption of bedding did not
142 contribute to nutrient intake. All steers were fed the Mixed diet before being adapted
143 to diets. Steers allocated the Concentrate diet, were adapted to the full concentrate
144 inclusion over four weeks. Forage to concentrate ratios were increased at weekly
145 intervals such that ratios of 38:62, 25:75, 13:87 and 8:92 were offered during
146 adaptation. During this period, steers were trained to use the electronic feed intake
147 recording equipment.

148

149 *56-day performance test*

150 After adaptation to the experimental diets, performance and feed efficiency were
151 characterised for all steers over a 56 day test period (day 0 to day 56). Animals were
152 maintained under controlled conditions, where group sizes within the pen remained
153 constant. Individual DM intakes (DMI, kg/day) were recorded for each animal using
154 the electronic feeding equipment and BW measured weekly before fresh feed was
155 offered using a calibrated weigh scale. Ultrasonic fat depth was obtained at the
156 12th/13th rib at the start (FD0) and end (FD1) of the 56 day test using industry-
157 standard equipment (Aloka 500, BCF Technology LTD, Scotland, UK). Images were
158 analysed using Matrox Inspector 8 software (Matrox Video and Imaging Technology
159 Europe Ltd., Middlesex, UK).

160

161 *Emissions measurement in respiration chambers*

162 Directly after the 56 day performance test, 72 steers were allocated to six respiration
163 chambers over a 12 week period using a randomised block design (six chambers
164 times four weeks) which was repeated three times. Within each block, each
165 treatment of the two × two factorial (breed × diet) experimental design was replicated
166 once in each respiration chamber. Steers were allocated to blocks to minimise
167 variation in BW (mean BW (kg) 617, SEM 6.6) on entry to the respiration chambers.
168 The steers remained in the respiration chambers for three days, during which time
169 they were fed once daily and had *ad-libitum* access to feed. Data for DMI during the
170 three day chamber measurement period were averaged per animal. One chamber
171 malfunctioned during weeks 6 to 10, which resulted in the requirement for an
172 additional week of chamber measurement; thus measurements were made from 73
173 steers.

174 Full details of the six indirect open-circuit respiration chambers (No Pollution
175 Industrial Systems Ltd., Edinburgh, UK) and their operation are given in Rooke *et al.*
176 (2014) and Troy *et al.* (2015). In addition to CH₄, CO₂ concentrations were also
177 measured by infrared absorption spectroscopy (MGA3000, Analytical Development
178 Co. Ltd., Hoddesdon, UK) after calibration with a gas mixture of known composition.
179 Prior to the beginning of the experiment, gas recoveries were measured by releasing
180 CO₂ at a constant rate into each chamber. To accustom the steers to the chamber
181 environment, six days prior to chamber measurements groups of steers were moved
182 to the building in which chambers were located and loose-housed in single pens (4 ×
183 3 m) of identical design to pens within the chambers. After six days, the steers were
184 then moved to the chambers and remained there for 72 h, with CH₄ and CO₂
185 measurements recorded in the final 48 h used in further analysis. Steers were fed (at
186 approximately 1.05 times average daily intake) once daily and weight of feed within
187 the bins recorded at 10 sec intervals using load cells. Front doors of chambers were
188 briefly opened at about 08.00 h daily to remove feed bins and again to replace bins
189 with fresh feed at approximately 09.00 h. The pens were cleaned daily between
190 08.00 and 09.00 h. The exact times when doors were opened were recorded.

191

192 *Rumen sampling, volatile fatty acid and microbial analyses*

193 Immediately after the steers (within two hours) left the respiration chambers, samples
194 of rumen fluid were obtained (one per animal) by inserting a tube (16 × 2700 mm
195 Equivet Stomach Tube, Jørgen Kruuse A/S, Langeskov, Denmark) nasally and
196 aspirating manually. Approximately 50 mL fluid were strained through two layers of
197 muslin and samples prepared for VFA analysis and DNA extraction prior to storage at
198 -20 °C as previously described (Rooke *et al.*, 2014). Similarly, DNA extraction was

199 carried out using a method based on repeated bead beating plus column filtration
200 and qPCR methodology to quantify relative abundance of microbial groups in rumen
201 samples (Rooke *et al.*, 2014).

