

Scotland's Rural College

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**Impact of adding nitrate or increasing the lipid content of two contrasting diets on blood methaemoglobin and performance of two breeds of finishing beef steers**

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Short title: Blood methaemoglobin and performance of cattle

26 **Abstract**

27 Adding nitrate to the diet or increasing the concentration of dietary lipid are effective  
28 strategies for reducing enteric methane emissions. This study investigated their  
29 effect on health and performance of finishing beef cattle. The experiment was a two  
30 × two × three factorial design comprising two breeds (CHX, crossbred Charolais; LU,  
31 Luining); two basal diets consisting of (g/kg dry matter (DM), forage to concentrate  
32 ratios) 520:480 (Mixed) or 84:916 (Concentrate); and three treatments: (i) control  
33 with rapeseed meal as the main protein source replaced with either (ii) calcium  
34 nitrate (18 g nitrate/kg diet DM) or (iii) rapeseed cake (increasing acid hydrolysed  
35 ether extract from 25 to 48 g/kg diet DM). Steers ( $n = 84$ ) were allocated to each of  
36 the six basal diet × treatments in equal numbers of each breed with feed offered *ad*  
37 *libitum*. Blood methaemoglobin (MetHb) concentrations (marker for nitrate poisoning)  
38 were monitored throughout the study in steers receiving nitrate. After dietary  
39 adaptation over 28 days, individual animal intake, performance and feed efficiency  
40 were recorded for a test period of 56 d. Blood MetHb concentrations were low and  
41 similar up to 14 g nitrate/kg diet DM but increased when nitrate increased to 18 g  
42 nitrate/kg diet DM ( $P < 0.001$ ). An interaction between basal diet and day ( $P < 0.001$ )  
43 indicated that MetHb% was consistently greater in Concentrate- than Mixed-fed  
44 steers at 18 g nitrate/kg diet DM. Maximum individual MetHb% was 15.4% (of total  
45 Hb), which is lower than considered clinically significant (30%). MetHb  
46 concentrations for individual steers remained consistent across time. Concentrate-  
47 fed steers were more efficient (lower residual feed intake (RFI) values) than Mixed-  
48 fed steers ( $P < 0.01$ ), with lower dry matter intake (DMI) (kg/d) ( $P < 0.001$ ) and  
49 similar average daily gain (ADG). CHx steers were more efficient (lower RFI;  $P <$   
50 0.01) than LU steers with greater ADG ( $P < 0.01$ ), lower DMI (/kg BW;  $P < 0.01$ ) and

51 lower fat depth ( $P < 0.001$ ). ADG, BW or DMI did not differ across dietary treatments  
52 ( $P > 0.05$ ). Neither basal diet nor treatment affected carcass quality ( $P > 0.05$ ), but  
53 CHX steers achieved a greater killing out proportion ( $P < 0.001$ ) than LU steers.  
54 Thus, adding nitrate to the diet or increasing the level of dietary lipid through the use  
55 of cold-pressed RSC, did not adversely affect health or performance of finishing beef  
56 steers when used within the diets studied.

57

58 **Keywords:** beef cattle, lipid, methaemoglobin, nitrate, performance

59

## 60 **Implications**

61 Adding nitrate to the diet or increasing the level of dietary lipid has been shown to  
62 reduce methane from cattle. These strategies should not adversely affect animal  
63 health or performance. The use of nitrate in ruminant diets has been limited due to  
64 the potential adverse effects on health and productivity. Following four weeks  
65 adaptation, neither the addition of nitrate to the diet (18 g nitrate/kg diet dry matter)  
66 nor increased dietary lipid (48 g acid hydrolysed ether extract/kg diet dry matter)  
67 adversely affected steer health or performance. These strategies provide the  
68 potential for reducing the environmental impact of beef enterprises.

69

## 70 **Introduction**

71 Livestock systems, in particular ruminant production, are under increasing political  
72 pressure to reduce their greenhouse gas outputs. Breeding, enterprise and systems  
73 management and diet formulation are all possible strategies to reduce methane  
74 ( $\text{CH}_4$ ) from cattle (Cottle *et al.*, 2011), with diet formulation representing one of the  
75 most practical and promising approaches. In addition to determining the

76 effectiveness of dietary strategies on CH<sub>4</sub>, it is important to report their implications  
77 on health, overall performance and efficiency.

78         Recent interest in the controlled feeding of nitrate has been stimulated  
79 because the reduction of nitrate to ammonium in the rumen of adapted animals  
80 provides an alternative hydrogen sink to the production of CH<sub>4</sub> (van Zijderveld *et al.*,  
81 2010). The reduction of nitrate to nitrite and then to ammonium provides an  
82 energetically more favourable route for disposal of metabolic hydrogen produced  
83 during fermentation of feed carbohydrates in the rumen than the production of CH<sub>4</sub>.  
84 Although nitrate has been shown in many studies to reduce CH<sub>4</sub> emissions from  
85 ruminants (Nolan *et al.*, 2010; van Zijderveld *et al.*, 2010; van Zijderveld *et al.*, 2011,  
86 Hulshof *et al.*, 2012; Li *et al.*, 2012), the potential for its use has been hindered due  
87 to the toxicity of the intermediate product (nitrite). In the rumen, microbes rapidly  
88 reduce nitrate to nitrite and then reduce nitrite to ammonia. However, in an animal  
89 that has not been previously exposed to nitrate, the rate of reduction of nitrite to  
90 ammonia is slower than the reduction of nitrate to nitrite resulting in the accumulation  
91 of nitrite in the rumen (van Zijderveld *et al.*, 2010; Jeyanathan *et al.*, 2014). Absorbed  
92 nitrite binds to haemoglobin (Hb) in the blood converting it to methaemoglobin  
93 (MetHb) which is not capable of transporting oxygen to tissues. High concentrations  
94 of MetHb can cause methaemoglobinaemia, in which the functional oxygen carrying  
95 capacity of the blood is reduced. Blood MetHb is used as a marker for nitrate  
96 poisoning with a value of 30% of total Hb associated with clinical symptoms  
97 (Bruning-Fann and Kaneene, 1993). Nitrate toxicity may reduce animal performance  
98 (feed intake, growth, loss of BW), but in more severe cases may be fatal (Cockburn  
99 *et al.*, 2013). Therefore, the use of nitrate in ruminant diets requires careful  
100 consideration.

