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Short title: Methane emissions from beef cows

Abstract

Increasing the concentration of dietary lipid is a promising strategy for reducing methane (CH_4) emissions from ruminants. This study investigated the effect of replacing grass silage with brewers' grains on CH_4 emissions of pregnant, non-lactating beef cows of two breeds. The experiment was a two x two factorial design comprising two breeds (LIMx, crossbred Limousin; and LUI, purebred Luing) and two diets consisting of (g/kg diet dry matter (DM)) barley straw (687) and grass silage (301, GS), or barley straw (763) and brewers' grains (226, BG), which were offered *ad libitum*. Replacing GS with BG increased the acid hydrolysed ether extract concentration from 21 to 37 g/kg diet DM. Cows (n=48) were group-housed in equal numbers of each breed across two pens and each diet was allocated to one pen. Prior to measurements of CH_4, individual dry matter intake (DMI), weekly BW and weekly body condition score were measured for a minimum of three weeks, following a four week period to acclimatise to the diets. Methane emissions were subsequently measured on one occasion from each cow using individual respiration chambers. Due to occasional equipment failures, CH_4 measurements were run over 9 weeks giving 10 observations for each breed x treatment combination (total n=40). There were no differences between diets for daily DMI measured in the chambers (9.92 *vs.* 9.86 kg/day for BG and GS, respectively; $P > 0.05$). Cows offered the BG diet produced less daily CH_4 than GS-fed cows (131 *vs.* 156 g/day: $P < 0.01$). When expressed either as g/kg DMI or kJ/MJ gross energy intake (GEI), BG-fed cows produced less CH_4 than GS-fed cows (13.5 *vs.* 16.4 g/kg DMI, $P < 0.05$; 39.2 *vs.* 48.6 kJ/MJ GEI, $P < 0.01$). Breed did not affect daily DMI or CH_4 expressed as g/day, g/kg DMI or kJ/MJ GEI ($P > 0.05$). However, when expressed as a proportion of metabolic BW ($BW^{0.75}$), LUI cows had greater DMI than LIMx cows (84.5 *vs.* 75.7

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51 g DMI/kg $BW^{0.75}$, $P < 0.05$) and produced more CH_4 per kg $BW^{0.75}$ than LIMx cows

52 (1.30 *vs.* 1.05 g CH_4/kg $BW^{0.75}$; $P < 0.01$). Molar proportions of acetate were higher

53 ($P < 0.001$) and propionate and butyrate lower ($P < 0.01$) in rumen fluid samples

54 from BG-fed compared to GS-fed cows. This study demonstrated that replacing GS

55 with BG in barley straw-based diets can effectively reduce CH_4 emissions from beef

56 cows, with no suppression of DMI.

57

58 **Keywords**: brewers' grains, cattle, greenhouse gas, methane, nutrition

59

60 **Implications**

61 Ruminant production contributes significantly to global greenhouse gas emissions.

62 Consequently, the identification of appropriate strategies to reduce methane is

63 becoming increasingly important. Diet formulation is one of the most promising

64 strategies for reducing methane production from ruminants. Increasing the

65 concentration of lipid in the diet of beef cows, by replacing grass silage with brewers'

66 grains, reduced methane emissions by 17%. As brewers' grains are a widely

67 available by-product feed, their use in ruminant diets provides a practical solution to

68 reduce the environmental impact of beef enterprises.

69

70 **Introduction**

71 Ruminants play a crucial role in food security, being able to convert forages and non-

72 human edible food into products for human consumption through enteric

73 fermentation of cellulosic carbohydrates. However, enteric fermentation is the main

74 source of ruminant emissions, where methane (**CH_4**) is an end product of the

75 microbial digestion process. Enteric CH_4 emissions represents a loss of feed energy

76 to the animal (estimated at 6-10%), which could be used by the animal for production

77 (e.g. deposition of lean meat) (Cottle *et al.,* 2011; Gerber *et al.,* 2013a and 2013b).