202

203 *Pre-slaughter measurements and carcass quality*

204 Other than for measurements of CH₄ emissions within the respiration chamber
205 facility, steers remained within the same pens from the end of the 56 day test to
206 slaughter. All steers remained on the same diet throughout the experiment. On the
207 day before slaughter, ultrasonic fat depth (FD2) at the 12th/13th rib was measured in
208 all steers as described above. Steers were slaughtered in five batches of 6, 21, 18,
209 15 and 19 steers on days 71, 92, 113, 134 and 155, respectively. Steers were
210 selected for slaughter based on BW and visual assessment of fatness. Steers had
211 access to feed until they left the premises. The steers were transported
212 (approximately 1 h) to a commercial abattoir and slaughtered within 2 h of arrival.
213 Cattle were stunned using a captive bolt, exsanguinated and subject to low voltage
214 electrical stimulation. Following hide removal, carcasses were split in half down the
215 mid-line and dressed to UK specification (see Meat and Livestock Commercial
216 Services Limited beef authentication manual, [www. mlcsl.co.uk](http://www.mlcsl.co.uk), for full description).
217 EUROP conformation and fat classifications (Fisher, 2007), based on the UK scale,
218 were allocated to all carcasses through visual assessment using a trained assessor.

219 Video Image Analysis (VIA) was used to estimate EUROP classifications
220 (conformation and fat), total lean (kg) and total fat (kg) content of the whole carcass.
221 The VIA systems in use in the EU are automatic machines that perform carcass
222 evaluation based on images of the half carcass. The VBS 2000 system used in this
223 study (E+V technology GmbH, Oranienburg, Germany) has been approved by the

224 Department for Environment, Food and Rural Affairs (Defra) for use in the UK since
225 2010. The system operated at the end of the slaughter line after all necessary
226 dressing and trimming had been completed. A pneumatically operated cradle
227 presented the left half side of each carcass for imaging. The VIA camera took two
228 images of the half carcass, a 2-dimensional image and a pseudo 3-dimensional
229 image using structured light (Craigie *et al.*, 2012). The VBS 2000 required
230 information on the category of the carcass (i.e., steer) and hot carcass weight (kg)
231 and, by combining this information with data automatically captured by the VIA
232 system (i.e., carcass dimensions, angles, areas, colour), predicted EUROP
233 classification and total lean and fat content of the whole carcass.

234

235 *Calculations and statistical analysis*

236 Data from two steers during the 56 day test period and one steer at slaughter were
237 unavailable as the animals were removed from the trial for health reasons
238 unconnected to the diets imposed. Growth was modelled by linear regression of BW
239 against test date, to obtain ADG, mid-test BW (mid-BW) and mid-test metabolic BW
240 (mid-MBW, $BW^{0.75}$). Mean DMI over the 56 day period was expressed as kg/day or
241 as a proportion of mid-BW and mid-MBW. Feed conversion ratio (FCR) was
242 calculated as average DMI per day (kg/d) divided by ADG. Residual feed intake (RFI)
243 was calculated as deviation of actual DMI (kg/d) from DMI predicted based on linear
244 regression of actual DMI on ADG, mid-MBW and FD1 (Basarab *et al.*, 2003). Cold
245 carcass weight (CCW) was calculated as a percentage of slaughter BW (SBW) to
246 determine killing out percentage (KO). To allow for statistical comparison, the
247 EUROP carcass classification values were expressed on the equivalent 15 point
248 scale (Kempster *et al.*, 1986). Statistical analyses of performance and carcass data

249 were conducted using the mixed procedure of SAS software with the fixed effects of
250 breed and diet, and the random effect of pen (and slaughter batch for carcass traits).
251 In addition, in the analysis of FD1 and FD2 the deviation from the breed mean of FD0
252 was included as a covariable. The interaction effects of breed x diet were included in
253 the model when these effects proved significant ($P<0.05$).

254 The respiration chamber measurements from three steers were discarded as
255 the DMI decreased substantially ($> 30\%$) whilst being housed in the respiration
256 chamber, leaving data from a total of 70 individual steers. Rumen fluid samples were
257 not obtained for two steers and therefore 68 individual animal observations were
258 available. Data were analysed using SAS software using linear mixed models. The
259 fixed effects were breed and diet, while the random effects were week and chamber.
260 The effect of the breed x diet interaction was also included in the model when this
261 proved significant ($P<0.05$).

