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

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ABSTRACT

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

Keywords

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

1. Introduction

Methane (CH_4) produced by fermentation of feed, predominantly in the rumen of ruminant livestock, contributes significantly to greenhouse gas emissions. In the United Kingdom in 2014 (Department of Energy and Climate Change, 2016), enteric CH_4 emissions were estimated to account for 23.8 Mt carbon dioxide equivalents or 48% of total greenhouse gas emissions from the agriculture sector. A reduction in CH_4 emissions from livestock is therefore part of international governmental strategies for reducing greenhouse gas emissions (Australian Government, 2017; Scottish Government, 2018).

Manipulation of the diet to reduce CH_4 emissions is an important strategy available to livestock farming (Hristov et al., 2013). Many such strategies have been tested but convincing evidence for long-term efficacy *in vivo* for many is lacking. Increasing dietary lipid and the inclusion of nitrate in the diet have been shown to be effective mitigation strategies (Hristov et al., 2013) and their use has been recently reviewed (Martin, Morgavi, & Doreau, 2010; Patra, 2014; Lee & Beauchemin, 2014; Yang, Rooke, Cabeza, & Wallace, 2016). The extent to which either lipid or nitrate can be included in the diet is limited by potential adverse effects such as a reduction in fibre digestion and consequently feed intake from increased lipid in the diet and nitrate / nitrite toxicity from adding nitrate. However, little attention has been paid to the effects the safe application of lipids and nitrate as CH_4 mitigation strategies have on product quality. For lipids, the focus has been on the effects feeding lipids protected from rumen biohydrogenation have on both the fatty acid composition of meat lipids and meat eating quality (Scollan et al., 2014. For nitrate, the main concern to date has been the potential transfer of nitrate or its metabolites (nitrite, nitrosamines) to meat with potential adverse consequences for consumer health.

As there have been no reports of the organoleptic quality of meat, particularly from nitrate-fed cattle, the present study reports the eating quality, as measured by a trained taste panel and the simulated retail display shelf life of beef obtained from two studies (Troy et al., 2015; Duthie et al., 2016, 2018) in which the lipid content was increased or nitrate included in the diets of finishing beef cattle to reduce CH_4 emissions.

2. Materials and methods

Both experiments were conducted at Scotland's Rural College (SRUC) Beef and Sheep Research Centre, UK. The experiments (ED AE 15/2013 and ED AE 08/2014) were approved by the Animal Experiment Committee of SRUC and conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986. For full details of experimental procedures see Troy et al. (2015) for CH_4 measurements and Duthie et al. (2016) for growth performance and carcass characteristics for Experiment 1. For Experiment 2, see Duthie et al. (2018) for both CH_4 measurements and growth performance.

2.1. Experiment 1. Experimental design, animals and diets

The experiment was of a two × two × three factorial design; comprising two breeds of steers (crossbred Charolais or purebred Luing; 6 sires per breed), two basal diets which included; the Mixed basal diet, 480 g concentrate / kg dry matter (DM), and the Concentrate diet, 920 g concentrate / kg DM, and three treatments selected for their potential as CH_4 mitigation strategies (Control, Nitrate or increased lipid in the form of rapeseed cake (RSC)). The Control treatment contained rapeseed meal as the main protein source which was replaced with either Nitrate in the form of calcium nitrate (Calcinit, Yara, Oslo, Norway; 18 g nitrate/kg diet DM) or RSC (a by-product from the production of rapeseed oil by cold-pressing). The ingredient and chemical compositions of the diets are given in Table 1.

In total, 84 steers (13 to 16 months of age at the start of performance trial; 42 of each breed type) were used. Thus, 14 animals were allocated to each of the 6 concentrate inclusion × treatment combinations (7 of each breed). The animals on each of the basal diet x treatment combinations were group-housed in one pen per combination (a total of 6 pens). All steers were offered feed individually *ad libitum* using electronic feeders (HOKO, Insentec, Marknesse, The Netherlands). Treatments were balanced for sire, age and live weight (LW) at the start of the experiment. Prior to the start of the experiment the steers were adapted to the experimental diets in two stages. In stage one, the steers were adapted to basal diets over a 4 week period. In stage two (also 4 weeks), steers were adapted to the mitigation treatments by progressively increasing the amounts of nitrate or RSC.