101           Increasing the concentration of dietary lipid has been shown to reduce CH<sub>4</sub>  
102 emissions from ruminants (Martin *et al.*, 2010; Grainger and Beauchemin, 2011;  
103 Patra, 2013). This is achieved through various mechanisms: fatty acids are not  
104 fermented in the rumen and therefore increasing dietary lipid concentration reduces  
105 the proportion of feed which is fermentable within the rumen; lipids can also reduce  
106 CH<sub>4</sub> production by coating fibre particles, reducing their digestibility, and by reducing  
107 the numbers and activity of the rumen methanogens and protozoa responsible for  
108 methanogenesis (Johnson and Johnson, 1995; Patra, 2013). Dietary lipid can be  
109 increased through the addition of pure fats or oils to the diet or through the use of by-  
110 products from distilleries, breweries or plant oil extraction as ingredients in the diet  
111 (Brask *et al.*, 2013). At concentrations greater than 6% (DM basis), lipid can  
112 negatively affect feed intake and productivity, but lipid concentrations lower than 6%  
113 can be used with no adverse effects (Brask *et al.*, 2013).

114           When considering mitigation strategies for beef cattle, studies have been  
115 mainly focussed on breeds that are managed more intensively, with less focus on  
116 breeds suited to extensive systems. The performance characteristics of hill and  
117 upland breeds, when managed more intensively, may be considerably different to  
118 that of intensively managed breeds, although the availability of performance data is  
119 limited. For example, baseline performance data of Luing cattle, a hill and upland  
120 breed, is unavailable in the literature, even though their popularity as suckler cows is  
121 increasing in the UK and consequently the numbers of Luing calves reaching  
122 finishing units for more intensive finishing is also rising. Calf registrations of Luing  
123 and crossbred Luing calves in the UK has increased from 6165 in 2011 to 6525 in  
124 2014 and is likely to increase further in 2015 (Agriculture and Horticulture  
125 Development Board, UK, 2015, personal communication). The large differences in

126 performance are likely a result of considerable genetic and physiological differences.  
127 It is important to consider the effect of mitigation strategies across different breeds,  
128 alongside their implications for health and productivity. If this differs across different  
129 breeds, the industry and beef producers need to understand this if a real change in  
130 CH<sub>4</sub> output is to be delivered in commercial practice.

131 The primary objective of the present study was to investigate the effect of  
132 adding nitrate, or increasing the concentration of dietary lipid, within two contrasting  
133 diets which are typical of industry practice, on the performance and carcass quality  
134 of finishing beef steers of two breeds. Due to the risks associated with feeding  
135 nitrate, a further objective was to investigate the effect of dietary nitrate on blood  
136 MetHb concentrations.

137

## 138 **Materials and methods**

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

143

### 144 *Experimental design, animals and diets*

145 The experiment was of a two × two × three factorial design, comprising two breeds  
146 (CHX, crossbred Charolais; LU, purebred Luing), two basal diets (concentrate-straw  
147 or silage-based) and three treatments selected for their potential CH<sub>4</sub> mitigation  
148 effects (Control, Nitrate or Rapeseed Cake (RSC)). The breed types were selected  
149 to represent two commercially relevant breeds where CHX cattle represent a  
150 common continental sired beef breed in the UK well known for fast growth and

151 excellent carcass conformation, whilst the LU breed is typical of a more extensively  
152 managed hardy hill and upland breed. The steers were fed one of two basal diets (as  
153 total mixed rations) using a diet mixing wagon, consisting of (g/kg dry matter (DM))  
154 forage to concentrate ratios of either 520:480 (Mixed) or 84:916 (Concentrate).  
155 Within each basal diet the steers were offered one of three treatments: (i) Control  
156 containing rapeseed meal as the main protein source which was replaced with either  
157 (ii) Nitrate in the form of calcium nitrate (Calcinit, Yara, Oslo, Norway; 18 g nitrate/kg  
158 diet DM) or (iii) an added source of lipid in the form of pelleted RSC which is a by-  
159 product from cold-pressing rapeseed (acid hydrolysed ether extract (AHEE)  
160 increased from 25 to 48 g AHEE/kg diet DM). The ingredient and chemical  
161 composition of the experimental diets are given in Table 1. The chemical  
162 composition of individual components is given in Table 2. The DM contents of  
163 individual components were determined on duplicate samples twice weekly and  
164 bulked feed samples (two per component) were analysed. Feed samples were  
165 analysed for DM, ash, CP, ADF, NDF, AHEE, starch and neutral cellulase and  
166 gammanase digestibility (Ministry of Agriculture Fisheries and Food, 1992) and gross  
167 energy by adiabatic bomb calorimetry. For the Nitrate and RSC-containing diets,  
168 calcium nitrate and RSC were incorporated firstly into a premix which contained the  
169 concentrate portion of the diet alongside minerals and molasses. Each batch of  
170 premix was mixed using a diet mixing wagon to produce a consistent premix. On a  
171 daily basis each premix was then mixed with the forage portion of the diet using the  
172 same mixing wagon to generate a consistent total mixed ration. Diets were mixed for  
173 a minimum duration of 20 minutes.

174 In total, 84 steers (42 of each breed) were used. Thus, 14 animals (seven of  
175 each breed) were allocated to each of the six basal diet × treatment combinations.



176 Due to the high risk of ill-health of unadapted animals gaining access to dietary  
177 nitrate, and the risks of forage-fed animals gaining access to large quantities of  
178 concentrate (e.g. acidosis), each diet x treatment combination was allocated to one  
179 pen (six pens in total). Treatments were balanced for sire within each breed, farm of  
180 origin and BW and were balanced across basal diets and treatment groups at the  
181 start of the experiment. Fresh water was provided *ad libitum* using a water trough,  
182 and diets were offered *ad libitum* to all steers using 32 electronic feeders (HOKO,  
183 Insentec, Marknesse, The Netherlands). Electronic feeders allow expression of  
184 performance in an environment close to on-farm conditions. All steers were bedded  
185 on wood fibre and sawdust to ensure that consumption of bedding did not contribute  
186 to nutrient intake.