262 Data are reported as means with their SEM unless indicated otherwise.
263 Differences between means were tested using a least square means comparison
264 test. Probability values were deemed significant where $P<0.05$ and indicated a
265 tendency when probability values were between $P=0.05$ and $P=0.1$. The numbers of
266 steers in treatments are given in each Table for clarity.

267

268 **Results**

269 *Performance test*

270 Although there were no differences in age at the start of the trial, CHX steers were
271 significantly ($P<0.001$) heavier than LU steers (Table 3). However, there were no
272 differences between breeds in daily DMI and therefore on a BW basis, LU steers
273 consumed more DM (g/kg BW or $\text{g/kg}^{0.75}$, $P<0.001$) than CHX steers. Compared to

274 LU steers, CHX steers had greater ADG ($P<0.05$) throughout the performance trial
275 and lower FD1 ($P<0.01$) at the end of the trial. CHX steers were more efficient than
276 LU steers as measured by numerically lower FCR and significantly ($P<0.001$) lower
277 RFI than LU steers.

278 Although steers consumed more of the Concentrate than Mixed diet ($P<0.01$),
279 there were no differences between diets in either ADG or feed efficiency (expressed
280 as either FCR or RFI). Fat depth (FD1) tended to be lower ($P=0.06$) on the
281 Concentrate than Mixed diet.

282

283 *Carcass traits*

284 CHX steers were superior to LU steers for most carcass traits recorded (Table 4).
285 Thus, CHX were heavier at slaughter with greater KO resulting in greater CCW (all
286 $P<0.001$). Regardless of measurement method, CHX steers had superior
287 conformation and fatness scores ($P<0.001$) which were reflected in greater carcass
288 meat and lower carcass fat yields (predicted by VIA).

289 Concentrate-fed steers were heavier at slaughter ($P<0.05$) and had greater
290 CCW than Mixed-fed steers ($P<0.001$). Although there were no differences in
291 carcass scores when visually assessed, the VIA system predicted superior
292 conformation scores ($P<0.05$) and meat yields ($P<0.01$) for Concentrate-fed steers.

293

294 *Methane and carbon dioxide production*

295 Breed of steer did not influence either CH₄ or CO₂ production. Methane production
296 (Table 5), whether expressed as g/day, g/kg DMI or kJ/MJ GE intake, was
297 substantially lower when the Concentrate rather than the Mixed diet was fed

298 ($P<0.001$). There were no differences between diets in total daily CO₂ production but
299 CO₂ production expressed as g/kg DMI was greater when the Mixed diet was fed.

300 The ratio of CH₄ to CO₂ production (mole/mole) was greater on the Mixed than
301 Concentrate diet ($P<0.001$). Although, there was a strong linear relationship between
302 CH₄ production (g/kg DMI) and CH₄ to CO₂ molar ratio ($P<0.001$) when all animals
303 were considered, this was largely due to between–diet differences as within diets, the
304 relationships were much weaker (Fig. 1). However, and irrespective of whether data
305 from all animals were considered together or within diets, essentially most of the
306 variation in CH₄ (g/kg DMI) was explained when both CH₄ to CO₂ ratio and CO₂
307 production (g/kg DMI) were included in models.

308

309 Overall: CH₄ (g/kgDMI) = 159 (16.3) CH₄ to CO₂ molar ratio + 0.0099 (0.00135) CO₂
310 (g/kg DMI); r^2 0.74, $P<0.001$.

311

312 *Rumen fluid VFA and microbial populations*

313 Rumen fluid from Concentrate-fed steers (Table 6) contained greater proportions of
314 propionic and valeric (both $P<0.001$) acids but lower proportions of acetic ($P<0.001$)
315 and butyric ($P<0.01$) acids than Mixed-fed steers. There were no differences in VFA
316 between breeds. Breed did not influence rumen microbial populations (Table 6).
317 Rumen fluid from Concentrate-fed steers had a lower abundance of archaea
318 ($P<0.01$) and protozoa ($P=0.09$) but more bacteria ($P<0.001$). There were no
319 differences between diets in abundance of *Clostridium* Cluster IV in rumen fluid, but
320 rumen fluid from Concentrate-fed steers contained more *Clostridium* Cluster XIVa
321 and *Bacteroides* plus *Prevotella* than Mixed-fed steers. When the relationship
322 between CH₄ emissions (g/kg DMI) and archaea populations (expressed as ratio of

323 archaea to total bacteria, Wallace *et al.*, 2014) was explored the relationship was
324 significant ($P<0.001$, Fig. 2) but when the Mixed and Concentrate diets were
325 considered individually the relationships were weaker and only significant ($P<0.05$)
326 for the Concentrate diet.

