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1 **Nutritional strategies to reduce methane emissions from cattle: effects on meat eating**
2 **quality and retail shelf life of loin steaks**

3 Ian Richardson^a, Carol-Anne Duthie^b, Jimmy Hyslop^c, John Rooke^{b,*}, Rainer Roehe^b

4

5 *^aDivision of Farm Animal Science, Department of Clinical Veterinary Medicine, University of*
6 *Bristol, Langford, BS40 5DU, UK*

7 *^bBeef and Sheep Research Centre, SRUC, West Mains Road, Edinburgh EH9 3JG, UK*

8 *^cBeef and Sheep Select, SAC Consulting Ltd., West Mains Road, Edinburgh, EH9 3JG, UK*

9

10 ** Corresponding author. E-mail address: john.rooke@sruc.ac.uk*

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12

13 ABSTRACT

14 Increasing the lipid concentration and/or inclusion of nitrate in the diet of ruminant livestock
15 have been proposed as effective strategies to reduce the contribution of methane from the
16 agricultural sector to greenhouse gas emissions. In this study, the effects of increased lipid
17 or added nitrate on beef eating quality were investigated in two experiments. In experiment
18 1, lipid and nitrate were fed alone with two different and contrasting basal diets to finishing
19 beef cattle. In the second experiment, lipid and nitrate were fed alone or in combination with
20 a single basal diet. The sensory properties and retail colour shelf life of loin muscle samples
21 obtained were then characterised. Overall, neither lipid nor nitrate had any adverse effects
22 on sensory properties or colour shelf life of loin muscle.

23 *Keywords*

24 Beef; methane emissions; nitrate; lipid; eating quality; shelf life

25 **1. Introduction**

26 Methane (CH₄) produced by fermentation of feed, predominantly in the rumen of
27 ruminant livestock, contributes significantly to greenhouse gas emissions. In the United
28 Kingdom in 2014 (Department of Energy and Climate Change, 2016), enteric CH₄ emissions
29 were estimated to account for 23.8 Mt carbon dioxide equivalents or 48% of total
30 greenhouse gas emissions from the agriculture sector. A reduction in CH₄ emissions from
31 livestock is therefore part of international governmental strategies for reducing greenhouse
32 gas emissions (Australian Government, 2017; Scottish Government, 2018).

33 Manipulation of the diet to reduce CH₄ emissions is an important strategy available to
34 livestock farming (Hristov et al., 2013). Many such strategies have been tested but
35 convincing evidence for long-term efficacy *in vivo* for many is lacking. Increasing dietary lipid
36 and the inclusion of nitrate in the diet have been shown to be effective mitigation strategies
37 (Hristov et al., 2013) and their use has been recently reviewed (Martin, Morgavi, & Doreau,
38 2010; Patra, 2014; Lee & Beauchemin, 2014; Yang, Rooke, Cabeza, & Wallace, 2016). The
39 extent to which either lipid or nitrate can be included in the diet is limited by potential adverse
40 effects such as a reduction in fibre digestion and consequently feed intake from increased
41 lipid in the diet and nitrate / nitrite toxicity from adding nitrate. However, little attention has
42 been paid to the effects the safe application of lipids and nitrate as CH₄ mitigation strategies
43 have on product quality. For lipids, the focus has been on the effects feeding lipids protected
44 from rumen biohydrogenation have on both the fatty acid composition of meat lipids and
45 meat eating quality (Scollan et al., 2014). For nitrate, the main concern to date has been the

46 potential transfer of nitrate or its metabolites (nitrite, nitrosamines) to meat with potential
47 adverse consequences for consumer health.

48 As there have been no reports of the organoleptic quality of meat, particularly from
49 nitrate-fed cattle, the present study reports the eating quality, as measured by a trained taste
50 panel and the simulated retail display shelf life of beef obtained from two studies (Troy et al.,
51 2015; Duthie et al., 2016, 2018) in which the lipid content was increased or nitrate included
52 in the diets of finishing beef cattle to reduce CH₄ emissions.

53

54 **2. Materials and methods**

55 Both experiments were conducted at Scotland's Rural College (SRUC) Beef and
56 Sheep Research Centre, UK. The experiments (ED AE 15/2013 and ED AE 08/2014) were
57 approved by the Animal Experiment Committee of SRUC and conducted in accordance with
58 the requirements of the UK Animals (Scientific Procedures) Act 1986. For full details of
59 experimental procedures see Troy et al. (2015) for CH₄ measurements and Duthie et al.
60 (2016) for growth performance and carcass characteristics for Experiment 1. For Experiment
61 2, see Duthie et al. (2018) for both CH₄ measurements and growth performance.

62 *2.1. Experiment 1. Experimental design, animals and diets*

63 The experiment was of a two × two × three factorial design; comprising two breeds of
64 steers (crossbred Charolais or purebred Luing; 6 sires per breed), two basal diets which
65 included; the Mixed basal diet, 480 g concentrate / kg dry matter (DM), and the Concentrate
66 diet, 920 g concentrate / kg DM, and three treatments selected for their potential as CH₄
67 mitigation strategies (Control, Nitrate or increased lipid in the form of rapeseed cake (RSC)).
68 The Control treatment contained rapeseed meal as the main protein source which was
69 replaced with either Nitrate in the form of calcium nitrate (Calcinit, Yara, Oslo, Norway; 18 g
70 nitrate/kg diet DM) or RSC (a by-product from the production of rapeseed oil by cold-
71 pressing). The ingredient and chemical compositions of the diets are given in Table 1.