187 Steers were adapted to the experimental diets in two stages. In stage one  
188 (day -56 to day -29), the animals were adapted to the basal diets. All steers were  
189 being fed the Mixed diet at the start of the adaptation period. Steers which were  
190 allocated the Concentrate diet, were adapted to the full concentrate inclusion over 4  
191 weeks. This was undertaken at weekly intervals where diets comprising (g/kg DM)  
192 forage to concentrate ratios of 38:62, 25:75, 13:87 and 8:92 were offered during  
193 weeks 1, 2, 3 and 4, respectively. During this period, steers were trained to use the  
194 electronic feed intake recording equipment. In stage two (day -28 to day 0), steers  
195 were adapted to the treatments over a second 4 week period. Treatments (Nitrate  
196 and RSC) were progressively incorporated into the diets at 25%, 50%, 75% and  
197 100% of the required level, on days -28, -21, -14 and -7, respectively.

198

199 *Blood methaemoglobin measurements*

200 All steers receiving dietary nitrate had blood samples taken weekly throughout the  
201 second treatment adaptation phase to monitor blood MetHb concentrations. Blood  
202 samples were taken when MetHb was expected to be greatest, i.e., 3 h after fresh  
203 feed was offered (van Zijderveld *et al.*, 2010), on the day after dietary nitrate was  
204 increased (days -27 (25%), -20 (50%), -13 (75%) and -6 (100%)) and then 15 days  
205 after maximum nitrate inclusion was achieved (day 8). To assess the long-term  
206 effects of feeding nitrate, blood samples were obtained at day 87 and day 101 (128  
207 days after initial inclusion of nitrate). Blood samples were taken from the caudal vein  
208 into an evacuated tube (Vacurette, Griener Bio One Ltd., Gloucestershire, UK)  
209 containing heparin. MetHb concentration in blood was measured within 2 h of  
210 sampling by co-oximetry (Stat Profile Critical Care Xpress, Nova Biomedical U.K.,  
211 Cheshire, UK). For each steer, dry matter intake (DMI) (kg/d) and weekly BW were  
212 assessed throughout adaptation.

213

#### 214 *56-d performance test*

215 After full adaptation to the experimental diets, performance and feed efficiency were  
216 characterised for all steers over a 56 d test period (day 0 to 56). Steers were  
217 maintained under controlled conditions, where group sizes within the pen remained  
218 constant. Individual DMI (kg/d) was recorded for each animal using the electronic  
219 feeding equipment and BW was measured weekly using a calibrated weigh scale.  
220 Measurements of BW were obtained before fresh feed was offered. For all steers,  
221 ultrasonic fat depth was obtained at the 12<sup>th</sup>/13<sup>th</sup> rib at the start (FD0) and end (FD1)  
222 of the 56 d test using an industry-standard Aloka 500 machine (BCF technology ltd.,  
223 Scotland, UK). Images were analysed using Matrox Inspector 8 software (Matrox  
224 Video and Imaging Technology Europe Ltd., Middlesex, UK). Hyslop *et al.* (2012)

225 assessed the consequences of alternative test lengths on the precision of average  
226 daily gain (ADG) and demonstrated that a 56-day measurement period, with weekly  
227 weighing is sufficient for characterising ADG of finishing beef cattle.

228

#### 229 *Pre-slaughter measurements and carcass quality*

230 Steers remained within the same pens and on the same diets from the end of the 56  
231 d test to slaughter. During this period CH<sub>4</sub> measurements were obtained from 76 of  
232 these steers and reported in Troy *et al.* (2015). On the day before slaughter,  
233 ultrasonic fat depth (FD2) at the 12<sup>th</sup>/13<sup>th</sup> rib was measured in all steers as described  
234 above. Steers were slaughtered in four batches of 17, 18, 21 and 25 steers on days  
235 85, 106, 127 and 148, respectively. Steers were selected for slaughter based on BW  
236 and visual assessment of fatness. The steers were transported (approximately 1 h)  
237 to a commercial abattoir and slaughtered within 2 h of arrival. Cattle were stunned  
238 using a captive bolt, exsanguinated and subject to low voltage electrical stimulation.  
239 Following hide removal, carcasses were split in half down the mid-line and dressed  
240 to UK specification (see Meat and Livestock Commercial Services Limited beef  
241 authentication manual, [www. mlcsl.co.uk](http://www.mlcsl.co.uk), for full description). EUROP conformation  
242 and fat classifications (Fisher, 2007), based on the UK scale, were allocated to all  
243 carcasses through visual assessment using a trained assessor.

244 Video Image Analysis (VIA) was used to estimate EUROP classifications  
245 (conformation and fat), total lean (kg) and total fat (kg) content of the whole carcass.  
246 The VIA systems in use in the EU are automatic machines that perform carcass  
247 evaluation based on images of the half carcass. The VBS 2000 system used in this  
248 study (E+V technology GmbH, Oranienburg, Germany) has been approved by the  
249 Department for Environment, Food and Rural Affairs (Defra) for use in the UK since

250 2010. The system operated at the end of the slaughter line after all necessary  
251 dressing and trimming had been completed. A pneumatically operated cradle  
252 presented the left half side of each carcass for imaging. The VIA camera took two  
253 images of the half carcass, a 2-dimensional image and a pseudo 3-dimensional  
254 image using structured light (Craigie *et al.*, 2012). The VBS 2000 required  
255 information on the category of the carcass (i.e., steer) and hot carcass weight (kg)  
256 and, by combining this information with data automatically captured by the VIA  
257 system (i.e., carcass dimensions, angles, areas, colour), predicted EUROP  
258 classification and total lean and fat content of the whole carcass.

259

#### 260 *Calculations and statistical analysis*

261 MetHb data were analysed using the mixed procedure of SAS software (SAS  
262 Institute Inc., Cary, NC, USA) using a repeated measures ANOVA including the  
263 effects of basal diet, sampling day and their interactions. Data are reported as  
264 means and standard errors of the mean (s.e.m.).