78   There is increasing interest internationally to develop sustainable approaches

79 to reduce CH_4 production from cattle. Breeding, enterprise or system management

80 and diet formulation are all useful strategies (Cottle *et al.,* 2011), with diet formulation

81 representing one of the most practical and promising approaches. It has been widely

82 demonstrated that the nutritional composition of the diet significantly affects CH_4

83 emissions (Cottle *et al.,* 2011). Dietary strategies to reduce CH_4 emissions are

84 generally based on one of the following principles: (i) reducing the production of

85 hydrogen during fermentation, (ii) direct inhibition of methanogenesis, or (iii)

86 providing alternative pathways for the use of hydrogen within the rumen (Martin *et*

87 *al.,* 2010). One promising approach, and the main focus of this paper, relates to

88 increasing the concentration of dietary lipid which has been demonstrated to

89 effectively reduce CH_4 emissions from ruminants (Martin *et al.,* 2010; Grainger and

90 Beauchemin, 2011; Hristov *et al.,* 2013; Patra, 2013). Lipids reduce CH_4 emissions

91 through various mechanisms: fatty acids are not fermented in the rumen and

92 therefore increasing their proportion in the diet reduces the proportion of feed which

93 is fermentable within the rumen; lipids can also reduce CH_4 production by coating

94 fibre particles, reducing their digestibility, and by reducing the numbers and activity

95 of the rumen methanogens and protozoa responsible for methanogenesis (Johnson

96 and Johnson, 1995; Patra, 2013). Dietary lipid can be increased through the addition

97 of plant oils to the diet or through the use of lipid-containing plant by-product feeds

98 from distilleries, breweries or plant oil extraction (Brask *et al.,* 2013). The use of by-

99 product feeds from these industries may be cost-effective and represents an

100 important energy source in ruminant diets. Cereals used for brewing beer or distilling

101   spirits predominantly use the starch portion for ethanol production with the resultant

102   by-product feed available to the ruminant feed market being proportionately higher in

103   fibre, protein and lipid. Brewers' grains are a widely available animal feed for both

104   beef and dairy cattle. Commonly, diets fed to housed beef cows in the winter include

105   large proportions of forages which are low in digestibility, for example barley straw.

106   Baseline data on CH_4 emissions from cows offered diets low in digestibility are

107   currently sparse, as is information on effective CH_4 mitigation strategies for these

108   diet types.

109   Evidence to support breed differences in CH_4 emissions is also limited. Most

110   studies have focussed on breeds of beef cattle that are typically managed more

111   intensively, but a small number have investigated breeds more suited to extensive

112   grazing systems (Fraser *et al.,* 2014; Richmond *et al.,* 2015). One could speculate

113   that breeds suited for hill and upland systems may have developed significant

114   physiological or behavioural differences to suit the harsher environments. They may

115   also differ in CH_4 production when offered a straw-based, poor quality diet in

116   comparison to breeds typically managed more intensively and selected for improved

117   growth and carcass yield.

118   The aim of this study was therefore to investigate the effect of increasing the

119   concentration of dietary lipid in a barley-straw based diet, typical of industry practice,

120   by replacing grass silage with brewers' grains, on CH_4 emissions of pregnant, non-

121   lactating spring calving beef cows of two breeds.

122

123   **Material and methods**

124   This study was conducted at the Beef and Sheep Research Centre, SRUC situated 6

125   miles south of Edinburgh UK. The experiment was approved by the Animal

126 Experiment Committee of SRUC and was conducted in accordance with the

127 requirements of the UK Animals (Scientific Procedures) Act 1986.

128

129 *Experimental design, diets and animals*

130 The experiment was of a two x two factorial design, comprising of two barley-straw

131 based diets with either (i) grass silage or (ii) brewers' grains as alternative protein

132 sources and two cow breed types (**LIMx**, crossbred Limousin; **LUI**, purebred Luing).

133 The two experimental diets consisted (g/kg dry matter (**DM**) basis) of (i) barley straw

134 (687) and grass silage (301; **GS**) or (ii) barley straw (763) and brewers' grains (226;

135 **BG**), which were offered as total mixed rations (TMR). The ingredient and chemical

136 composition of the experimental diets are given in Table 1. The chemical

137 composition of individual dietary components is given in Table 2. The DM contents of

138 individual components were determined twice weekly and bulked feed samples

139 (three per component) were analysed. Feed samples were analysed for DM, ash,

140 crude protein, acid detergent fibre, neutral detergent fibre, acid hydrolysed ether

141 extract (AHEE), water soluble carbohydrate, starch and neutral cellulose and

142 gamminase digestibility (Ministry of Agriculture Fisheries and Food, 1992) and gross

143 energy by adiabatic bomb calorimetry. The LIMx cows were all Limousin-sired from a

144 2-breed (Limousin and Aberdeen Angus) reciprocal crossing program whilst the

145 Luings were all purebred Luing cows. The breeds were selected to represent two

146 commercially relevant breeds where crossbred Limousin cows represent the most

147 common continental sired beef breed in the UK, whilst the LUI breed is typical of a

148 more extensively managed hardy hill and upland breed.