72 In total, 84 steers (13 to 16 months of age at the start of performance trial; 42 of each
73 breed type) were used. Thus, 14 animals were allocated to each of the 6 concentrate
74 inclusion × treatment combinations (7 of each breed). The animals on each of the basal diet
75 × treatment combinations were group-housed in one pen per combination (a total of 6 pens).
76 All steers were offered feed individually *ad libitum* using electronic feeders (HOKO, Insentec,
77 Marknesse, The Netherlands). Treatments were balanced for sire, age and live weight (LW)
78 at the start of the experiment. Prior to the start of the experiment the steers were adapted to

79 the experimental diets in two stages. In stage one, the steers were adapted to basal diets
80 over a 4 week period. In stage two (also 4 weeks), steers were adapted to the mitigation
81 treatments by progressively increasing the amounts of nitrate or RSC.

82 *2.2. Experiment 2. Experimental design, animals and diets*

83 Except where otherwise stated, the experimental procedures were the same as
84 Experiment 1. The experiment was a two (breed) × four (treatment) factorial design. The
85 basal diet contained 450 g of concentrate /kg DM. The four treatments were assigned
86 according to a 2 x 2 factorial arrangement where the Control treatment contained rapeseed
87 meal as the main protein source which was replaced either with Nitrate (21.5 g nitrate/kg
88 DM) or maize distiller's dark grains (MDDG), to increase lipid concentration (Lipid), or with
89 both nitrate and MDDG (Combined). The ingredient and nutritional compositions of each
90 treatment are given in Table 2. The 80 cross-bred steers (5 sires per breed; 13 to 15 months
91 of age at start of performance trial) used were from a rotational cross between pure-bred
92 Aberdeen Angus or Limousin sires and cross-bred dams of those breeds. Thus, 20 steers
93 (10 of each breed type) were allocated to each dietary treatment. Treatments were balanced
94 for sire, age and LW at the start of the experiment.

95 *2.3. Performance test and slaughter*

96 Growth, performance and feed conversion were characterized for all steers over a
97 56-day period. Dry matter intake (DMI, kg/d) was recorded daily for each animal and LW
98 weekly. At the end of the performance test, steers remained on the same diets until
99 slaughter and DMI and LW measurements continued throughout. Before slaughter, CH₄
100 production was measured (6 steers per week) over 13 weeks (Troy et al., 2015; Duthie et al.,
101 2018) for both experiments). In each week of CH₄ measurement, steers selected were
102 balanced for concentrate inclusion and treatment and so that when subsequently sent for
103 slaughter, variation in LW and visual assessment of fatness between slaughter groups was
104 minimized and steers achieved commercially acceptable conformation and fat
105 classifications. Age at slaughter therefore varied; in Experiment 1, steers were slaughtered
106 in 4 batches on days 85, 106, 127 and 148 after the start of the performance trial. Similarly,
107 in Experiment 2 slaughter took place 99, 120, 141 and 162 days after the start of the
108 performance trial. The steers were transported (approximately 1 h) to a commercial abattoir
109 and slaughtered within 2 h of arrival. Cattle were stunned using a captive bolt,
110 exsanguinated and subject to low voltage electrical stimulation. Following hide removal,
111 carcasses were split in half down the mid-line and dressed to UK specifications (see Meat
112 and Livestock Commercial Services Limited beef authentication manual, www.mlcsl.co.uk,

113 for full description). EUROP conformation and fat classifications (Fisher, 2007), based on the
114 UK scale, were allocated to all carcasses through visual assessment using a trained Meat
115 and Livestock Commercial assessor.

116 At 48 h *post-mortem*, samples from the loin eye muscle, *M. longissimus thoracis* (LT)
117 were obtained from all carcasses, vacuum-packed and delivered, using chilled transport, to
118 the University of Bristol for assessment of sensory characteristics, colour stability under retail
119 display conditions and vitamin E content (MacKintosh et al., 2017). All samples were chilled
120 and conditioned at 0 ± 1 °C for 10 days. Then two, 20 mm thick steaks were individually
121 packaged in modified atmosphere packaging (MAP, 80% oxygen: 20% carbon dioxide) and
122 displayed in a chiller under simulated retail display conditions (3 °C, 16 h light: 8 h dark, 700
123 lx). Finally, a 75 mm section was vacuum packed, conditioned for a further 2 days (to a total
124 of 14 days from slaughter) and then frozen for subsequent analysis by a trained sensory
125 taste panel.

126 2.4. Meat colour and chemical analysis

127 The colour of duplicate steaks packed in MAP was measured daily at 3 positions on
128 the meat surface, through the film lid of the pack using a Minolta CR400 (Minolta camera
129 Company, Milton Keynes, UK) with an open cone for measuring through the package
130 surface. Illuminant D65 0/45 standard observer 10 °C as per recommendations of the expert
131 working group (Cassens et al., 1995). A white tile covered by the film lid of MAP was used to
132 standardise the chromameter. Colour shelf life was measured daily until a chroma of ≤ 18
133 was obtained, which is a critical threshold at which consumers can detect discolouration
134 (Hood & Riordan, 1973; MacDougall, 1982). Colour saturation (chroma) was calculated as

$$135 \quad \text{Chroma} = [(a^*)^2 + (b^*)^2]^{0.5}$$

136 The vitamin E content of meat was measured according to the methodology
137 described by Arnold et al. (1993). Rac-5,7-dimethyl-tocol solution was used as the internal
138 standard, and 4% (v/v) dioxane in hexane was used as the mobile phase for HPLC.