327

328 **Discussion**

329 *Performance*

330 *Diets.* There were few differences in performance traits between the Mixed (500 g
331 concentrate DM / kg total DM) and Concentrate diets in the present study. Feed
332 intake was significantly and ADG numerically greater for the Concentrate than Mixed
333 diet but neither FCR nor RFI differed between diets. Since there were also few
334 differences in carcass composition, after differences in slaughter weight were
335 accounted for, there was little evidence for any underlying differences between diets
336 in the energy content of deposited tissue. These results are similar to the study of
337 Duthie *et al.* (2016) who used the same breeds and similar diets and experimental
338 protocols to the present study. Thus, FCR did not differ between diets and there was
339 little evidence of differences in carcass composition particularly fat content in either
340 study and therefore, there was no advantage to the Concentrate diet in animal
341 performance either in BW, CCW or energetic terms. This lack of difference between
342 diets is in contrast to the expectation from the literature. For example, Lovett *et al.*
343 (2003) reported that heifers offered a concentrate diet (900 g concentrate / kg DM)
344 consumed similar DMI but grew faster (1.1 v. 0.8 kg/d) and had superior FCR (8.5 v.
345 11.4 kg DMI/ kg ADG) than heifers fed a 600 g concentrate / kg DM diet. The
346 predicted efficiencies of utilisation of metabolisable energy for growth (AFRC 1993;
347 0.50 and 0.54 for Mixed and Concentrate diets) would suggest that the Concentrate

348 diet could support superior performance and the higher molar proportion of propionic
349 acid on the Concentrate diet would have supplied more precursors for
350 gluconeogenesis and lean tissue deposition. A likely explanation for the lack of
351 difference between the two diets is that the numerically greater ADG for steers fed
352 the Concentrate diet were the maximum ADG possible.

353

354 *Breeds.* The differences in performance between CHX and LU in the present study
355 were similar to Duthie *et al.* (2016). That is, the CHX steers had greater daily ADG
356 and superior FCR. The differences between breeds in slaughter characteristics were
357 also similar between studies; CHX had greater carcass weights and superior EUROP
358 conformation (visually assessed or predicted from VIA) and lower fat depth. In the
359 present study, the quantitative differences between the breeds in performance were
360 lower. In particular, in Duthie *et al.* (2016) LU steers had greater DMI than CHX, but
361 there were no differences in the present study. The reason for this difference is likely
362 that in Duthie *et al.* (2016), steers entered the performance study at the same BW but
363 LU steers were approximately 30 days older and thus nearer maturity especially
364 since LU steers would reach maturity at a younger age than CHX. In this context, if
365 LU are classified as a medium maturing cattle type compared to the CHX, a late
366 maturing type, then from AFRC (1993) the energy value of gain would be 22.2 and
367 23.3 MJ/kg ADG for CHX and LU respectively. Using these values, the net energy
368 requirements for the observed ADG of 36.8 and 36.6 MJ net energy / day for CHX
369 and LU respectively are little different. Thus in terms of energy efficiency there is little
370 difference between the breeds.