149 In total 48 cows (n=24 of each breed type) were group-housed in equal

150 numbers of each breed type across two pens, and each diet type was allocated to

151   one pen. Thus, 12 animals were allocated to each diet x breed combination.

152   Treatments were balanced for age at the start of the experiment, number of days into

153   pregnancy and BW. In the group-pens all cows were bedded on wood fibre and

154   sawdust to ensure that consumption of bedding did not contribute to nutrient intake.

155   Fresh water was provided *ad libitum* using a water trough, and both TMR diets were

156   offered *ad libitum* to all cows twice daily using electronic feeders (HOKO, Insentec,

157   Marknesse, The Netherlands). The TMR's were formulated to meet the cow's

158   average nutrient requirements for maintenance and pregnancy according to AFRC

159   (1993). Prior to measurements using respiration chambers, feed intake and weekly

160   BW and body condition score (**BCS**) had been measured for a minimum of three

161   weeks, following a four week adaptation period to acclimatise to the diets.

162

163   *Emissions Measurement in Respiration Chambers*

164   The cows were originally allocated to six respiration chambers over an eight week

165   period, using a replicated (two times) randomised block design (each block

166   consisting of four weeks). Thus each component of the two x two factorial (breed x

167   diet) experimental design was allocated twice to each respiration chamber. The cows

168   were allocated to minimise variation in stage of pregnancy and BW on entry into the

169   respiration chambers. Prior to entry to the respiration chamber, cows were loose-

170   housed for a period of six days individually in training pens within the same building

171   of identical size (4 x 3 m) and shape to the pens within the respiration chambers to

172   allow them to acclimatise to being housed individually. The cows were then moved to

173   individual respiration chambers where they remained for three days. Cows were fed

174   once daily with *ad libitum* access to their respective TMR's and feed consumption

175   was monitored from weigh cells located in feed bins with records made at 10 s

7

176 intervals. Data for DMI during the 3 d chamber measurement period were averaged

177 per animal. Front doors of chambers were briefly opened and closed at

178 approximately 08.00 h daily to remove feed bins and again to replace bins with fresh

179 feed at approximately 09.00 h. The pens were cleaned daily between 08.00 and

180 09.00 h. Exact times when doors were opened and closed were recorded.

181 The methodology for measuring emissions using respiration chambers has

182 been previously described in Rooke *et al.* (2014). Briefly, six indirect open-circuit

183 respiration chambers were used (No Pollution Industrial Systems Ltd., Edinburgh,

184 UK). The total chamber volume (76 m^3) was ventilated to give approximately 2.5 air

185 changes/h. Temperature and relative humidity were set at 15°C and 60%,

186 respectively. Total air flow, temperature and humidity were recorded at 5 s intervals.

187 Chambers were operated under negative pressure (50 N/m^2). Methane

188 concentrations were measured by infrared absorption spectroscopy (MGA3000,

189 Analytical Development Co. Ltd., Hoddesdon, UK). The analyser was calibrated

190 before and after each three day chamber measurement period using calibration

191 gases for zero (99.998% Nitrogen, BOC Ltd., Surrey, UK) and span (500 ppmv CH_4,

192 1975 ppmv CO_2, 20.9% O_2, BOC Ltd., Surrey, UK). Gas concentrations were

193 recorded for each chamber and for inlet air every six min. Prior to the beginning of

194 the experiment, gas recoveries were measured by releasing carbon dioxide at a

195 constant rate into each chamber. The mean recovery was 98% (SEM 3.0) which was

196 not different from 100%. The final 48 h of a 72 h measurement period were used to

197 calculate daily gas production. To minimise bias caused by the entry of air when the

198 doors to the chamber were opened for feeding, and as cows did not have access to

199 feed at this time, gas concentrations measured during this period were not used for

200 further analysis. Instead, and to minimise bias, these values were replaced by the

mean value of measurements made in the last hour before the doors were opened. If a cow had consumed feed during that period, mean values for the hour preceding feed consumption was used. During the 8 week period, because of failures in air recirculation (n=3) within chambers and with gas analysis (n=6), and poor DMI from one cow (n=1), data from only 38 cows were obtained and therefore an extra set of measurements were obtained in a ninth week (for 5 of 10 cows above) to bring total number of observations to 43. On completion of the experiment, critical appraisal of the data caused three of these measurements to be rejected because of gas analysis problems and therefore there were 40 observations available for analysis (n=10 for each breed x treatment combination).