139 2.5. Sensory assessment

140 The sensory analysis was performed for each animal by a 10-person trained
141 professional taste panel, using the same people for the duration of each experiment (British
142 Standards Institution, 1993). The loin was thawed overnight at 4 °C and cut into 20 mm thick
143 steaks. Steaks were grilled to an internal temperature of 74 °C, measured using a
144 thermocouple probe (Testo Limited, Alton, UK). Following cooking, all fat and connective

145 tissue was removed and the steak cut into 2 cm³ cubes. The samples were placed into pre-
146 labelled foils and placed in a heated incubator at 65 °C. Assessors tasted the samples in an
147 order based on the designs outlined by MacFie, Bratchell, Greenhoff, & Vallis (1989) for
148 balancing carryover effects between samples. All sensory assessments were completed
149 under red light in a purpose-built sensory suite where each tasting booth was equipped with
150 computer terminals linked to a fileserver running a sensory software programme (Fizz v
151 2.20h, Biosystemes, Couternon, France). Each panellist assessed one sample from each
152 diet per session (six samples for experiment 1 and four samples for experiment 2), with four
153 sessions in a morning and animals from each of the slaughter dates represented in a
154 morning. Steaks were scored against 0–100 mm unstructured intensity line scales for a
155 consensually agreed texture profile, where 0 = nil and 100 = extreme, and 8-point category
156 scales for tenderness (1 = extremely tough to 8 = extremely tender), juiciness (1 = extremely
157 dry to 8 = extremely juicy), beefy flavour and abnormal beef flavour intensities (1 = extremely
158 weak to 8 = extremely strong). The hedonic scale served as an indication of preference by
159 the panel, but it cannot be used to infer consumer acceptance since the results are based on
160 10 assessors who can no longer be considered as typical consumers because of the training
161 they have received in meat assessment.

162

163 *2.6. Calculations and statistical analysis*

164 All statistical analysis was performed using GenStat software, 16th Edition. Analyses
165 of performance and carcass data were conducted using linear mixed models of the REML
166 procedure with fixed effects of breed (both experiments), concentrate inclusion (experiment
167 1 only), and treatment (both experiments). Interaction effects of breed, concentrate inclusion
168 and treatment were included in the models where applicable and significant ($P < 0.05$). For
169 data recorded after slaughter, age at slaughter and the length of time experimental
170 treatments were fed were tested as covariates and included where significant. Changes in
171 chroma during simulated retail display data were analysed using the repeated measures
172 procedure of REML and fixed effects were as above with the addition of measurement day.
173 For sensory characteristics, assessor and sensory sessions were additionally included as
174 fixed effects without interactions with the other fixed effects. The standard error of the
175 difference (sed) from the analyses is shown, and a P value of < 0.05 was taken as significant
176 for all statistical analysis.

177

178 **3. Results**

179 *3.1. Experiment 1. Performance and carcass data*

180 Steers offered the Mixed basal diet had greater DMI ($P<0.001$) and LW gains
181 ($P=0.002$) than those offered the Concentrate basal diet (Table 3) but feed to gain ratio did
182 not differ between basal diets ($P=0.56$). There were no differences in performance between
183 the CH₄ mitigation treatments. Steers did not differ between treatments in age at slaughter,
184 but Mixed basal diet steers had greater slaughter ($P=0.028$) and carcass weights ($P=0.001$)
185 than those fed the Concentrate basal diet. Methane mitigation treatments did not influence
186 slaughter or carcass weights. Nutritional treatments imposed had no effect (Table 3) on
187 carcass conformation or fatness ($P>0.05$). Charolais steers grew faster and had superior
188 feed conversion ratios ($P<0.001$) than Luing steers. Carcass weights ($P<0.001$) were greater
189 and conformation ($P<0.001$) and fat scores ($P=0.019$) superior for Charolais steers. There
190 were no interactions between breed and nutritional treatments ($P>0.05$).

191 *3.2. Experiment 1. Eating quality and simulated retail display*

192 Loin steaks from steers offered the Mixed basal (Table 4) diet were tougher
193 ($P=0.009$) but had lower abnormal flavour intensity scores ($P=0.022$) than steaks from steers
194 fed the Concentrate basal diet. Methane mitigation treatments had no effect on eating quality
195 ($P>0.05$). Steaks from Luing steers were overall liked better than those from Charolais
196 steers ($P<0.001$) as a result of better scores for juiciness, tenderness (both $P<0.001$) and
197 beef flavour ($P=0.002$). There were no interactions between breed and nutritional treatments
198 ($P>0.05$).

199 Colour chroma declined ($P<0.001$; Fig. 1) as display progressed reaching a value of
200 18 after 16 – 18 days display. Chroma of Concentrate basal diet steaks were lower than
201 those of Mixed basal diet steaks ($P<0.001$) and as a result these animals reached a value of
202 18 earlier than Mixed basal diet samples (Table 4). The rate of chroma decline did not differ
203 between basal diets (time x basal diet, $P>0.05$). Again, CH₄ mitigation treatment did not
204 affect meat chroma. There were no significant differences between breed in meat chroma
205 ($P>0.05$) or interactions between breed and nutritional treatments ($P>0.05$).