2.2. Experiment 2. Experimental design, animals and diets

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

2.3. Performance test and slaughter

Growth, performance and feed conversion were characterized for all steers over a 56-day period. Dry matter intake (DMI, kg/d) was recorded daily for each animal and LW weekly. At the end of the performance test, steers remained on the same diets until slaughter and DMI and LW measurements continued throughout. Before slaughter, CH_4 production was measured (6 steers per week) over 13 weeks (Troy et al., 2015; Duthie et al., 2018) for both experiments). In each week of CH_4 measurement, steers selected were balanced for concentrate inclusion and treatment and so that when subsequently sent for slaughter, variation in LW and visual assessment of fatness between slaughter groups was minimized and steers achieved commercially acceptable conformation and fat classifications. Age at slaughter therefore varied; in Experiment 1, steers were slaughtered in 4 batches on days 85, 106, 127 and 148 after the start of the performance trial. Similarly, in Experiment 2 slaughter took place 99, 120, 141 and 162 days after the start of the performance trial. The steers were transported (approximately 1 h) to a commercial abattoir and slaughtered within 2 h of arrival. Cattle were stunned using a captive bolt, exsanguinated and subject to low voltage electrical stimulation. Following hide removal, carcasses were split in half down the mid-line and dressed to UK specifications (see Meat and Livestock Commercial Services Limited beef authentication manual, www.mlcsl.co.uk, for full description). EUROP conformation and fat classifications (Fisher, 2007), based on the UK scale, were allocated to all carcasses through visual assessment using a trained Meat and Livestock Commercial assessor.

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

2.4. Meat colour and chemical analysis

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

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

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

2.5. Sensory assessment

The sensory analysis was performed for each animal by a 10-person trained professional taste panel, using the same people for the duration of each experiment (British Standards Institution, 1993). The loin was thawed overnight at 4 °C and cut into 20 mm thick steaks. Steaks were grilled to an internal temperature of 74 °C, measured using a thermocouple probe (Testo Limited, Alton, UK). Following cooking, all fat and connective tissue was removed and the steak cut into 2 cm^3 cubes. The samples were placed into pre-labelled foils and placed in a heated incubator at 65 °C. Assessors tasted the samples in an order based on the designs outlined by MacFie, Bratchell, Greenhoff, & Vallis (1989) for balancing carryover effects between samples. All sensory assessments were completed under red light in a purpose-built sensory suite where each tasting booth was equipped with computer terminals linked to a fileserver running a sensory software programme (Fizz v 2.20h, Biosystemes, Couternon, France). Each panellist assessed one sample from each diet per session (six samples for experiment 1 and four samples for experiment 2), with four sessions in a morning and animals from each of the slaughter dates represented in a morning. Steaks were scored against 0–100 mm unstructured intensity line scales for a consensually agreed texture profile, where 0 = nil and 100 = extreme, and 8-point category scales for tenderness (1 = extremely tough to 8 = extremely tender), juiciness (1 = extremely dry to 8 = extremely juicy), beefy flavour and abnormal beef flavour intensities (1 = extremely weak to 8 = extremely strong). The hedonic scale served as an indication of preference by the panel, but it cannot be used to infer consumer acceptance since the results are based on 10 assessors who can no longer be considered as typical consumers because of the training they have received in meat assessment.

2.6. Calculations and statistical analysis

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

3. **Results**

3.1. Experiment 1. Performance and carcass data

Steers offered the Mixed basal diet had greater DMI (*P*<0.001) and LW gains (*P*=0.002) than those offered the Concentrate basal diet (Table 3) but feed to gain ratio did not differ between basal diets (*P*=0.56). There were no differences in performance between the CH_4 mitigation treatments. Steers did not differ between treatments in age at slaughter, but Mixed basal diet steers had greater slaughter (*P*=0.028) and carcass weights (*P*=0.001) than those fed the Concentrate basal diet. Methane mitigation treatments did not influence slaughter or carcass weights. Nutritional treatments imposed had no effect (Table 3) on carcass conformation or fatness (*P*>0.05). Charolais steers grew faster and had superior feed conversion ratios (*P*<0.001) than Luing steers. Carcass weights (*P*<0.001) were greater and conformation (*P*<0.001) and fat scores (*P*=0.019) superior for Charolais steers. There were no interactions between breed and nutritional treatments (*P*>0.05).