Rumen sampling and volatile fatty acid analysis

Rumen fluid samples were taken from each animal within 2 h of animals leaving the respiration chambers. Animals had *ad libitum* access to feed until removal from chambers at 09.00 h (no fresh feed was provided on the morning of removal). Approximately 50 mL of rumen liquid were taken by inserting a stomach tube (16 x 2700 mm Equivet Stomach Tube, JørgenKruuse A/S, Langeskov, Denmark) nasally and aspirating manually. This liquid was filtered through two layers of muslin. A 5 mL sample of the filtered liquid was deproteinised by adding 1 mL metaphosphoric acid (215 g/L) and 0.5 ml methylvaleric acid (10 g/L) was added as an internal standard. These samples were stored at -20 °C between collection and analysis. Volatile fatty acid (VFA) concentrations were determined by HPLC as described in Rooke *et al.* (1990).

Statistical analysis

226 For all traits other than DMI (kg/day) statistical analyses were conducted using the

227 mixed procedure of SAS software (SAS Inst. Inc., Cary, North Carolina). The fixed

228 effects were diet (BG and GS) and breed (LUI and LIMx), and random effects

229 included were week (instead of block to account for the extra week of chamber

230 measurements) and chamber. The interaction between diet and breed was also

231 included in the model when these effects proved significant ($P < 0.05$). For

232 comparison of daily DMI at different measurement periods, data were analysed using

233 the mixed procedure of SAS software using a repeated measures ANOVA including

234 the effects of diet (BG and GS), breed (LUI and LIMx) and measurement period

235 (group pen, training pen, chamber). There were no interactions between diet, breed

236 and period and thus no interaction terms were included in the model. Probability

237 values were deemed as significant where $P < 0.05$. Data are reported as means with

238 their standard errors of the mean (**SEM**).

239

240 **Results**

241 *Body weight and body condition score*

242 Mean values for BW and BCS parameters determined in this study are presented in

243 Table 3. Due to cow allocation to treatments there were no diet or breed differences

244 for either age or number of days into pregnancy on entry to the chamber ($P > 0.05$).

245 Furthermore, there were no between-diet differences for BW or BCS on entry to the

246 chamber ($P > 0.05$). Body weight was affected by breed where LUI cows had lower

247 BW than LIMx cows (572 *vs.* 668 kg; $P < 0.01$). Body condition score was affected

248 by breed where LUI cows had poorer BCS than LIMx cows (2.5 *vs.* 3.1; $P < 0.001$).

249

250 *Dry matter intake*

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251 No diet or breed differences were observed for DMI expressed as kg per day (Table

252 3; P > 0.05). Dry matter intake differed between measurement periods where cows

253 had lower DMI (kg/day) within the group-pen environment compared to DMI

254 measured in the training pens and respiration chambers (group pen = 9.10 kg/day,

255 training pen = 9.66 kg/day, chamber = 9.88 kg/day; P < 0.05; SEM = 0.259). There

256 was no interaction of measurement period with diet or breed (P > 0.05). However,

257 when expressed as a proportion of metabolic BW ($BW^{0.75}$) LUI cows had greater DMI

258 within the chambers than LIMx cows (84.5 $vs.$ 75.7 g DMI/kg $BW^{0.75}$; P < 0.05).

259

260 *Methane emissions*

261 Cows offered the BG diet (Table 3) produced less CH_4 per day than GS-fed cows

262 (131 $vs.$ 156 g/day; P < 0.01). Whether expressed as g/kg DMI (13.5 $vs.$ 16.4 g/kg

263 DMI; P < 0.05) or kJ/MJ GEI (39.2 $vs.$ 48.6 kJ/MJ GEI; P < 0.01) BG-fed cows

264 produced less CH_4 than cows offered the GS diet. Luing cows consistently produced

265 more CH_4 (g/day, g/kg DMI and kJ/MJ GEI) than LIMx cows although the breed

266 effect was not significant (P > 0.05). However, when CH_4 emission was expressed

267 as a proportion of metabolic BW, LUI cows produced more CH_4 than LIMx cows

268 (1.30 $vs.$ 1.05 g CH_4/kg $BW^{0.75}$; P < 0.01).

269

270 *Volatile fatty acid molar proportions*

271 Molar proportions of acetate (Table 4) were higher in rumen fluid samples from cows

272 fed BG compared to GS (769 $vs.$ 737 mmol/mol; P < 0.001), while the proportions

273 were lower for both propionate (146 $vs.$ 162 mmol/mol; P < 0.01) and butyrate (65

274 $vs.$ 80 mmol/mol; P < 0.01). The proportions of valerate did not differ between diet

275 types (P > 0.05). Thus the acetate to propionate ratio was greater in cows fed the BG

11

276   than the GS diet (5.5 *vs.* 4.6; $P < 0.001$). There was no difference between the two

277   breeds for volatile fatty acid molar proportions ($P > 0.05$).