206 Vitamin E concentrations in loin steaks were greater for Mixed basal diet samples
207 ($P<0.001$; Table 4) and within concentrate inclusion, greater for Lipid than Control or Nitrate
208 treatments ($P<0.001$). Steaks from Luing steers had greater vitamin E concentrations than
209 steaks from Charolais steers ($P<0.001$). As vitamin E is more concentrated in fat than lean
210 tissues, this would result from the Luing having fatter carcasses.

211 3.3. *Experiment 2. Performance and carcass data*

212 Increasing dietary lipid had no effects on either performance or carcass
213 characteristics (Table 5, $P>0.05$); there were also no interactions between increased lipid or
214 inclusion of nitrate. However, steers consuming nitrate grew more slowly ($P=0.008$) and had
215 poorer feed to gain ratios ($P=0.013$) than steers not fed nitrate. Feeding nitrate (Table 5) had
216 no effect on age at slaughter, or slaughter or carcass weights, but nitrate-fed steers had
217 poorer conformation scores ($P=0.016$) than steers not fed nitrate. Aberdeen Angus
218 crossbred steers had greater DMI and LW gain than Limousin crossbred steers ($P<0.001$)
219 and thus were heavier at slaughter ($P=0.011$). However, there were no differences in feed
220 conversion ratio, carcass weights, conformation or fat scores between breeds ($P>0.05$).
221 There were no interactions between breed and nutritional treatments ($P>0.05$).

222 3.4. *Experiment 2. Eating quality and simulated retail display*

223 Increased dietary lipid or feeding nitrate (Table 6) had no effect on eating quality or
224 vitamin E content of loin steaks. Steaks from Aberdeen Angus crossbred steers had greater
225 overall liking scores ($P=0.011$) than those from Limousin crossbred steers which was
226 associated with higher scores for juiciness and tenderness (both $P<0.001$). Vitamin E
227 concentrations were greater for steaks from Aberdeen Angus crossbred steers ($P=0.017$).
228 There were no interactions between breed and nutritional treatments ($P>0.05$).

229 Colour chroma decreased with time ($P<0.001$) of display (Fig. 2) reaching a chroma
230 of 18 between 15 and 17 days of display. Increased lipid concentration had no effect on
231 chroma. However, inclusion of nitrate extended shelf life by approximately 1 day (Table 6;
232 $P=0.005$) because the rate of decline of chroma (time x nitrate interaction, $P<0.001$) was
233 greater for steaks from steers that were not fed nitrate. Breed had no effect on chroma
234 change in meat ($P>0.05$).

235

236 4. *Discussion*

237 The primary aim of these experiments was to quantify the efficacy of added nitrate
238 or increasing dietary lipid as strategies to reduce enteric CH₄ emissions within different
239 nutritional and genetic backgrounds. The different genetic backgrounds were included to
240 determine whether breed had any influence on CH₄ emissions (which it did not). In
241 Experiment 1, a comparison was made between breeds with very different characteristics
242 Charolais, known for fast growth and excellent carcass composition and the Luings, a more

243 extensively managed, hardy hill and upland breed. In Experiment 2, cross-bred Angus x
244 Limousin cattle, extensively used commercially in the UK and intermediate between
245 Charolais and Luing, were used. However, an important secondary aim, which is the subject
246 of this paper, was to determine whether these mitigation strategies had any adverse effects
247 on meat/product quality; a strategy that adversely impacted the quality of the final product
248 could not be recommended. Whilst adding nitrate to the Concentrate basal diet (Experiment
249 1, Troy et al., 2015) did not reduce CH₄ emissions (Control v Nitrate, 14.7 v 15.4 g CH₄ / kg
250 DMI), CH₄ was reduced from 25.1 to 20.6 g/kg DMI when the Mixed basal diet was fed.
251 Similarly, increasing dietary lipid had no effect on CH₄ emissions when the Concentrate
252 basal diet was fed (Control v Lipid, 14.7 v 15.7 g/kg DMI) but reduced CH₄ (25.1 v 23.1 g/kg
253 DMI) when the Mixed basal diet was fed albeit to a lesser extent than Nitrate. In experiment
254 2 (Duthie et al. 2018) where only the Mixed basal diet was fed, both nitrate and increased
255 lipid reduced CH₄ emissions and their effects were additive (Control, 24.0, Nitrate, 22.1,
256 Lipid, 23.4, Combined 20.9 g /kg DMI). The efficacy of nitrate in reducing CH₄ was less in
257 Experiment 2 than Experiment 1 (45 v 80% of theoretical maximum reduction). To provide
258 context to results concerning meat quality, the performance and carcass characteristics of
259 each experiment (Experiment 1, Duthie et al., 2015; Experiment 2 (performance only),
260 Duthie et al., 2018) were reproduced in Tables 3 and 5.