265 Data from three steers were unavailable as the animals were removed from  
266 the trial during the 56-d test period for health reasons unconnected to the diets and  
267 treatments imposed. Growth was modelled by linear regression of BW against test  
268 date, to obtain ADG, mid-test BW (mid-BW) and mid-test metabolic BW (mid-MBW =  
269  $BW^{0.75}$ ). Mean DMI over the 56 d period was expressed as kg per day or as a  
270 proportion of mid-BW and mid-MBW. Feed conversion ratio (FCR) was calculated as  
271 average DMI per day (kg/d)/ADG. Residual feed intake (RFI) was calculated as  
272 deviation of actual DMI (kg/d) from DMI predicted based on linear regression of  
273 actual DMI on ADG, mid-MBW and FD1 (Basarab *et al.*, 2003). Cold carcass weight  
274 (CCW) was calculated as a percentage of slaughter BW (SBW) to determine killing

275 out percentage (KO). To allow for statistical comparison, the EUROP carcass  
276 classification values were expressed on the equivalent 15 point scale (Kempster *et*  
277 *al.*, 1986). Statistical analyses of performance and carcass data were conducted  
278 using the mixed procedure of SAS software with the fixed effects of breed, basal diet  
279 and treatment, and the random effect of pen (and slaughter batch for carcass traits).  
280 In addition, in the analysis of FD1 and FD2 the deviation from the breed mean of  
281 FD0 was included as a covariable. The interaction effects of breed × basal diet,  
282 basal diet × treatment, breed × treatment and breed × basal diet × treatment were  
283 included in the model when these effects proved significant ( $P < 0.05$ ). Data are  
284 reported as means with their s.e.m. Differences between means were tested using a  
285 least square means comparison test (PDIF option of SAS). Probability values were  
286 deemed significant where  $P < 0.05$  and indicated a tendency when probability values  
287 were between  $P = 0.05$  and  $P = 0.1$ .

288

## 289 **Results**

### 290 *Blood met-haemoglobin response to dietary nitrate*

291 During the adaptation period (Table 3), blood MetHb concentrations were similar  
292 when feed contained up to 75% of total nitrate (up to -13 d) but increased when  
293 nitrate was included at the 100% level (18 g nitrate/kg diet DM) on both basal diets.  
294 During adaptation there was no difference ( $P > 0.05$ ) in MetHb between the basal  
295 diets but blood MetHb concentrations of steers offered the Concentrate diet were  
296 consistently greater (day × basal diet interaction,  $P < 0.001$ ) than those offered the  
297 Mixed diet from day 8 onwards.

298         There was a consistent individual animal response across sampling days in  
299 MetHb concentrations when animals were offered the maximum dietary nitrate

300 (100%, day -6 to 101). Of 28 steers, six always had MetHb concentrations less than  
301 the median MetHb for each sampling day whilst nine steers consistently had MetHb  
302 concentrations greater than the upper quartile. Figure 1 shows individual values for  
303 five steers with the smallest mean MetHb concentration and the five steers with the  
304 greatest mean concentrations and demonstrates consistency of steer response  
305 across time (from day -6 onwards, when 100% nitrate was offered). Maximum values  
306 for blood MetHb concentration (Table 3) were always less than 30% of total Hb. The  
307 greatest individual MetHb concentration value was 15.4%. There was no significant  
308 effect of breed on blood MetHb concentrations ( $P > 0.05$ ).

309

#### 310 *56-d performance test*

311 Neither age at the start (AgeST) nor Mid-BW differed between basal diets ( $P > 0.05$ ;  
312 Table 4). Although not significant, the greater ADG in Mixed-fed steers (1.54 v. 1.41  
313 kg/d;  $P > 0.05$ ) was associated with greater daily DMI than Concentrate-fed steers  
314 (12.0 v. 11.0 kg/day;  $P < 0.001$ ). Basal diet did not affect DMI per kg BW ( $P > 0.05$ ).  
315 Concentrate-fed steers were more efficient (lower RFI) than Mixed-fed steers (-0.24  
316 v. 0.22 kg;  $P < 0.01$ ) due to lower daily DMI. Basal diet did not affect FD1 ( $P > 0.05$ ).

317 Mid-BW, ADG, DMI and FD1 (kg/day or g/kg BW) did not differ across  
318 treatments ( $P > 0.05$ ). An interaction between basal diet and treatment was identified  
319 for FCR and RFI ( $P < 0.05$ ). For concentrate-fed steers, FCR did not differ between  
320 RSC and Control treatments ( $P > 0.05$ ). There was, however, a tendency for steers  
321 offered Nitrate to have improved (lower) FCR values compared to steers offered the  
322 Control (7.40 v. 8.17 kg/kg;  $P = 0.07$ ). Similarly nitrate-fed steers achieved lower RFI  
323 values than steers offered the Control treatment but this was not significant ( $P >$

324 0.05). When offered the Mixed basal diet, neither Nitrate nor RSC treatments differed  
325 to the Control for FCR or RFI ( $P > 0.05$ ).

326 To balance for BW, CHX steers were younger than LU steers at the start of  
327 test (442 v. 476 d;  $P < 0.001$ ). Mid-BW did not differ between breeds ( $P > 0.05$ ). CHX  
328 steers achieved greater ADG than LU steers (1.56 v. 1.39 kg/day;  $P < 0.01$ ) with  
329 similar levels of daily DMI (11.4 v. 11.7 kg/day;  $P > 0.05$ ) and lower DMI per kg BW  
330 (18.98 v. 19.98 g/kg BW;  $P < 0.01$ ) to LU steers. Furthermore, FD1 was lower in CHx  
331 steers than LU steers (6.41 v. 8.28 mm;  $P < 0.001$ ). Thus, CHx steers were more  
332 efficient than LU steers with lower FCR (7.39 v. 8.57 kg, kg;  $P < 0.001$ ) and RFI (-0.2  
333 v. 0.22 kg;  $P < 0.01$ ) values.