278

279   **Discussion**

280

281   *Diet effects on methane emissions*

282   Increasing the concentration of lipid in ruminant diets reduces CH_4 emissions (Martin

283   *et al.,* 2010; Grainger and Beauchemin, 2011; Hristov *et al.,* 2013; Patra, 2013).

284   However, the effectiveness of dietary lipid depends on the type and amount of lipid

285   added to the diet (Brask *et al.,* 2013; Hristov *et al.,* 2013). However, less attention

286   has been paid to the nature of the basal diet. The present study demonstrated that

287   incorporating brewers' grains into a straw-based diet reduced CH_4 emissions from

288   beef cows; replacing grass silage with brewers' grains increased the lipid

289   concentration from 20 to 37 g AHEE/kg diet DM and reduced CH_4 yield (g/kg DMI) by

290   17%. In recent reviews, Grainger and Beauchemin (2011) found that CH_4 yield

291   decreased 1 g/kg DMI for every 10 g/kg DM increase in dietary lipid, and Martin *et al.*

292   (2010) reported that CH_4 yield decreased by 3.8% with every 10 g lipid/kg DM

293   increase*.* In the present study CH_4 yield decreased by 1.6 g/kg DMI or 10% for every

294   10 g AHEE/kg diet DM increase upon inclusion of brewers' grains which is greater

295   than the above reports. In the present study, however, cows were observed to

296   attempt to select brewers' grains from the mixed feed. This would result in a higher

297   proportion of brewers' grains consumed compared to that offered. It was not

298   anticipated that the cows would attempt to select specific dietary constituents, thus it

299   is important to consider the potential differences in the composition of the consumed

300   diet. To explore the potential difference in dietary lipid consumed, a corrected

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301 estimate of the ratio of barley straw to brewers' grains was calculated based on the

302 assumption that all refusals consisted solely of barley straw and did not contain

303 brewers' grains. Based on 216 observations, the consumed ration was calculated as

304 (g/kg DM basis) 334 brewers' grains and 666 straw instead of the formulated ration

305 of 226 brewers' grains and 763 straw. Based on this corrected ratio, the lipid content

306 of the diet would have increased to 49 g AHEE/kg diet DM compared to 37 g

307 AHEE/kg diet DM in the formulated ration. This brings the results in line with the

308 findings of Grainger and Beauchemin (2011) where at this corrected concentration a

309 1 g/kg reduction in CH_4 for every 10 g/kg increase in dietary lipid was observed.

310 Furthermore, the effect of dietary lipid may be greater within a diet containing a high

311 proportion of low digestible fibre such as barley straw. Martin *et al.* (2010) reported

312 that the effects of dietary lipid were greater on a hay diet than a maize silage diet

313 and previous findings have demonstrated greater reductions in CH_4 production on

314 forage than concentrate-based diets (Lovett *et al.,* 2003; Troy *et al.,* 2015).

315   The use of by-products containing dietary lipid can be an effective strategy for

316 reducing CH_4 emissions from cattle. Troy *et al.* (2015) investigated the addition of

317 cold-pressed rapeseed cake to the diet of finishing beef steers and found that the

318 addition of rapeseed cake, which is higher in lipid than brewers' grains (174 g

319 AHEE/kg DM), to a mixed forage and concentrate diet (52 g AHEE/kg diet DM)

320 resulted in a reduction in CH_4 yield of 3.3% (0.83 g/kg DMI) per 10 g/kg DM increase

321 in dietary lipid, which is slightly lower than the results reported here. Brask *et al.,*

322 (2013) added rapeseed cake (173 g crude fat/kg DM) to the diet of dairy cows and

323 found a greater CH_4 yield reduction of 4.6% for every 10 g/kg DM increase in dietary

324 lipid. Relatively few studies have reported the effects of including brewers' grains in

325 the diet on CH_4 production. However, Moate *et al.* (2011) used brewers' grains,

326 hominy meal and a combination of hominy meal and cold pressed rapeseed in dairy

327 cow diets, where the diets contained 51, 65 and 52 g crude fat/kg diet DM,

328 respectively (compared to the control which contained 26 g crude fat /kg diet DM).