261 *4.1 Concentrate inclusion (Experiment 1)*

262 Mixed basal diet-fed steers produced loin steaks which tended to be preferred by
263 the taste panel compared to steaks from cattle fed the Concentrate basal diet. This was
264 associated with a lower occurrence of abnormal flavours but tougher meat. Although many
265 studies have reported effects on meat quality of varying the proportion of concentrate in the
266 diet, responses have been variable. This is probably due to factors which include a wide
267 range in proportions of concentrate compared, the composition of the diet and differences in
268 perception of taste in the panels in different countries (Realini, Duckett, Brito, Dalla Rizza, &
269 De Mattos, 2004). Focussing on studies which used broadly similar concentrate inclusions to
270 the current study, French et al. (2001) found no differences in meat quality or colour when
271 concentrate proportion was varied. However, Aviles, Martinez, Domenech, & Pena (2015)
272 found, similar to the current experiment, that meat derived from cattle offered 600 g
273 concentrates / kg total DM was tougher (mechanical testing) than meat from cattle fed a high
274 concentrate diet. Aviles, Martinez, Domenech, & Pena (2015) also reported differences in
275 colour parameters between treatments: meat from cattle fed a high concentrate diet had
276 greater L* and a* and lower b* values than meat from cattle offered 600 g/kg concentrates.

277 The concentrations of the fat soluble vitamin E in loin steaks were measured
278 because of the positive association between vitamin E concentration and shelf life as
279 measured by changes in colour chroma (Wood et al., 2008, Scollan et al., 2014) and
280 therefore to aid interpretation. Meat from Mixed basal diet steers contained higher
281 concentrations of vitamin E (2.8 v 1.7 µg/kg Mixed v Concentrate) and had approximately
282 one day longer shelf life in simulated retail display than Concentrate basal diet samples. This
283 longer shelf life may be associated with the higher vitamin E concentrations in the Mixed
284 samples which may well be derived from the grass silage in the Mixed basal diet
285 (Mackintosh et al., 2017). It is also noteworthy that meat vitamin E concentration from both
286 diets was less than the value of 3.0 mg/kg reported as optimum for colour stability by Liu,
287 Scheller, Arp, Schaefer, & Williams, (1996). However, as the rate of decline in chroma did
288 not differ between basal diets, differences in stability between treatments may relate more to
289 differences in chroma at the start of simulated display which may be unrelated to vitamin E
290 concentration.

291

292 4.2. Nitrate

293 The present study extends the findings on the efficacy of nitrate in reducing CH₄
294 production to aspects of meat quality. In studies using similar dietary concentrations (around
295 20 g nitrate / kg diet DM) to the present study, nitrate has had few negative impacts on
296 animal performance (see reviews by Lee & Beauchemin, 2014; Yang, Rooke, Cabeza, &
297 Wallace, 2016). The poorer feed conversion ratio in Experiment 2 when nitrate was fed is an
298 exception. In terms of negative impacts, the potential toxicity of nitrate to the animal mainly
299 through formation of Met-haemoglobin from nitrite absorbed from the rumen as a product of
300 nitrate reduction has been most studied. As found in the current studies (see Duthie et al.,
301 2016, 2018) after careful adaptation of animals to nitrate, no potentially toxic Met-
302 haemoglobin concentrations were found. More recently, Hegarty et al. (2016) and Lee,
303 Araujo, Koenig, & Beauchemin, (2017) found no adverse effects of adding nitrate to diets on
304 carcass characteristics. Similarly, in the present experiments, carcass characteristics, with
305 the exception of a poorer carcass conformation in experiment 2, were not affected by nitrate.
306 Sensory meat quality was not influenced by addition of nitrate to diets irrespective of basal
307 diet or whether nitrate was fed alone or with increased lipid in the diet. Thus, this experiment
308 extends the evidence that dietary nitrate when used appropriately has no adverse effects on
309 product quality.

310 Addition of nitrate to the diet had no effect on simulated retail display in Experiment 1
311 but improved shelf life by around 1 day in Experiment 2. This improvement in Experiment 2
312 appeared unrelated to vitamin E concentrations which did not differ in the presence or
313 absence of nitrate. It is possible that elevated nitrate or nitrite in meat in Experiment 2 might
314 have provided the extra stability. When the data for Medium concentrate diets in
315 Experiments 1 and 2 were compared, the major difference was that in experiment 2, nitrate
316 was less effective in reducing CH₄ emissions. As noted above, in Experiment 1, the
317 reduction in CH₄ emissions was 80% of the theoretical maximum if all nitrate fed was
318 reduced to ammonia in the rumen but only 42% in Experiment 2. This implies that 20
319 (Experiment 1) and 58% (Experiment 2) of the nitrate fed may have been absorbed and
320 excreted either as nitrate *per se* or after metabolism. Potential metabolites of nitrate are N
321 containing gases, nitrite or nitrosamines. Of these, nitrate, nitrite and nitrosamines would be
322 of concern if elevated in meat. Guyader et al. (2016) did not detect nitrate in milk from
323 nitrate-fed cows, nor did Hegarty et al. (2016) find elevated nitrate in meat from nitrate-fed
324 cattle and nitrosamines were below the level of detection. Lee, Araujo, Koenig, &
325 Beauchemin (2017) did report an increase in nitrate (from 0.1 in control to 0.6 mg/kg in
326 muscle from nitrate-fed steers) but pointed out that these concentrations were below the
327 level of concern for human diets. In the current study, concentrations of nitrate in meat from
328 Experiment 1 were below the limit of detection of the assay employed (data not reported).
329 Although the above evidence suggests that increased nitrate / nitrite concentrations in meat
330 are unlikely, because 58% of the nitrate fed in experiment 2 could not be accounted for by
331 ammonia formation in the rumen, this possibility can not be ruled out.