334

#### 335 *Pre-slaughter measurements and carcass traits*

336 Carcass traits were not affected by basal diet (Table 5), except for fat score  
337 (determined by VIA) where Concentrate-fed steers had lower fat scores than Mixed-  
338 fed steers (8.02 v. 9.08;  $P < 0.001$ ). There was no difference between treatments for  
339 any carcass quality trait other than for FD2, where steers offered the RSC treatment  
340 had greater FD2 compared to the Control treatment (10.07 v. 8.48 mm;  $P < 0.05$ ).

341 Compared to LU steers, CHX steers had lower FD2 (6.99 v. 10.79 mm;  $P <$   
342 0.001), greater SBW (723 v. 701 kg;  $P = 0.051$ ), greater CCW (415 v. 369 kg;  $P <$   
343 0.001) and greater KO (57.5 v. 52.8%;  $P < 0.001$ ). LU steers offered the Concentrate  
344 diet had lower CCW than those offered the Mixed diet (357 v. 379 kg;  $P < 0.05$ ). For  
345 visually assigned EUROP classifications, CHX steers achieved greater conformation  
346 grades (9.90 v. 8.05;  $P < 0.001$ ) and lower fat grades (9.50 v. 11.03;  $P < 0.001$ )  
347 compared to the LU steers which are in agreement with the VIA data. LU steers had  
348 greater total fat content (51.4 v. 40.4 kg;  $P < 0.01$ ) and lower total meat content

349 (258.8 v. 305.7 kg;  $P < 0.001$ ) determined by VIA than CHX steers. There were  
350 neither any treatment nor breed  $\times$  treatment interaction effects for any performance  
351 or carcass-related trait ( $P > 0.05$ ).

352

## 353 **Discussion**

### 354 *Methaemoglobin response to dietary nitrate*

355 Blood MetHb concentrations were monitored in Nitrate-fed steers for 128 d after  
356 introduction of nitrate to the diet: mean concentrations ranged from 2-7% of total Hb  
357 over this period with values in Concentrate-fed steers being consistently greater than  
358 in Mixed-fed steers. No individual MetHb measurement was greater than 15% of  
359 total Hb which was substantially less than 30% total Hb, the value associated with  
360 clinical symptoms of methaemoglobinemia (Bruning-Fann and Kaneene, 1993). This  
361 agrees with most studies in which animals were adapted slowly to dietary nitrate by  
362 increasing nitrate intakes over a period of weeks (cattle, Hulshof *et al.*, 2012, van  
363 Zijderveld *et al.*, 2011; sheep, Li *et al.*, 2012, van Zijderveld *et al.*, 2010). Slow  
364 adaptation to dietary nitrate allows the rate of reduction of nitrite to ammonia by the  
365 rumen microflora to increase and prevents accumulation of nitrite in the rumen and  
366 absorption from the rumen and thereby avoids conversion of haemoglobin to MetHb  
367 (Lee and Beauchemin, 2014). Only where nitrate was administered directly into the  
368 rumen (Takahashi *et al.*, 1998; Sar *et al.*, 2004) were greater MetHb concentrations  
369 than those found in the present study observed (34.3% and 18.37% in each of the  
370 studies, respectively), presumably because of transiently high concentrations of  
371 nitrite in the rumen generated by the method of administration. Recently, Newbold *et*  
372 *al.* (2014) removed steers from an experiment because MetHb concentrations in  
373 excess of 20% were observed during adaptation to nitrate. Although most steers



374 removed (8 of 9) were fed higher dietary nitrate concentrations (24 and 30 g  
375 nitrate/kg diet DM) than used in the present study, one steer removed was fed 18 g  
376 nitrate/kg diet DM. There was no evidence in the current experiment for adverse  
377 effects of dietary nitrate over the 128 d monitoring period.

378 Measurement of blood MetHb over a 128 d period in the present study has  
379 demonstrated (i) that after adaptation to nitrate, MetHb concentrations remained  
380 elevated and (ii) that individual steers were consistent in their response to nitrate  
381 across time, i.e., some steers always had elevated MetHb concentrations. Thus,  
382 although there was no association between MetHb and animal performance in the  
383 present study, in assessing the risk of methaemoglobinemia, this consistent  
384 difference in response to nitrate between individual animals, together with the  
385 observations of Newbold *et al.* (2014) should be noted. There was however no  
386 evidence for any association between meal size and MetHb concentrations in the  
387 present experiment and therefore the differences between individual animals in  
388 MetHb response to nitrate are more likely to be explained by differences between  
389 animals in rumen microflora, rates of absorption of nitrite from the rumen and the  
390 metabolism of absorbed nitrite.

391

#### 392 *Basal diet and treatment effects*

393 Feeding diets containing a high proportion of cereals has been shown to reduce  
394 enteric CH<sub>4</sub> production compared to forage-based diets (Johnson and Johnson,  
395 1995; Moss *et al.*, 1995; Mc Geough *et al.*, 2010; Rooke *et al.*, 2014). This strategy  
396 is attractive, in that accompanying improvements in animal performance and  
397 efficiency has been demonstrated (Lovett *et al.*, 2003; Mc Geough *et al.*, 2010). In  
398 the present study, steers offered a higher proportion of concentrate in the diet

399 expressed better feed efficiency (RFI) than those offered a mixed forage:concentrate  
400 diet. These steers were also shown to have lower levels of CH<sub>4</sub> production  
401 compared to steers offered higher quantities of forage (Troy *et al.*, 2015).