371

372 *Methane emissions*

373 *Diets*. In an experiment of similar design to that reported here (Rooke *et al.*, 2014)
374 but using different breeds of cattle, mean CH₄ emissions (g/kg DMI) were similar
375 (present experiment *v.* Rooke *et al.*, 2014; Concentrate, 13.9 *v.* 13.6; Mixed, 20.4 *v.*
376 21.8). This difference between diets was consistent with both the literature (Hristov *et*
377 *al.*, 2013) and the observed changes in VFA proportions: increased molar proportions
378 of propionate (hydrogen consuming) and decrease proportions of acetate (hydrogen
379 producing) on the Concentrate diet. Based on many studies, equations to predict CH₄
380 yield which include the proportion of concentrate in the diet have been developed.
381 The equation of Sauvant and Giger-Reverdin (2009) predicted CH₄ yields (expressed
382 as kJ CH₄ / MJ total GE) of 48 and 79 kJ CH₄ /MJ GE intake for the Concentrate and
383 Mixed diets respectively compared to observed means of 42 and 60. The more
384 recent equation for non-lactating cattle developed by Hristov *et al.* (2013) produced
385 values of 59 and 65 kJ CH₄ / MJ GE intake. Both equations thus over-predicted CH₄
386 produced from the Concentrate diet. This may be because of under-representation or
387 absence of high concentrate diets from the prediction data sets. Rooke *et al.* (2014)
388 noted that the value of 39 kJ CH₄ / MJ GE for the Concentrate was higher than
389 values observed for North American feedlot diets (20 – 30 kJ MJ CH₄ / MJ GE) based
390 on maize grain and that this was due to the greater cell wall concentration in barley
391 grain (Beauchemin *et al.*, 2005; Doreau *et al.*, 2011). For the Mixed diet, the value
392 predicted by Hristov *et al.* (2013) was in closer agreement with the observed value
393 than that from Sauvant and Giger-Reverdin (2009) likely because the Hristov *et al.*
394 (2013) equation included terms for NDF and ether extract which more accurately
395 described the nutrient composition of the diet.

396 Breed had no overall effect on CH₄ yield in the present experiment. This was
397 in agreement with our own (Rooke *et al.*, 2014; Duthie *et al.*, 2015; Troy *et al.*, 2015)

398 and other previous studies (Boadi and Wittenberg 2002; Fraser *et al.*, 2014;
399 Richmond *et al.*, 2015) using different breeds. However, Hristov *et al.* (2013) have
400 argued that emissions intensity (CH₄ produced per unit animal product) most
401 accurately represented the potential of a mitigation strategy. Since detailed animal
402 performance records and CH₄ emissions were measured in this experiment, it was
403 appropriate to estimate emissions intensities for the diets fed. In so-doing the
404 limitations imposed by recording animal performance, CH₄ emissions and carcass
405 characteristics consecutively should be noted. As an example, feed intakes
406 expressed as a proportion of BW were greater during the performance trial than the
407 CH₄ measurement period and therefore CH₄ emissions (g/kg DMI) during the
408 performance measurement would likely have been less than those measured later
409 (e.g. Sauvant and Giger-Reverdin 2009). Table 7 shows that whilst the difference
410 between diets within breed remained relatively independent of the method of
411 measurement, the effect of breed was substantial particularly when CH₄ emissions
412 were based on carcass and estimated meat weights with the LU cattle fed the Mixed
413 diet producing nearly twice the amount of CH₄ on a carcass meat basis than CHX
414 cattle fed the Concentrate diet.

415

416 *Rumen microbiota.* In Rooke *et al.* (2014), there was a significant relationship
417 between archaea populations (ratio of archaea to total bacteria) and CH₄ emissions
418 (Wallace *et al.*, 2014) and there were also differences in rumen microbiota between
419 breeds (Rooke *et al.*, 2014). In the present study, there was a similar relationship
420 between CH₄ emissions and archaeal populations (Fig. 2) to Wallace *et al.* (2014)
421 where the relationship was positive and significant for the Concentrate but not the
422 Mixed diet, suggesting that the archaea populations and CH₄ emissions were limited

423 by available hydrogen on the Concentrate diet (Janssen, 2010). However, in contrast
424 to Rooke *et al.* (2014) there were no differences in rumen microbiota or CH₄
425 emissions between breeds of cattle. This was despite the fact that the breeds used in
426 the present experiment (CHX and LU) were more genetically divergent than the
427 genotypes used by Rooke *et al.* (2014; Limousin x Aberdeen Angus and Aberdeen
428 Angus x Limousin). A possible explanation for this difference may be the source of
429 the cattle used. Whereas the steers used by Rooke *et al.* (2014) were raised on the
430 farm in which the experiment was carried out, in the present experiment, steers were
431 obtained from nine different farms. It is thus possible that the different farm
432 environments the cattle used in the present experiment were derived from had a
433 greater effect on rumen microbiota than differences between breeds.