332 4.3 Lipids

333 The concentration of lipid in the diet was increased from 25 in the Control diets to 48
334 and 37 g / kg DM respectively in Experiments 1 and 2 respectively. These concentrations
335 were less than the 60 g / kg DM, above which disturbances to rumen function are likely
336 (Brask et al., 2013). The increases in lipid concentration were limited to avoid excessive
337 increases in diet crude protein concentration and consequent increases in nitrogen excretion
338 with potentially adverse environmental consequences. The lipid sources used were by-
339 products of cold pressed rapeseed oil production in Experiment 1 and the distilling industry
340 in Experiment 2 to avoid utilising lipid destined for the human food industry. Both rapeseed
341 (approximately 60% monounsaturated and 30% polyunsaturated) and maize (27%
342 monounsaturated and 48% polyunsaturated) contain substantial amounts of unsaturated
343 fatty acids. However, this lipid was not protected from biohydrogenation in the rumen
344 because diversion of hydrogen from CH₄ formation to biohydrogenation is one of the

345 mechanisms by which lipids reduce CH₄ formation (Martin, Morgavi, & Doreau, 2010). Thus,
346 in contrast to situations where lipid sources protected from rumen metabolism and
347 containing high concentrations of polyunsaturated fatty acids are fed (see review by Scollan
348 et al., 2014), the combination of small increases in dietary lipid and biohydrogenation in the
349 present experiment, suggests that amounts of unsaturated fatty acid absorbed from the
350 small intestine and incorporated into meat would be limited. As increases in unsaturated fatty
351 acid concentrations in meat are a main factor influencing sensory traits (Vatansever et al.,
352 2000), the absence of any effect of lipid on the sensory qualities of meat in the current
353 experiments is not surprising. Similarly, there was no effect of lipid on simulated display shelf
354 life. The only notable effect of lipid on meat characteristics was an increase in vitamin E
355 concentrations in experiment 1. This may be related to increased fat concentrations in the
356 meat; the absence of an increase in vitamin E in meat in experiment 2 may be related to the
357 lesser increase in dietary lipid in that experiment.

358 **5. Conclusions**

359 Although basal diet (Experiment 1) and breed (both experiments) had significant
360 effects on eating quality, in neither experiment did increased lipid or nitrate added to the diet
361 of beef cattle have a negative effect on eating quality. Similarly, in neither experiment did
362 CH₄ mitigation treatments reduce the colour shelf life of loin samples although in experiment
363 2 nitrate did significantly increase colour shelf life. Vitamin E concentrations in loin muscle
364 were increased significantly by lipid in experiment 1 but there was no difference in
365 experiment 2; nitrate had no effect on vitamin E concentrations. Overall the nutritional
366 treatments explored here, which reduced CH₄ emissions, had no adverse effects on meat
367 quality, although it must be noted that only one cut of meat was assessed and conclusions
368 may not necessarily apply to other cuts.

369 **Conflict of interest statement**

370 The authors declare no conflict of interest.

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377 Research Platform.

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Table 1

Experiment 1. Ingredient and chemical composition (both g/kg DM unless otherwise stated) of different basal (Mixed, 480 g concentrate /kg DM) and Concentrate (916 g concentrate /kg DM) diets Adapted from Duthie et al. (2016).

Basal diet Treatment	Mixed (480)			Concentrate (916)		
	Control	Nitrate	Lipid	Control	Nitrate	Lipid
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 ^a		24			24	
Molasses	19	21	20	21	21	21
Mineral/vitamin premix ^b	9	10	9	10	10	10
Chemical composition						
Dry matter (g/kg fresh weight)	543	539	541	863	860	865
Crude protein	143	148	145	133	138	136
Acid detergent fibre	252	240	253	145	130	143
Neutral detergent fibre	376	361	367	237	220	223
Starch	234	257	211	430	458	408
Ether extract	24	23	44	27	27	51
Ash	48	44	50	36	31	37
Metabolisable Energy (MJ/ kg DM)	11.6	11.4	12.1	12.0	11.9	12.7

^aContained (g/kg DM): nitrate, 769; Ca, 229.

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

Table 2

Experiment 2. Ingredient and chemical composition (both g/kg DM unless otherwise stated) of diets in which rapeseed meal was replaced with nitrate, lipid concentration (using maize distillers dark grains) increased, or both nitrate included and lipid increased (Combined). Adapted from Duthie et al. (2018)

Treatment	Control	Nitrate	Lipid	Combined
Barley	336	388	289	263
Grass silage	210	211	209	210
Whole crop barley silage	347	347	346	346
Rapeseed meal	79	0	0	0
Calcinit ^a	0	25	0	24
Maize distiller's dark grains	0	0	128	127
Molasses	19	20	19	19
Minerals ^b	9	9	9	9
Chemical Composition				
DM (g/kg fresh weight)	533	531	533	533
Crude protein	135	141	136	162
Acid detergent fibre	184	166	184	183
Neutral detergent fibre	308	295	317	313
Starch	281	308	264	250
Ether extract	25	23	37	36
Ash	52	48	51	51
Metabolisable Energy (MJ/kg DM)	11.7	11.5	12.0	11.7

^aContained (g/kg DM): nitrate, 757; Ca, 225.