402 Consistent results in the literature demonstrate, over the short-term, that  
403 dietary nitrate can be successfully administered at levels capable of reducing CH<sub>4</sub>  
404 with no adverse effects on performance in sheep (van Zijderveld *et al.*, 2010; Li *et*  
405 *al.*, 2012; El-Zaiat *et al.*, 2014), goats (Nguyen *et al.*, 2010), dairy cows (van  
406 Zijderveld *et al.*, 2011) and beef cattle (Ngoc Huyen *et al.*, 2010). However, a  
407 comprehensive review by Bruning-Fann and Kaneene (1993) reported a reduction in  
408 feed intake when nitrate was included in the diet at 10 g nitrate/kg DM (cattle) and 30  
409 g nitrate/kg DM (sheep). In agreement, Hulshof *et al.* (2012) reported a tendency for  
410 calcium nitrate (fed at 22 g nitrate/kg DM) to reduce DMI by 6% in beef cattle fed a  
411 sugarcane-based diet. In contrast, Sangkhom *et al.* (2012) reported improved growth  
412 rates and feed conversion efficiency in growing cattle when potassium nitrate (fed at  
413 36.8 g nitrate/kg DM) was included in the diet. In agreement with most studies to  
414 date, feeding 18 g nitrate/kg diet DM had no adverse effects on DMI or ADG in the  
415 present study. Furthermore, the Nitrate treatment tended to improve FCR of steers  
416 offered the Concentrate basal diet but not the Mixed diet.

417 Van Zijderveld *et al.* (2011) reported the persistency of the effects of dietary  
418 nitrate in dairy cows fed a mixed forage and concentrate diet, in which  
419 measurements were obtained over four successive 24 d periods. No adverse effects  
420 on milk yield or energy balance were identified, but the reductions in energy losses  
421 as CH<sub>4</sub> were not associated with any improvements in productivity. In the present  
422 study steers received the experimental diets for a minimum of 120 d and maximum  
423 of 176 d. No differences in SBW, CCW, carcass grades or yields were observed

424 between treatments, and thus long-term feeding of nitrate did not adversely affect  
425 the level of production.

426 In the wider study from which this experiment is drawn, nitrate was shown to  
427 reduce CH<sub>4</sub> (i.e., reduced energy loss) from steers offered the Mixed diet (Troy *et al.*,  
428 2015); however no response was observed in the present study on FCR or RFI. The  
429 benefits in reduced energy loss as CH<sub>4</sub> observed by Troy *et al.* (2015) may have  
430 been counter-balanced by sub-clinical effects of nitrate. In contrast, nitrate improved  
431 the FCR of steers offered the Concentrate diet, but with no aligned reduction in CH<sub>4</sub>  
432 (Troy *et al.*, 2015).

433 Although increased concentrations of dietary lipid has been shown to reduce  
434 CH<sub>4</sub> from ruminants (Martin *et al.*, 2010; Grainger and Beauchemin, 2011; Patra,  
435 2013), at high concentrations in the diet lipid can negatively affect DMI and  
436 productivity (Brask *et al.*, 2013). Based on a meta-analysis, Patra (2013)  
437 demonstrated that dietary lipid concentrations in excess of 6% cause problems with  
438 productivity. Such diets with high lipid levels which negatively affect productivity are  
439 unsuitable for livestock producers due to their adverse consequences on the  
440 profitability of the enterprise. However, since the AHEE level in the RSC treatment  
441 was only 48 g/kg DM this dietary lipid concentration did not suppress DMI and no  
442 adverse effects were observed for any performance or carcass-related trait. The  
443 RSC treatment was shown to positively reduce CH<sub>4</sub> production by 7.5% from steers  
444 offered the Mixed basal diet but no reduction in CH<sub>4</sub> was observed on the  
445 Concentrate diet (Troy *et al.*, 2015).

446

447 *Breed effects*

448 Data on the performance and efficiency of native hill breeds in direct comparison to  
449 the more common breeds of finishing cattle are sparse in the literature. The  
450 performance of the LU breed on the two finishing basal diets and dietary treatments  
451 considered here have not been reported to date, and thus provides novel insight into  
452 the performance of this breed when managed within indoor finishing units. When  
453 given diets typical of indoor finishing systems in the UK, considerable differences in  
454 performance characteristics between LU and CHx steers were determined. CHx  
455 steers expressed greater rates of ADG, consumed lower DMI (/day and /kg BW),  
456 thus had better feed efficiency compared to the LU steers. This inefficiency will have  
457 considerable impact on profitability. Although both breeds reached similar SBW, CHx  
458 cattle yielded greater CCW and better EUROP classifications, both of which are  
459 incorporated into current payment schemes in the UK. These differences are not  
460 unexpected given the selection history of these breeds. Here LU cattle are being  
461 compared with a breed intensively selected for fast growth, however in comparison  
462 to the average 2014 Scottish figures for ADG of cereal-based finishing enterprises  
463 (1.34 kg/d) (QMS, 2014), the Concentrate-fed LU cattle performed well (1.32 kg/d).  
464 Given the animal performance results reported here, it is anticipated that the dietary  
465 mitigation strategies considered will not adversely affect health or performance of  
466 either breed type. Consequently, the same practical advice with regard to dietary  
467 mitigation strategies can be given to commercial beef finishers looking to reduce  
468 CH<sub>4</sub> regardless of the breed types being finished.

469

## 470 **Conclusions**

471 This study demonstrated that (i) the addition of nitrate to the diet or (ii) increasing the  
472 level of dietary lipid through the use of cold-pressed RSC, does not adversely affect

473 either the performance or feed efficiency of finishing beef steers when used within  
474 either a Mixed forage/concentrate diet or a high Concentrate diet. The use of nitrate  
475 in the diet of ruminants has been limited to date due to the potential toxicity of the  
476 intermediate product (nitrite) which, at high levels, can severely impact animal health  
477 and productivity. The present study demonstrated that, following an appropriate  
478 adaptation period (four weeks), feeding of nitrate at the level considered here (18 g  
479 nitrate/kg diet DM) together with the basal diet types studied did not provide  
480 measureable adverse effects, in terms of blood MetHb response (where the  
481 maximum level reached was 15% of total Hb), animal performance and carcass  
482 characteristics. This study demonstrated that the use of RSC to increase the level of  
483 dietary lipid from 25 to 48 g AHEE/kg diet DM did not suppress DMI or ADG.  
484 Furthermore, based on the same steers Troy *et al.* (2015) demonstrated the  
485 effectiveness of these dietary treatments within a Mixed diet for reducing CH<sub>4</sub> (CH<sub>4</sub>  
486 yield was reduced by 17% and 7.5% through the use of nitrate and RSC treatments,  
487 respectively). Therefore, it is concluded that these are appropriate strategies on  
488 Mixed diets. Although the use of these mitigation strategies within a high concentrate  
489 diet was shown in the present study to provide no adverse effects on performance,  
490 they were not effective at reducing CH<sub>4</sub> yield (Troy *et al.*, 2015) and therefore cannot  
491 be recommended for use within high concentrate diets.