434

435 *Methane and carbon dioxide emissions*

436 Quantifying CH₄ emissions using respiration chambers is a costly and relatively low
437 throughput procedure and there is therefore considerable interest in establishing
438 proxy procedures which are low cost, more rapid and more applicable to the normal
439 farm environment. A possible option within dairy systems is the measurement of CH₄
440 and CO₂ concurrently from sampling points for example in the dairy parlour (Lassen
441 *et al.*, 2012; Bell *et al.*, 2014b). Both the above studies concluded that the CH₄ to
442 CO₂ phenotype was repeatable. It was proposed by Madsen *et al.* (2010) that by
443 calculating heat production by the animal and converting heat production to CO₂
444 production, CH₄ to CO₂ ratios could be converted to daily CH₄ emissions. However
445 Bell *et al.* (2014b) found only a poor relationship between average CO₂ production
446 estimated according to Madsen *et al.* (2010) and measured CO₂ concentrations.
447 Factors proposed to explain this lack of agreement by Bell *et al.* (2014b) were animal

448 to animal variation including differences in diurnal pattern of CH₄ to CO₂ ratio, feed
449 intake and fasting heat production itself. This is confirmed in the present study where
450 measurements were made over a 48 h period thus excluding short-term changes in
451 breath CH₄ and CO₂ concentration. Further, since all animals were gaining weight,
452 CO₂ derived from body tissue mobilisation would not have influenced the results. The
453 diets fed influenced CO₂ production and therefore CH₄ to CO₂ ratio with CO₂
454 production (g/kg DMI) being greater for the Mixed diet as expected from differences
455 in VFA pattern. More importantly and particularly within diets, the correlation between
456 CH₄ production (g/kg DMI) and CH₄ to CO₂ ratio was poor (Fig. 1) but variation in
457 CO₂ production in conjunction with CH₄ to CO₂ ratio explained most of the variation in
458 CH₄ production. Thus although the phenotype of CH₄ to CO₂ ratio may be
459 repeatable, the present experiment suggests that it may not relate well to daily CH₄
460 production because of animal to animal variation in extent of digestion, efficiency of
461 utilisation of absorbed nutrients and tissue CO₂ turnover.

462

463 **Conclusions**

464 This large scale, integrative study reported animal performance including carcass
465 characteristics together with measurement of CH₄ emissions and characterised
466 rumen VFA and microbial abundance. In agreement with previous studies (Rooke *et*
467 *al.*, 2014; Duthie *et al.*, 2016) CH₄ emissions were less (0.68 of mixed diet) when a
468 high concentrate diet was fed compared to a mixed forage:concentrate diet.
469 However, although energy lost as CH₄ was reduced by 18 KJ/MJ gross energy
470 intake, there were no differences in animal performance or carcass characteristics
471 between the diets fed. Although breed of steer had no effect on CH₄ emissions, ADG
472 was less and feed conversion efficiency was poorer for LU compared to CHX steers.

473 Assessment of the CH₄ to CO₂ ratio as a proxy measurement for CH₄ emissions
474 made using respiration chambers, suggests that the ratio may not relate well to daily
475 CH₄ production because of animal to animal variation in digestion and utilisation of
476 feed.

477

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486

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621 **Table 1** *Ingredient composition and calculated chemical composition of experimental diets*

Diet	Mixed	Concentrate
Ingredient composition, g/kg DM ¹		
Grass silage	215	-
Whole crop barley silage	285	-
Barley straw	-	79
Barley	388	713
Wheat Distillers Dark Grains	103	175
Molasses	-	23
Minerals ²	9	10
Chemical composition, g/kg DM ³		
Dry matter (g/kg)	437	862
CP	138	135
ADF	207	112
NDF	337	248
AHEE	39	47
Starch	284	415
Ash	53	32
ME (MJ/kg DM)	12.0	12.8
GE (MJ/kg DM)	19.2	18.6

622 ¹Ingredient composition is the mean of the daily diets received by the animals across the
 623 experiment.

624 ²Contained (mg/kg): Fe, 6036; Mn, 2200; Zn, 2600; Iodine, 200; Co, 90; Cu, 2500; Se 30;
 625 (µg/kg): vitamin E, 2000; vitamin B12, 1000; vitamin A, 151515; vitamin D, 2500

626 ³Chemical composition is the mean of 4 analyses per diet, apart from DM which is the mean
 627 of 44 analyses.

628 CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; AHEE, acid
 629 hydrolysed ether extract; ME, metabolisable energy; GE, gross energy.