3.2. Experiment 1. Eating quality and simulated retail display

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

Colour chroma declined (*P*<0.001; Fig. 1) as display progressed reaching a value of 18 after 16 – 18 days display. Chroma of Concentrate basal diet steaks were lower than those of Mixed basal diet steaks (*P*<0.001) and as a result these animals reached a value of 18 earlier than Mixed basal diet samples (Table 4). The rate of chroma decline did not differ between basal diets (time x basal diet, *P*>0.05). Again, CH_4 mitigation treatment did not affect meat chroma. There were no significant differences between breed in meat chroma (*P*>0.05) or interactions between breed and nutritional treatments (*P*>0.05).

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

3.3. Experiment 2. Performance and carcass data

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

3.4. Experiment 2. Eating quality and simulated retail display

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

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

4. Discussion

The primary aim of these experiments was to quantify the efficacy of added nitrate or increasing dietary lipid as strategies to reduce enteric CH_4 emissions within different nutritional and genetic backgrounds. The different genetic backgrounds were included to determine whether breed had any influence on CH_4 emissions (which it did not). In Experiment 1, a comparison was made between breeds with very different characteristics Charolais, known for fast growth and excellent carcass composition and the Luing, a more extensively managed, hardy hill and upland breed. In Experiment 2, cross-bred Angus x Limousin cattle, extensively used commercially in the UK and intermediate between Charolais and Luing, were used. However, an important secondary aim, which is the subject of this paper, was to determine whether these mitigation strategies had any adverse effects on meat/product quality; a strategy that adversely impacted the quality of the final product could not be recommended. Whilst adding nitrate to the Concentrate basal diet (Experiment 1, Troy et al., 2015) did not reduce CH_4 emissions (Control v Nitrate, 14.7 v 15.4 g CH_4 / kg DMI), CH_4 was reduced from 25.1 to 20.6 g/kg DMI when the Mixed basal diet was fed. Similarly, increasing dietary lipid had no effect on CH_4 emissions when the Concentrate basal diet was fed (Control v Lipid, 14.7 v 15.7 g/kg DMI) but reduced CH_4 (25.1 v 23.1 g/kg DMI) when the Mixed basal diet was fed albeit to a lesser extent than Nitrate. In experiment 2 (Duthie et al. 2018) where only the Mixed basal diet was fed, both nitrate and increased lipid reduced CH_4 emissions and their effects were additive (Control, 24.0, Nitrate, 22.1, Lipid, 23.4, Combined 20.9 g /kg DMI). The efficacy of nitrate in reducing CH_4 was less in Experiment 2 than Experiment 1 (45 v 80% of theoretical maximum reduction). To provide context to results concerning meat quality, the performance and carcass characteristics of each experiment (Experiment 1, Duthie et al., 2015; Experiment 2 (performance only), Duthie et al., 2018) were reproduced in Tables 3 and 5.

4.1 Concentrate inclusion (Experiment 1)

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

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

4.2. Nitrate

The present study extends the findings on the efficacy of nitrate in reducing CH_4 production to aspects of meat quality. In studies using similar dietary concentrations (around 20 g nitrate / kg diet DM) to the present study, nitrate has had few negative impacts on animal performance (see reviews by Lee & Beauchemin, 2014; Yang, Rooke, Cabeza, & Wallace, 2016). The poorer feed conversion ratio in Experiment 2 when nitrate was fed is an exception. In terms of negative impacts, the potential toxicity of nitrate to the animal mainly through formation of Met-haemoglobin from nitrite absorbed from the rumen as a product of nitrate reduction has been most studied. As found in the current studies (see Duthie et al., 2016, 2018) after careful adaptation of animals to nitrate, no potentially toxic Met-haemoglobin concentrations were found. More recently, Hegarty et al. (2016) and Lee, Araujo, Koenig, & Beauchemin, (2017) found no adverse effects of adding nitrate to diets on carcass characteristics. Similarly, in the present experiments, carcass characteristics, with the exception of a poorer carcass conformation in experiment 2, were not affected by nitrate. Sensory meat quality was not influenced by addition of nitrate to diets irrespective of basal diet or whether nitrate was fed alone or with increased lipid in the diet. Thus, this experiment extends the evidence that dietary nitrate when used appropriately has no adverse effects on product quality.