492

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502

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

Basal Diet Treatment	Mixed			Concentrate		
	Control	Nitrate	RSC	Control	Nitrate	RSC
Ingredient composition, g/kg DM <sup>1</sup>						
Grass silage	189	193	192			
Whole crop barley silage	331	334	334			
Barley straw				84	84	83
Barley	328	374	287	740	797	700
Rapeseed meal	123	45	16	145	63	19
Rapeseed cake			142			167
Calcinit <sup>2</sup>		24			24	
Molasses	19	21	20	21	21	21
Minerals <sup>3</sup>	9	10	9	10	10	10
Chemical composition, g/kg DM <sup>4</sup>						
Dry matter (g/kg)	543	539	541	863	860	865
CP	143	148	145	133	138	136
ADF	252	240	253	145	130	143
NDF	376	361	367	237	220	223
Starch	234	257	211	430	458	408
AHEE	23.9	23.4	44.1	27.0	26.6	51.0
Ash	48	44	50	36	31	37
ME (MJ/kg DM)	11.6	11.4	12.1	12.0	11.9	12.7
GE (MJ /kg DM)	17.7	17.2	18.1	18.1	17.7	18.7

632 <sup>1</sup>Ingredient composition is the mean of the daily diets received by the animals across the  
633 experimental period.

634 <sup>2</sup>Contained (g/kg DM): nitrate, 769; Ca, 229.

635 <sup>3</sup>Contained (mg/kg): Fe, 6036; Mn, 2200; Zn, 2600; Iodine, 200; Co, 90; Cu, 2500; Se 30;  
636 (µg/kg): vitamin E, 2000; vitamin B12, 1000; vitamin A, 151515; vitamin D, 2500

637 <sup>4</sup>Chemical composition is the mean of 2 analyses per treatment, apart from DM which is the  
638 mean of 32 analyses.

639 RSC, Rapeseed Cake; AHEE, acid hydrolysed ether extract; ME, metabolisable energy; GE,  
640 gross energy

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642

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

	Grass Silage	WCBS	Straw	Barley	RSM	RSC	Molasses
DM (g/kg)	273	557	807	867	896	901	971
CP	150	103	16	104	367	318	67
NDF	486	575	826	163	326	209	0
ADF	345	390	551	86	243	197	0
Starch	5.7	122	0	571	52	41	0
AHEE	36	12.6	14	30	27	170	0
Ash	80	41	63	22	79	75	147
NCGD (% DM)			44	88	73	78	0
ME (MJ /kg DM)	11.4	10.75	6.5	13.05	10.9	15.15	12.7
GE (MJ /kg DM)	19.1	16.0	15.0	18.7	19.3	22.4	14.25
pH	4.1	5.3					

644 WCBS, whole crop barley silage; RSM, rapeseed meal; RSC, rapeseed cake; DM, dry  
 645 matter; AHEE, acid hydrolysed ether extract; NCGD, neutral cellulase and gammanase  
 646 digestibility; ME, metabolisable energy; GE, gross energy  
 647 ME values (Thomas, 2004), were either estimated from near infra red spectroscopy (silage  
 648 and WCBS), from NCDG and AHEE (barley, RSM and RSC) or from tabulated values for  
 649 feed composition (straw and molasses).

650

651 **Table 3** Changes in mean and maximum individual blood MetHb concentration (% total Hb) in relation to nitrate intake and long-term nitrate  
 652 feeding

Day <sup>1</sup>	-27	-20	-13	-6	8	87	101		Significance		
Nitrate (%) <sup>2</sup>	25	50	75	100	100	100	100	SEM	Day	Diet	Day*Diet
Mixed	0.26 <sup>a</sup>	0.78 <sup>ab</sup>	0.80 <sup>ab</sup>	3.50 <sup>c</sup>	2.16 <sup>bc</sup>	1.29 <sup>ab</sup>	3.60 <sup>c</sup>	0.61	***	*	***
Concentrate	0.32 <sup>a</sup>	0.62 <sup>a</sup>	0.98 <sup>a</sup>	2.80 <sup>b</sup>	4.53 <sup>bc</sup>	6.46 <sup>d</sup>	4.61 <sup>c</sup>				
Maximum	0.60	2.00	3.20	9.50	11.60	15.40	10.30				

653 Number of steers = 28

654 <sup>1</sup>Day relative to start of 56 day performance period.

655 <sup>2</sup>Nitrate as percentage of maximum level of intake (100% = 18 g/kg DM).

656 Within a row, means without a common superscript differ ( $P < 0.05$ ).

657 \* $P < 0.05$ ; \*\*\* $P < 0.001$ .

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670 **Table 4** Effect of breed (B), basal diet (D) and treatment (T) on growth, feed intake and feed efficiency of Charolais-sired (CHX) and purebred  
 671 *Lu*ing (LU) steers fed either a Mixed- or Concentrate-based diet containing one of three treatments: Control, Nitrate or Rapeseed cake (RSC)