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633 **Table 2** *Chemical composition of feeding stuffs (g/kg DM)*

	Grass silage	WCBS	Straw	Barley	WDG	Molasses
DM (g/kg)	288	298	830	862	851	786
CP	149	111	16	106	321	89
ADF	337	336	547	60	149	0
NDF	393	535	867	169	339	0
Starch	6.0	199.8	16.0	574.3	26.4	0.0
AHEE	37	17	14	33	126	0
Ash	91	66	37	22	58	134
NCGD (% DM)			45	89	78	
ME (MJ /kg DM)	11.9	9.9	6.3	13.3	14.1	12.7
GE (MJ /kg DM)	20.6	19.2	18.1	18.2	22.1	15.5
pH	4.2	4.3				

634 WCBS, whole crop barley silage; WDG, Wheat Distillers Dark Grains; DM, dry matter; CP,

635 crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; AHEE, acid

636 hydrolysed ether extract; NCGD, neutral cellulase and gammanase digestibility; ME,

637 metabolisable energy; GE, gross energy

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642 **Table 3** Effect of breed (B), diet (D) on growth, feed intake and feed efficiency of Charolais-sired (CHX) and purebred Luining (LU) steers fed
 643 either a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diet during a 56-day performance trial

Diet	Mixed		Concentrate		SEM	Significance			
	CHX	LU	CHX	LU		B	D	B × D	
n of steers	19	19	21	19					
AgeST (days)	394	393	391	391	6.8				
Mid-test BW (kg)	540	476	560	477	13.3	***			
Mid-test MBW (kg ^{0.75})	112	102	115	102	2.1	***			
ADG (kg/day)	1.59	1.49	1.73	1.63	0.228	*			
DMI (kg/day)	10.61	10.67	11.73	11.15	0.256		**		
DMI / BW (g/kg)	19.66	22.58	20.95	23.51	1.067	***			
DMI / MBW (g/kg ^{0.75})	94.67	105.08	101.87	109.48	4.212	***			
FCR (kg DMI/ kg ADG)	6.74	7.26	6.84	6.97	0.210				
RFI (kg)	-0.643	0.091	0.148	0.427	0.4833	***		†	
FD1 (mm) ¹	6.60	7.74	5.98	7.05	0.341	**	†		

644 AgeST, Age at start of test; MBW, mid-test metabolic BW; ADG, average daily gain; DMI, dry matter intake; FCR, feed conversion ratio; RFI,
 645 residual feed intake; FD1, fat depth at the 12/13th[†] rib at the end of the 56 d test; B×D, breed × diet

646 ¹Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable

647 ***, P<0.001; **, P<0.01; *, P<0.05; †, P<0.1.

648

649 **Table 4** Effect of breed (B) and, diet (D) and their interaction on carcass traits of Charolais-sired (CHX) and purebred Luing (LU) steers fed
 650 either mixed forage-concentrate (Mixed) or high concentrate-based (Concentrate) diets

Diet	Mixed		Concentrate		SEM	Significance		
	CHX	LU	CHX	LU		B	D	B × D
n of steers	19	20	21	19				
FD2 (mm) ¹	6.92	9.50	7.57	10.4	0.42	***	†	
CCW (kg)	378	305	401	312	7.6	***	***	†
KO (%)	57.3	51.9	57.9	52.3	2.11	***		
SBW (kg)	661	588	694	597	9.3	***	*	
CONF	9.6	7.7	9.6	7.8	0.51	***		
FAT	8.6	10.6	9.3	10.5	0.64	***		†
CONF (VIA)	10.3	7.6	10.8	8.0	0.23	***	*	
FAT (VIA)	6.5	9.3	6.9	8.7	0.75	***		†
TOTAL FAT (kg)	28.03	36.18	34.14	33.75	3.771	*		*
TOTAL MEAT (kg)	270.2	204.7	283.5	214.8	8.95	***	**	

651 FD2, pre-slaughter fat depth at the 12/13th rib; CCW, cold carcass weight; KO, killing out %; SBW, slaughter BW; CONF, EUROP conformation
 652 (15 pt scale) assigned by visual assessor; FAT, EUROP fatness (15pt scale) assigned by visual assessor; CONF (VIA), conformation grade
 653 (15pt scale) assigned by VIA; FAT (VIA), fatness grade (15pt scale) assigned by VIA; TOTAL FAT; total fat content predicted by VIA; TOTAL
 654 MEAT, total meat content predicted by VIA.

655 ¹Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable

656 ***, P<0.001; **, P<0.01; *, P<0.05; †, P<0.1.