329 Moate *et al.* (2011) observed a 5% reduction of CH_4 yield on both the brewers'

330 grains and combined hominy meal and rapeseed treatments, and 12% on the

331 hominy meal treatment; the greater reduction on the hominy meal treatment was

332 likely due to the higher lipid concentration in the diet. They demonstrated for each 10

333 g/kg DM increase in dietary lipid concentration, CH_4 emissions were reduced by

334 3.5%. Although the majority of studies to date have not investigated the persistency

335 of the effects of lipid on suppressing CH_4 production, Moate *et al.* (2011)

336 demonstrated a persistency of their dietary effects over more than 7 weeks. The

337 effect of lipid persisted throughout the current experiment (9 weeks) as there was no

338 effect of measurement week ($P = 0.50$) on CH_4 production.

339   One of the mechanisms by which lipid is thought to suppress CH_4 production

340 is through increased production of propionate versus acetate and thus reduction in

341 the amounts of hydrogen generated through fermentation. The meta-analysis of

342 Patra (2013) demonstrated that although total VFA concentrations were not altered

343 by increasing the dietary lipid content, the proportion of propionate to acetate

344 increased and the proportion of butyrate decreased with increasing concentration of

345 lipid which supports the above mechanism. In contrast, an increase in acetate and

346 decrease in propionate on the BG diet was observed in the present study alongside

347 a reduction in butyrate. Increasing the concentrate proportion of the diet is normally

348 associated with increases in propionate molar proportions, although the response is

349 likely to depend on the nutrients supplied by the diet. In the present study, 964 g/kg

350 DM in diet BG was accounted for by neutral detergent fibre, crude protein, AHEE

14

351 and ash whereas only 649 g/kg DM was accounted for by these constituents in diet

352 GS. The constituents unaccounted for in GS include fermentation acids, particularly

353 lactic acid. Since there is a positive correlation between silage lactic acid

354 concentration and rumen propionic acid molar proportion (Martin *et al.* 1994), the

355 greater propionic acid molar proportions in the GS diet most likely reflected the lactic

356 acid in the silage and the low concentrations of starch in the brewers' grains. From

357 the above, decreased hydrogen supply to the rumen archaea from increased

358 production of propionic acid does not appear to be the most likely mechanism of

359 action of lipid in the present experiment. More likely mechanisms may be physical

360 coating of fibre by the lipid and the reduction in rumen-fermentable substrates as a

361 result of lipid addition. Furthermore, apart from the increased lipid content of the diet,

362 the other main changes in the composition of the diet when brewers' grains replaced

363 grass silage were increased NDF and CP contents. The greater acetate to

364 propionate ratio observed for diet BG is consistent with the increase in NDF but there

365 was no increase in branched chain VFA on diet BG which might be expected from

366 increased protein degradation. However, the increase in acetate to propionate ratio

367 on diet BG was not associated as might be expected with an increase in CH_4

368 emissions and therefore it is likely that the increase in dietary lipid was the major

369 factor underlying the reduction in CH_4 emissions observed when BG rather than GS

370 was fed.

371   At high concentrations in the diet, lipid can negatively affect DMI and

372 productivity, but low concentration of dietary lipid can be used with no adverse

373 effects (Brask *et al.,* 2013). Based on a meta-analysis, Patra (2013) demonstrated

374 that lipid supplementation in excess of 6% causes problems with productivity. Diets

375 which negatively affect productivity are unsuitable for livestock producers, and

376 therefore, in the present study the concentration of lipid in the BG diet was 37 g

377 AHEE/kg diet DM in the formulated ration. As expected, and consistent with the

378 literature, this concentration of dietary lipid did not suppress DMI. Even if we assume

379 the animals preferentially selected the diet as observed, the diet would still not have

380 reached a concentration of lipid expected to suppress DMI. However, at this

381 corrected level of BG inclusion a marked decrease in CH_4 production was observed

382 without adverse effects on DMI. Therefore, the use of by-products such as brewers'

383 grains, represents an attractive strategy for use in beef cow diets from both an

384 animal productivity and CH_4 mitigation perspective.

385 　　In the present study mean CH_4 yields for each of the BG and GS diets were

386 0.039 and 0.049 MJ/MJ GEI respectively, considerably lower than the value

387 currently adopted by the IPCC (2006) (0·065 MJ/MJ GEI). The IPCC (2006)

388 approach does not account for differences in the digestibility of diets and as a result

389 over-estimates CH_4 yield from diets containing large proportions of forages which

390 are low in digestibility.