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

Table 3

Experiment 1. Effect of added nitrate or increased lipid in basal diets containing different (g/kg DM) concentrate inclusions (Mixed, 480 and Concentrate, 916) on performance and slaughter characteristics of Charolais (CH) and Luining (LUI) steers. Adapted from Duthie et al. (2016).

	Breed		Mixed			Concentrate			SED	<i>P</i> -value ^b		
	CH	LUI	Control	Lipid	Nitrate	Control	Lipid	Nitrate		Breed	Basal diet	Treatment
Daily gain (kg/day)	1.51	1.41	1.52	1.56	1.53	1.36	1.28	1.48	0.099	<0.001	0.002	0.665
Dry matter intake (kg/day)	11.2	11.8	12.1	11.8	12.1	11.1	10.9	10.8	0.49	0.130	<0.001	0.769
Feed to gain (kg/kg)	7.6	8.8	8.1	7.6	8.1	8.2	8.0	7.4	0.42	<0.001	0.562	0.362
Age at slaughter (days)	565	599	585	578	579	587	584	579	10.1	<0.001	0.625	0.614
Slaughter weight (kg)	723	698	724	718	719	704	700	701	19.2	0.010	0.028	0.883
Carcass weight (kg)	414	369	400	395	395	391	386	383	9.3	<0.001	0.001	0.578
Conformation ^a	9.9	8.0	9.1	9.0	9.0	9.0	8.7	9.0	0.34	<0.001	0.456	0.635
Fatness ^a	10.4	11.2	10.3	11.5	10.4	10.6	10.7	11.4	0.36	0.019	0.552	0.249

^a 15 point EAAP scale for classification of beef carcasses based on conformation and fatness (Fisher, 2007)

^b There were no significant ($P>0.05$) basal diet x treatment interactions. SED given for basal diet x treatment, n=14.

Table 4

Experiment 1. Effect of added nitrate or increased lipid in basal diets containing different (g/kg DM) concentrate inclusions (Mixed, 480 and Concentrate, 916) on eating quality of grilled beef loin steaks from either Charolais (CH) or Luïng (LUI) steers, cooked to 74°C internal endpoint temperature.

	Breed		Mixed			Concentrate			SED	<i>P</i> -value ^a		
	CH	LUI	Control	Lipid	Nitrate	Control	Lipid	Nitrate		Breed	Basal diet	Treatment
Tenderness	5.0	6.0	5.5	5.3	5.3	5.6	5.9	5.6	0.21	<0.001	0.009	0.411
Juiciness	5.1	5.5	5.4	5.4	5.3	5.3	5.4	5.2	0.13	<0.001	0.399	0.463
Beef flavour intensity	4.4	4.5	4.3	4.4	4.4	4.6	4.6	4.5	0.11	0.002	0.157	0.774
Abnormal flavour intensity	2.4	2.1	2.1	2.2	2.2	2.2	2.4	2.2	0.10	<0.001	0.022	0.516
Overall liking	5.0	5.7	5.5	5.4	5.3	5.2	5.3	5.3	0.14	<0.001	0.093	0.876
Colour												
Days to a chroma value of 18	16.9	16.5	17.5	17.1	16.9	16.3	16.2	16.3	0.88	0.760	0.049	0.876
Vitamin E (µg/g loin)	2.00	2.48	2.67	3.13	2.59	1.37	2.07	1.62	0.191	<0.001	<0.001	<0.001

^a There were no significant ($P>0.05$) basal diet x treatment interactions. SED given for basal diet x treatment, n=14.

Table 5

Experiment 2. Effect of diets in which either rapeseed meal was replaced with nitrate, or lipid concentration increased, or both nitrate and lipid (Combined) on growth, and carcass characteristics of Aberdeen Angus (AAx) or Limousin (LIMx) crossbred steers (daily gain, Dry matter intake and feed to gain data adapted from Duthie et al. (2018)).

	Breed		Treatment				SED	<i>P</i> -value ^b		
	AAx	LIMx	Control	Nitrate	Lipid	Combined		Breed	Nitrate	Lipid
Daily gain (kg/day)	1.75	1.56	1.74	1.54	1.72	1.63	0.076	<0.001	0.008	0.445
Dry matter intake (kg/day)	12.1	11.1	11.8	11.4	11.8	11.5	0.39	<0.001	0.257	0.971
Feed to gain (kg/kg)	7.02	7.23	6.85	7.52	6.90	7.18	0.269	0.329	0.013	0.460
Age at slaughter (days)	549	546	548	548	547	547	7.7	0.331	0.876	0.978
Slaughter weight (kg)	687	670	687	675	675	677	12.2	0.011	0.639	0.639
Carcass weight (kg)	381	386	391	380	383	380	7.0	0.631	0.198	0.413
Conformation ^a	9.4	9.7	10.1	9.2	9.6	9.3	0.34	0.412	0.016	0.413
Fatness ^a	10.5	10.3	10.5	10.0	10.4	10.7	0.31	0.232	0.651	0.177

^a15 point EAAP scale for classification of beef carcasses based on conformation and fatness (Fisher, 2007)

^b There were no significant interactions ($P>0.05$) between nitrate and lipid; SED for treatment (n=20)

Table 6

Experiment 2. Effect of diets in which rapeseed meal was replaced with nitrate, lipid concentration increased, or both nitrate and lipid increased (Combined) on eating quality of grilled beef loin steaks from crossbred Aberdeen Angus (AAx) or Limousin (LIMx) steers, cooked to 74°C internal endpoint temperature.