657 **Table 5** Dry matter intakes and methane production from Charolais-sired (CHX) and
 658 purebred Luing (LU) steers fed either a high concentrate (Concentrate) or mixed
 659 forage:concentrate (Mixed) diets

Diet (D)	Mixed		Concentrate		SEM	Significance		
	CHX	LU	CHX	LU		B	D	B x D
No of steers	17	19	18	16				
DMI								
kg/day	9.0	9.0	11.0	9.9	0.49		***	†
g/kg BW	14.2	15.8	16.2	16.9	0.78	*	**	
Methane								
g/day	193	184	144	150	11.0		***	
g/kg DMI	20.2	20.7	13.2	14.7	0.64		***	
kJ/MJ GEI	59.1	60.6	39.4	43.6	1.88		***	
Carbon dioxide								
g/day	7468	7034	7685	7376	548.5			
g/kg DMI	788	795	710	730	62.2		*	
Molar ratio								
CH ₄ :CO ₂	0.071	0.072	0.052	0.056	0.004		***	

660 DMI, dry matter intake; GEI, gross energy intake; CH₄, methane; CO₂, carbon dioxide

661 ***, P<0.001; **, P<0.01; *, P<0.05; †, P<0.1.

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664 **Table 6** Volatile fatty acid molar proportions (mmol/mol) and microbial abundance in
 665 rumen fluid samples obtained from Charolais-sired (CHX) and purebred Luing (LU) steers
 666 fed either a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diets

Diet (D)	Mixed		Concentrate		Significance			
	CHX	LU	CHX	LU	SEM	B	D	B x D
No of steers	17	19	18	16				
Acetic	645	657	561	577	9.0		***	
Propionic	174	178	293	257	20.7		***	
Butyric	130	118	95	112	17.7		**	†
Valeric	14	14	17	18	0.8		***	
Branched chain ^A	38	34	34	36	10.0			
Copy number (x 10 ³) / ng DNA								
Archaea	15.4	11.6	7.4	8.3	3.16		**	
Protozoa	45.8	47.2	34.2	40.5	11.35		†	
Total bacteria	501	565	980	964	69.8		***	
<i>Clostridium</i>								
Cluster IV	156	178	211	289	101.1			
Cluster XIVa	147	174	241	320	87.0		**	
<i>Bacteroides</i> plus <i>Prevotella</i>	374	435	994	854	64.4		***	

667 ^ABranched chain: iso-butyric plus isovaleric acids

668 Significance, ***, P<0.001; **, P<0.01; *, P<0.05; †, P<0.1.

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675 **Table 7** *The effect of different metrics on methane emissions from Charolais-sired (CHX)*
 676 *and purebred Luing (LU) steers fed either a high concentrate (Concentrate) or mixed*
 677 *forage:concentrate (Mixed) diets. Values expressed as a proportion of those for CHX*
 678 *steers fed the Mixed diet are given in brackets.*

Diet	Mixed		Concentrate	
Breed	CHX	LU	CHX	LU
Methane				
g / kg DMI	20.2 (1.53)	20.7 (1.57)	13.2 (1.00)	14.7 (1.11)
g/ kg LWG	134 (1.51)	148 (1.66)	90 (1.00)	102 (1.12)
g/kg cold carcass weight	0.567 (1.47)	0.724 (1.88)	0.386 (1.00)	0.525 (1.36)
g/kg total carcass meat	0.794 (1.46)	1.083 (1.99)	0.545 (1.00)	0.762 (1.40)

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682 **Figure Captions**

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684 **Figure 1** Relationships between methane production (g/kg DM intake) and methane to
685 carbon dioxide molar ratio for steers fed Concentrate (solid line and solid circles; $\text{CH}_4 =$
686 $7.23 + 124 \text{ CH}_4 / \text{CO}_2$ molar ratio, r^2 0.22, $P=0.005$) and Mixed (broken line and open
687 circles, $\text{CH}_4 = 10.3 + 141 \text{ CH}_4/\text{CO}_2$ molar ratio, r^2 0.10, $P=0.060$) diets.

688

689 **Figure 2** Relationships between methane yield and archaea to bacteria ratio for samples
690 from cattle fed Concentrate (solid line and solid circles $\text{CH}_4 = 12.5 + 160$ Archaea to
691 Bacteria ratio, r^2 0.10, $P<0.05$) and Mixed (open circles, $P>0.05$) diets.

692