391 　　Respiration chambers are generally considered to be the most accurate

392 technology for measuring CH_4 emissions from ruminants. However, one of the major

393 challenges associated with this technology is avoiding a reduction in feed intake

394 within the chamber environment, where the animals are individually housed. This is

395 necessary for CH_4 emissions data to be representative of normal feeding behaviour

396 in a group-housed environment (Garnsworthy *et al.*, 2012; Bickell *et al.,* 2014). In the

397 present study, no reduction in DMI was observed from group-housing to respiration

398 chambers, but there was a small increase in DMI per day of 9%.

399

400 *Breed effects on methane emissions*

16

401   There is limited experimental evidence to support differences in CH_4 emissions

402   between breeds. Rooke *et al.* (2014) examined CH_4 emissions from crossbred

403   Limousin and crossbred Aberdeen Angus, and found no difference between these

404   breeds in methane yield whether expressed per level of DMI or GEI. Troy *et al.*

405   (2015) compared two breeds of finishing beef steers (purebred Luing and crossbred

406   Charolais) and reported no differences between breeds in CH_4 whether expressed

407   as g/day, g/kg DMI or kJ/MJ GEI. These studies were both conducted using the

408   same respiration chambers and methodologies to those used in the current study.

409   Differences in grazing behaviour between breeds is likely to have a large

410   impact on CH_4 emissions, as demonstrated in a modelling study by Ricci *et al.*

411   (2014). Based on the potential for differences in animal physiology or behaviour to

412   influence CH_4 production, a number of recent studies have been conducted within

413   outdoor grazing environments to examine CH_4 emissions from breeds which are

414   suited to extensive grazing systems compared to more intensively managed breeds

415   selected for increased growth and carcass yields. Measurements of CH_4 in grazing

416   environments are possible using the SF6 tracer technique (Deighton *et al.*, 2014). In

417   the studies of Fraser *et al.* (2014) and Richmond *et al.* (2015), where two breeds

418   were studied on two pasture types (lowland *vs.* upland pasture), no difference

419   between breeds (or interactions with pasture type) were identified for CH_4 expressed

420   on a daily, DMI or GEI basis. Rooke *et al.* (2015) reported CH_4 emissions of lactating

421   beef cows of the same two breeds considered in the present study on either

422   reseeded predominantly perennial ryegrass pasture or rough hill grazing. Consistent

423   with previous findings, daily CH_4 emissions were influenced by pasture type, but not

424   breed. The results of the present study, although measured in chamber

425   environments, were consistent with Rooke *et al.* (2015) where these breeds of beef

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426 cow did not influence CH_4 when expressed as g/day, g/kg DMI or kJ/MJ GEI. These

427 expressions of CH_4, however do not take account of the differences in BW of these

428 two breed types. Within the present study, LUI cows were considerably smaller than

429 the LIMx cows (572 *vs.* 668 kg BW) and produced greater levels of CH_4 per kg

430 metabolic BW compared to the LIMx cows. This greater level of CH_4 is driven by the

431 differences in DMI/kg $BW^{0.75}$, where LUI cows consumed greater DMI per kg

432 metabolic BW than LIMx cows. When considering the difference between breeds, it

433 is important to take additional characteristics of the animals (such as DMI/kg $BW^{0.75}$)

434 into account as these have an important influence on CH_4 production.

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436 Increasing the dietary lipid concentration has been shown to effectively reduce CH_4

437 production from ruminants provided the amount fed is less than that which adversely

438 affects digestion and feed intake. However, the practicality and sustainability of this

439 approach is dependent on the type of lipid used. Pure oils that could be used for

440 human food are of high cost, or indeed where production is controversial (palm oil)

441 may not represent the best sustainable solution. The use of by-products from ethanol

442 production (biodiesel and alcoholic beverages) or oil extraction produces feeds that

443 are better balanced in protein and lipid/energy supply than the parent feeds and are

444 well established for use within ruminant diets. Thus the use of by-products, such as

445 brewers' grains represents a cost-effective and sustainable solution for mitigation of

446 CH_4 from ruminant systems.

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557 **Table 1** *Component composition and calculated chemical composition of experimental diets*

558 *(BG, Barley straw- brewers' grains; GS, Barley straw-grass silage)*

Diets	BG	GS
Components (g/kg dry matter)		
Barley straw	763	687
Grass silage		301
Brewers' grains	226	
Mineral / vitamin mix[1]	11	12
Composition (g/kg dry matter)		
Dry matter (g/kg)	550	533
Crude protein	73	59
NDF[2]	771	693
ADF[2]	516	499
Starch	6	0
WSC[2]	6	20
AHEE[2]	37	20
Ash	38	48
Metabolisable energy (MJ /kg DM)	7.4	8.1
Gross energy (MJ /kg DM)	19.4	18.9

559 [1]mineral / vitamin mix (Norvite, Insch, Aberdeenshire, UK) supplied (mg /kg unless stated
560 otherwise) vitamin A, 500000 international units (IU); Vitamin D 100000 iu; Vitamin E 4000;
561 Fe, 5271; Mn, 5000; Zn, 3600; I, 1000; Co, 90; Cu, 3000; Se, 35.