	Breed		Treatment				SED	<i>P</i> -value ^a		
	AAx	LIMx	Control	Nitrate	Lipid	Combined		Breed	Nitrate	Lipid
Tenderness	6.0	5.4	5.6	5.7	5.8	5.7	0.21	<0.001	0.904	0.456
Juiciness	5.5	5.2	5.4	5.5	5.3	5.3	0.12	<0.001	0.559	0.250
Beef flavour intensity	4.6	4.5	4.5	4.5	4.5	4.6	0.11	0.271	0.799	0.613
Abnormal flavour intensity	2.2	2.2	2.3	2.2	2.2	2.2	0.10	0.679	0.981	0.472
Overall liking	5.5	5.3	5.4	5.2	5.4	5.4	0.13	0.011	0.342	0.401
Colour										
Days to a chroma value of 18	16.2	16.4	15.7	17.0	15.7	16.8	0.60	0.992	0.005	0.896
Vitamin E	3.47	3.19	3.25	3.26	3.43	3.28	0.017	0.017	0.873	0.205

^a There were no significant interactions ($P>0.05$) between nitrate and lipid; SED for Treatment, n=20

Legends to Figures.

Fig. 1. Experiment 1. The change in colour chroma over 17 days simulated retail display of *M. longissimus* steaks in modified atmosphere from steers fed Mixed (M, 480) or Concentrate (C, 916) basal diets (g concentrate/kg DM) and added nitrate or increased lipid concentration. A chroma value of 18 indicates the threshold for consumer acceptability. SE of difference for n=14 was 0.682.

Fig. 2. Experiment 2. The change in colour chroma over 16 days simulated retail display of *M. longissimus* steaks in modified atmosphere from steers fed diets in which rapeseed meal was replaced with nitrate, lipid concentration increased, or both nitrate included and lipid increased (Combined). A chroma value of 18 indicates the threshold for consumer acceptability. SE of difference for n=18 was 0.489.

Fig. 1. Experiment 1. The change in colour chroma over 17 days simulated retail display of *M. longissimus* steaks in modified atmosphere from steers fed Mixed (M, 480) or Concentrate (C, 916) basal diets (g concentrate/kg DM) and added nitrate or increased lipid concentration. A chroma value of 18 indicates the threshold for consumer acceptability.

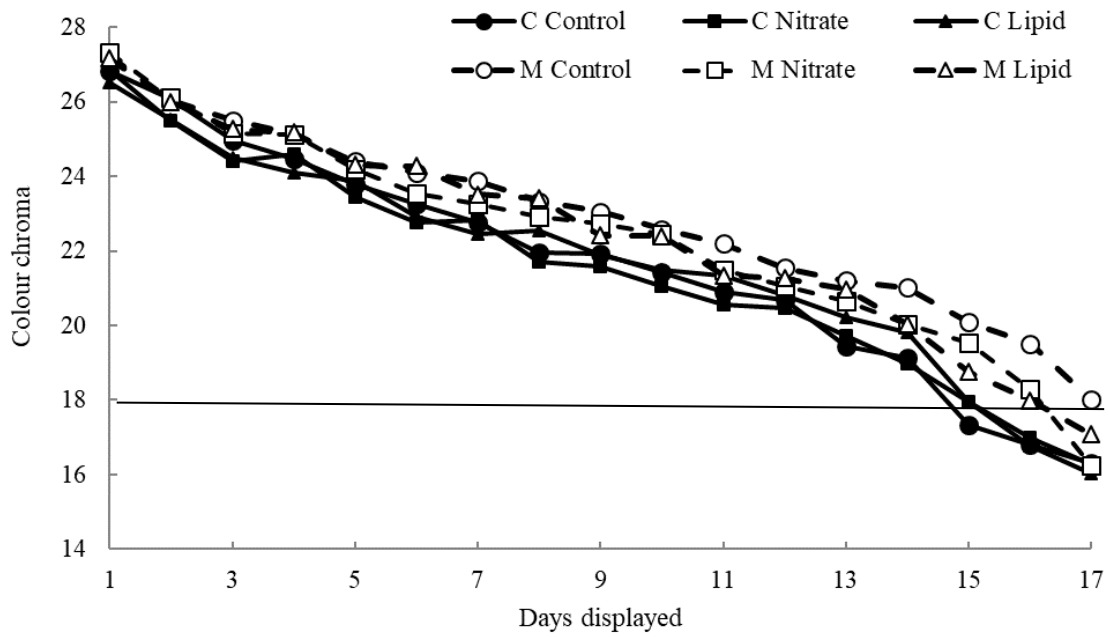


Fig. 2. Experiment 2. The change in colour chroma over 16 days simulated retail display of *M. longissimus* steaks in modified atmosphere from steers fed diets in which rapeseed meal was replaced with nitrate, lipid concentration increased, or both nitrate included and lipid increased (Combined). A chroma value of 18 indicates the threshold for consumer acceptability.