Basal Diet	Mixed						Concentrate						SEM	Significance <sup>1</sup>		
	Control		RSC		Nitrate		Control		RSC		Nitrate			B	D	T
Breed	CHX	LU	CHX	LU	CHX	LU	CHX	LU	CHX	LU	CHX	LU				
AgeST (days)	445	478	434	474	437	474	449	481	444	482	441	465	7.9	***	ns	ns
Mid-BW (kg)	611	601	591	594	605	596	594	567	588	573	602	571	22.2	ns	ns	ns
Mid-MBW (kg)	123	121	120	120	122	121	120	116	119	117	121	117	3.4	ns	ns	ns
ADG (kg/day)	1.56	1.48	1.71	1.42	1.61	1.46	1.47	1.32	1.46	1.19	1.53	1.44	0.092	**	ns	ns
DMI (kg/day)	11.4	12.8	11.7	11.8	12.1	12.2	11.1	11.2	11.1	10.9	10.7	11.0	0.50	ns	***	ns
DMI/BW(g/kg)	18.7	21.2	19.8	19.9	19.9	20.5	18.8	19.8	18.8	19.0	17.8	19.1	0.49	**	ns	ns
DMI/MBW(g/kg)	93.0	105.0	97.7	98.0	98.7	101.3	92.5	96.7	92.7	92.8	88.0	93.5	2.52	**	ns	ns
FCR (kg, kg) <sup>2</sup>	7.45	8.69	6.86	8.39	7.61	8.49	7.59	8.85	7.70	9.33	7.16	7.67	0.421	****	ns	ns
RFI (kg) <sup>3</sup>	-0.27	0.76	-0.15	-0.06	0.44	0.62	-0.27	0.12	-0.22	-0.10	-0.71	-0.18	0.228	**	**	ns
FD1 (mm) <sup>4</sup>	6.31	8.83	6.87	9.12	5.89	7.53	6.85	8.34	6.65	8.49	5.87	7.25	0.650	***	ns	ns

672 Number of animals = 81; AgeST, Age at start of test; Mid-BW, mid-test BW; Mid-MBW, mid-test metabolic BW; ADG, average daily gain at the  
 673 end of the 56 d test; FCR, feed conversion ratio; RFI, residual feed intake; FD1, fat depth at the 12/13<sup>th</sup> rib at the end of the 56 d test

674 <sup>1</sup>Breed × Diet and Breed × Treatment interaction effects were not significant for all variables ( $P > 0.05$ )

675 <sup>2</sup>Diet × Treatment interaction ( $P < 0.05$ ): Concentrate-Nitrate different to Concentrate-RSC ( $P < 0.05$ ); Concentrate-Control different to  
 676 Concentrate-Nitrate ( $P = 0.07$ )

677 <sup>3</sup>Diet × Treatment interaction ( $P < 0.05$ ): Mixed-Nitrate different to Mixed-RSC ( $P < 0.01$ )

678 <sup>4</sup>Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable

679 \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

680 **Table 5** Effect of breed (B), basal diet (D) and treatment (T) on carcass traits of Charolais-sired (CHX) and purebred Luing (LU) steers fed  
 681 either a Mixed- or Concentrate-based diet containing one of three treatments: Control, Nitrate or Rapeseed cake (RSC)

Basal Diet	Mixed						Concentrate						SEM	Significance <sup>1</sup>		
	Control		RSC		Nitrate		Control		RSC		Nitrate					
Breed	CHX	LU	CHX	LU	CHX	LU	CHX	LU	CHX	LU	CHX	LU		B	D	T
FD2 (mm) <sup>2</sup>	6.31	10.17	7.84	12.90	6.47	9.28	6.60	10.83	8.03	11.50	6.67	10.06	0.907	***	ns	*
CCW (kg) <sup>3</sup>	430	370	406	385	408	382	417	365	411	361	418	346	9.4	***	ns	ns
KO (%)	58.4	52.3	56.7	53.7	57.0	53.1	58.4	52.8	57.3	53.0	57.4	51.5	0.88	***	ns	ns
SBW (kg)	738	710	717	719	717	720	713	694	718	682	729	672	19.5	ns	ns	ns
CONF	10.3	8.0	9.7	8.3	9.7	8.3	10.0	8.0	9.4	8.0	10.3	7.7	0.34	***	ns	ns
FAT	10.0	10.6	10.0	12.0	8.7	10.6	9.4	10.7	9.4	11.0	9.4	11.3	0.44	***	ns	ns
CONF (VIA)	10.7	8.0	9.8	8.0	9.6	7.6	10.3	7.4	9.9	6.9	9.8	6.7	0.53	***	ns	ns
FAT (VIA)	7.9	10.7	8.3	10.2	7.5	10.0	7.6	8.7	7.6	9.3	6.6	8.7	0.47	***	***	ns
TOTFat (kg)	46.4	51.3	42.1	70.7	38.1	50.1	41.8	44.5	34.7	45.9	37.8	42.8	5.95	**	ns	ns
TOTMeat (kg)	314.0	256.6	294.2	261.9	299.0	270.0	308.9	260.7	306.3	259.2	312.0	244.5	8.09	***	ns	ns

682 Number of animals = 81; FD2, pre-slaughter fat depth at the 12/13<sup>th</sup> rib; CCW, cold carcass weight; KO, killing out %; SBW, slaughter BW;  
 683 CONF, EUROP conformation (15 pt scale) assigned by visual assessor; FAT, EUROP fatness (15pt scale) assigned by visual assessor; CONF  
 684 (VIA), conformation grade (15pt scale) assigned by VIA; FAT (VIA), fatness grade (15pt scale) assigned by VIA; TOTFat; total fat content  
 685 predicted by VIA; TOTMeat, total meat content predicted by VIA.

686 <sup>1</sup>Breed × Treatment and Basal Diet × Treatment interaction effects were not significant for all variables ( $P > 0.05$ ).

687 <sup>2</sup>Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable

688 <sup>3</sup>Breed × Diet interaction ( $P < 0.05$ ): CHX-Concentrate different from LU-Concentrate and LU-Mixed ( $P < 0.001$ ); CHX-Mixed different from LU-  
 689 Concentrate and LU-Mixed ( $P < 0.001$ ); LU-Mixed different from LU-Concentrate ( $P < 0.01$ ). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

690 **List of Figure Captions**

691 **Figure 1** Changes in Met-haemoglobin (MetHb) concentrations (% total blood Hb) when fed  
692 100% dietary nitrate (18 g nitrate/kg DM) for 5 steers with overall smallest and overall  
693 greatest mean MetHb concentrations. Solid lines and dashed lines represent the Mixed and  
694 Concentrate basal diets, respectively. Samples 1 to 4 refer to sampling days -6, 8, 87 and  
695 101, respectively. Each line represents an individual animal. Sample 4 was not present for 3  
696 animals as had been already been sent for slaughter before day 101.

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