562 [2]NDF, neutral detergent fibre; ADF, acid detergent fibre; WSC, water soluble carbohydrate;
563 AHEE, acid hydrolysed ether extract

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568 **Table 2** *Chemical composition of components (g/kg DM)*

	Barley straw	Grass silage	Brewers' grains
Dry matter (g/kg)	805	298	263
Crude protein	20	150	255
NDF[1]	847	370	553
ADF[1]	593	303	279
Starch	0	0	26
WSC[1]	7	50	3
AHEE[1]	14	36	118
Ash	38	73	38
NCGD[1]	308	0	567
Metabolisable energy (MJ /kg DM)	6.5	12.1	10.9
Gross energy (MJ /kg DM)	18.8	19.8	22.5
pH		4.3	

569 [1]NDF, neutral detergent fibre; ADF, acid detergent fibre; WSC, water soluble carbohydrate;
570 AHEE, acid hydrolysed ether extract; NCGD, neutral cellulose and gamanase digestibility

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583 **Table 3** *Age, BW and body condition score (BCS) of cows at allocation, intakes and CH_4*

584 *production as measured from the respiration chambers (means with average SEM)*

Diet[1]	BG		GS			Significance[4]	
Breed	LIMx	LUI	LIMx	LUI	SEM	Breed	Diet
On entry to chamber							
Age (years)	5.2	5.7	6.1	4.6	1.33	ns	ns
Days pregnant[2]	212	224	226	212	4.11	ns	ns
BW (kg)	697	587	638	557	32.58	**	ns
BCS	3.2	2.6	3.0	2.4	0.12	***	ns
DMI							
Group pen kg/d[3]	9.62	8.65	9.28	8.84	0.60	ns	ns
Training pen kg/d	9.70	9.36	10.32	9.26	0.60	ns	ns
Chamber kg/d	10.06	9.77	9.90	9.81	0.82	ns	ns
Chamber g/kg $BW^{0.75}$	74.1	82.8	79.0	85.8	6.45	*	ns
CH_4							
g/d	129	133	143	169	11.17	ns	**
g/kg DMI	13.2	13.9	14.7	18.0	1.48	ns	*
kJ/MJ GEI	38.2	40.3	43.8	53.4	4.36	ns	**
g/kg $BW^{0.75}$	0.95	1.12	1.14	1.48	0.09	**	***

585 *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

586 DMI, dry matter intake; GEI, gross energy intake; LIMx, crossbred Limousin; LUI, purebred
587 Luing

588 [1]BG, Barley Straw-Brewers' Grains; GS, Barley Straw-Grass Silage

589 [2]Three animals were identified as not in calf (all LUI, one allocated to BG and 2 allocated to
590 GS diet)

591 [3]Measured throughout 1 week prior to entry to training pen

592 [4]The interaction effect of breed x diet was not significant for any trait except days pregnant
593 ($P<0.01$).

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595 **Table 4** *Volatile fatty acid molar proportions (mmol/mol) in rumen fluid samples taken on exit*

596 *from respiration chambers (means and average SEM are given for effects of breed and diet)*

Diet[1]	BG		GS			Significance[3]	
Breed	LIMx	LUI	LIMx	LUI	SEM	Breed	Diet
Acetate	769	770	738	736	9.2	ns	***
Propionate	145	146	162	162	9.0	ns	**
Butyrate	65	65	78	81	4.2	ns	***
Valerate	6	6	6	6	0.6	ns	ns
Branched Chain[2]	15	13	16	15	1.2	ns	ns
Acetate:propionate ratio	5.5	5.4	4.6	4.6	0.4	ns	***

597 *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

598 LIMx, crossbred Limousin; LUI, purebred Luing

599 [1]BG, Barley Straw-Brewers' Grains; GS, Barley Straw-Grass Silage

600 [2]Branched chain is sum of iso-butyrate and iso-valerate

601 [3]The interaction effect of breed x diet was not significant for any trait

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