Addition of nitrate to the diet had no effect on simulated retail display in Experiment 1 but improved shelf life by around 1 day in Experiment 2. This improvement in Experiment 2 appeared unrelated to vitamin E concentrations which did not differ in the presence or absence of nitrate. It is possible that elevated nitrate or nitrite in meat in Experiment 2 might have provided the extra stability. When the data for Medium concentrate diets in Experiments 1 and 2 were compared, the major difference was that in experiment 2, nitrate was less effective in reducing CH_4 emissions. As noted above, in Experiment 1, the reduction in CH_4 emissions was 80% of the theoretical maximum if all nitrate fed was reduced to ammonia in the rumen but only 42% in Experiment 2. This implies that 20 (Experiment 1) and 58% (Experiment 2) of the nitrate fed may have been absorbed and excreted either as nitrate *per se* or after metabolism. Potential metabolites of nitrate are N containing gases, nitrite or nitrosamines. Of these, nitrate, nitrite and nitrosamines would be of concern if elevated in meat. Guyader et al. (2016) did not detect nitrate in milk from nitrate-fed cows, nor did Hegarty et al. (2016) find elevated nitrate in meat from nitrate-fed cattle and nitrosamines were below the level of detection. Lee, Araujo, Koenig, & Beauchemin (2017) did report an increase in nitrate (from 0.1 in control to 0.6 mg/kg in muscle from nitrate-fed steers) but pointed out that these concentrations were below the level of concern for human diets. In the current study, concentrations of nitrate in meat from Experiment 1 were below the limit of detection of the assay employed (data not reported). Although the above evidence suggests that increased nitrate / nitrite concentrations in meat are unlikely, because 58% of the nitrate fed in experiment 2 could not be accounted for by ammonia formation in the rumen, this possibility can not be ruled out.

4.3 Lipids

The concentration of lipid in the diet was increased from 25 in the Control diets to 48 and 37 g / kg DM respectively in Experiments 1 and 2 respectively. These concentrations were less than the 60 g / kg DM, above which disturbances to rumen function are likely (Brask et al., 2013). The increases in lipid concentration were limited to avoid excessive increases in diet crude protein concentration and consequent increases in nitrogen excretion with potentially adverse environmental consequences. The lipid sources used were by-products of cold pressed rapeseed oil production in Experiment 1 and the distilling industry in Experiment 2 to avoid utilising lipid destined for the human food industry. Both rapeseed (approximately 60% monounsaturated and 30% polyunsaturated) and maize (27% monounsaturated and 48% polyunsaturated) contain substantial amounts of unsaturated fatty acids. However, this lipid was not protected from biohydrogenation in the rumen because diversion of hydrogen from CH_4 formation to biohydrogenation is one of the mechanisms by which lipids reduce CH_4 formation (Martin, Morgavi, & Doreau, 2010). Thus, in contrast to situations where lipid sources protected from rumen metabolism and containing high concentrations of polyunsaturated fatty acids are fed (see review by Scollan et al., 2014), the combination of small increases in dietary lipid and biohydrogenation in the present experiment, suggests that amounts of unsaturated fatty acid absorbed from the small intestine and incorporated into meat would be limited. As increases in unsaturated fatty acid concentrations in meat are a main factor influencing sensory traits (Vatansever et al., 2000), the absence of any effect of lipid on the sensory qualities of meat in the current experiments is not surprising. Similarly, there was no effect of lipid on simulated display shelf life. The only notable effect of lipid on meat characteristics was an increase in vitamin E concentrations in experiment 1. This may be related to increased fat concentrations in the meat; the absence of an increase in vitamin E in meat in experiment 2 may be related to the lesser increase in dietary lipid in that experiment.

5. Conclusions

Although basal diet (Experiment 1) and breed (both experiments) had significant effects on eating quality, in neither experiment did increased lipid or nitrate added to the diet of beef cattle have a negative effect on eating quality. Similarly, in neither experiment did CH_4 mitigation treatments reduce the colour shelf life of loin samples although in experiment 2 nitrate did significantly increase colour shelf life. Vitamin E concentrations in loin muscle were increased significantly by lipid in experiment 1 but there was no difference in experiment 2; nitrate had no effect on vitamin E concentrations. Overall the nutritional treatments explored here, which reduced CH_4 emissions, had no adverse effects on meat quality, although it must be noted that only one cut of meat was assessed and conclusions may not necessarily apply to other cuts.

Conflict of interest statement

The authors declare no conflict of interest.

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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	Mixed (480)			Concentrate (916)		
Treatment	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

[a] Contained (g/kg DM): nitrate, 769; Ca, 229.

[b] Contained (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

[a] Contained (g/kg DM): nitrate, 757; Ca, 225.

[b] Contained (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 Luing (LUI) steers. Adapted from Duthie et al. (2016).

	Breed		Mixed			Concentrate				*P*-value[b]		
	CH	LUI	Control	Lipid	Nitrate	Control	Lipid	Nitrate	SED	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 Luing (LUI) steers, cooked to 74^{0}C internal endpoint temperature.

	Breed		Mixed			Concentrate				*P*-value[a]		
	CH	LUI	Control	Lipid	Nitrate	Control	Lipid	Nitrate	SED	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					*P*-value[b]		
	AAx	LIMx	Control	Nitrate	Lipid	Combined	SED	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

[a] 15 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 74^0C internal endpoint temperature.

	Breed		Treatment					*P*-value[a]		
	AAx	LIMx	Control	Nitrate	Lipid	Combined	SED	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	0017	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.

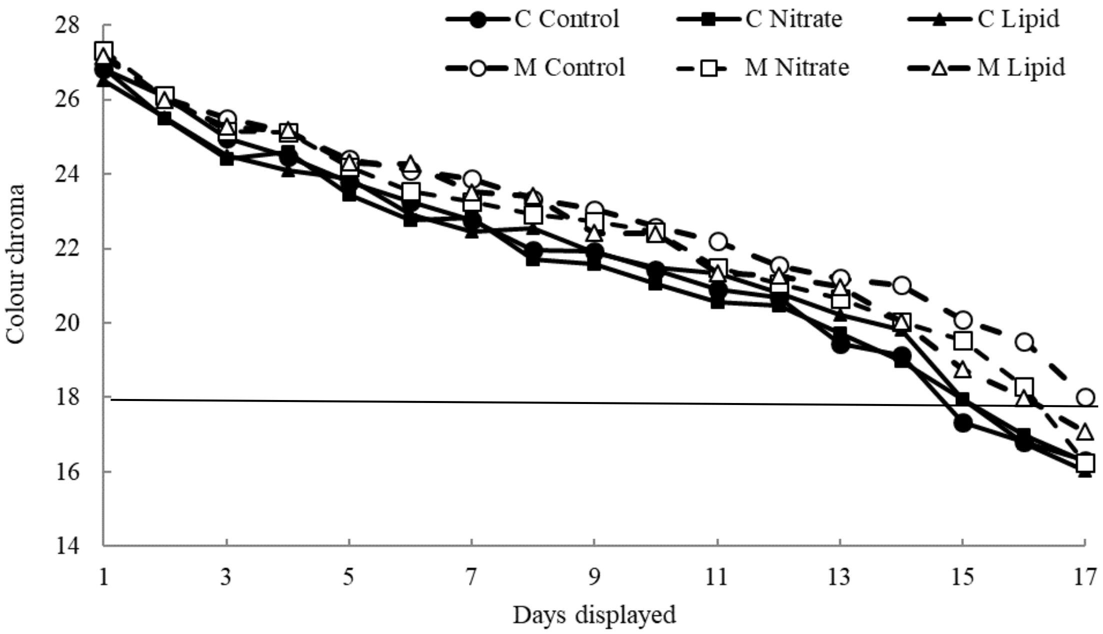

Fig. 2. Experiment 2. The change in colour chroma over 16 days simulated retail display of *M. longissimus* steaks in modified atmosphere from steers fed diets in which rapeseed meal was replaced with nitrate, lipid concentration increased, or both nitrate included and lipid increased (Combined). A chroma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