# "NUTRIBEEF"

# Nutritional improvements using diets and novel feed additives to enhance overall efficiency of beef production including meat quality and mitigation of greenhouse gas emissions as identified by characterisation of the rumen microbial population

**FINAL REPORT**

**AHDB BEEF AND LAMB (Project Number: 66714)**

**Professor Rainer Roehe[1], Dr Carol-Anne Duthie[1], Mr Dave Ross[1], Dr John Rooke[1], Dr Shane Troy[1], Dr Jimmy Hyslop[1], Dr Tony Waterhouse[1], Professor Richard Dewhurst[1], Dr Ian Richardson[2], and Professor John Wallace[3]**

[1]Future Farming Systems and Animal and Veterinary Sciences Groups, Research Division, SRUC, Kings Buildings, Edinburgh, EH9 3JG

[2]Division of Food Animal Science, University of Bristol, Langford, Bristol, BS40 5DU

[3]Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, AB21 9SB

**November 2015**

**CONTENTS**

**List of Tables…… 3**

**List of Figures……...6**

**1. MEMBERS OF THE NUTRIBEEF PROJECT MANAGEMENT GROUP……..... 8**

**2. EXECUTIVE SUMMARY……. 8**

**3. INTRODUCTION……... 12**

**4. PROJECT OBJECTIVES…….. 14**

**5. EVALUATION STUDY: MATERIAL AND METHODS…… 15**
5.1 Experimental design ……15
5.2 Breeds…….. 15
5.3 Experimental diets……... 16
5.4 Experimental protocol…… 17
5.4.1 Adaptation…….... 18
5.4.2 Performance test……18
5.4.3 Methane measurements……19
5.4.4 Slaughter……... 20
5.5 Sample collection and laboratory analyses……..21
5.5.1 Blood samples for methaemoglobin…… 21
5.5.2 Rumen samples……..... 22
5.5.3 Loin muscle samples……..... 23
5.6 Calculations and statistical analysis……25
5.6.1 Blood methaemoglobin response to dietary nitrate…….. 25
5.6.2 Performance and slaughter traits……...25
5.6.3 Methane and hydrogen emissions……26
5.6.4 Rumen microbial populations and VFA……..... 26
5.6.5 Meat quality…….. 26

**6. EVALUATION STUDY: RESULTS…… 27**
6.1 Blood methaemoglobin response to dietary nitrate…… 27
6.2 Performance test…….. 28
6.3 Carcass characteristics…… 29
6.4 Methane and hydrogen emissions…… 32
6.5 Volatile fatty acids……... 35
6.6 Rumen microbial analysis…… 39
6.6.1. Changes in microbial populations with time……..... 39
6.6.2. Quantification of microbial species implicated in nitrate reduction……
in the rumen……...42
6.7 Meat quality…… 43
6.7.1 Vitamin E content……..... 43
6.7.2 Colour shelf life……..... 44
6.7.3 Nitrate and nitrite levels in the meat…… 45
6.7.4 Sensory taste panel assessment…… 45
6.7.5 Fatty acids…… 46

**7. EVALUATION STUDY: MAIN CONCLUSIONS……..56**

**8. VALIDATION STUDY: MATERIAL AND METHODS……... 58**
8.1 Experimental design…… 58
8.2 Breeds…….. 58
8.3 Experimental diets……... 59
8.4 Experimental protocol…… 60
8.5. Sample collection and laboratory analyses……61
8.6 Calculations and statistical analysis……62
8.6.1 Blood methaemoglobin response to dietary nitrate…….. 62
8.6.2 Performance and slaughter traits…….. 62
8.6.3 Methane and hydrogen emissions……63
8.6.4 Rumen volatile fatty acids…… 63
8.6.5 Meat quality…….. 63

**9. VALIDATION STUDY: RESULTS……..... 64**
9.1 Blood methaemoglobin response to dietary nitrate…… 64
9.2 Performance test ……..... 66
9.3 Carcass characteristics …… 67
9.4 Methane and hydrogen emissions……70
9.5 Volatile fatty acids……... 71
9.6 Meat quality……77
9.6.1 Vitamin E content……..... 77
9.6.2 Colour shelf life……..... 78
9.6.3 Sensory taste panel assessment……79
9.6.4 Fatty acids……81

**10. VALIDATION STUDY: MAIN CONCLUSIONS……86**

**11. REPORT ON METHANE HOODS: EVALUATION AND VALIDATION TRIALS…... 88**
11.1 Material and Methods…….. 88
11.2 Results……... 90
11.2.1 Repeatability of the Hood system…….. 90
11.2.2 Comparison of dietary effects across both methods…….. 91
11.2.3 Prediction of individual animal emissions from $CH_4$ Hood system…….. 94
11.3 Conclusions……... 97

**12. PROFITABILITY, ECONOMIC ANALYSIS AND RECOMMENDATIONS……..98**
12.1 Approaches taken to calculate profitability and economic analysis……..... 98
12.1.1 Gross Margin calculations……..... 98
12.1.2 Net Margin calculations……..... 99
12.2 Financial analysis results…….. 99
12.2.1 Evaluation study (2013): evaluation of Nitrate (Calcinit)……..
and Lipid (Rapeseed cake) as dietary $CH_4$ mitigation strategies……... 100
12.2.2 Validation study (2104): evaluation of Nitrate and Lipid either…….
alone or in combination in finishing diets for steers…….. 106
12.3 Discussion, conclusions and key financial recommendations……... 113

**13. LIST OF PROJECT OUTPUTS: REFEREED PUBLICATIONS…….. 115**

**14. REFERENCES……...116**

## LIST OF TABLES


**Table 5.1.** Experimental design of the evaluation study.

**Table 5.2.** Ingredient composition of mixed forage: concentrate (Mixed) and high-concentrate (Concentrate) diets (g/kg DM).

**Table 5.3.** Chemical composition of feed components of the mixed forage: concentrate (Mixed) and high-concentrate (Concentrate) diets*.

**Table 5.4.** Chemical composition of the mixed forage: concentrate (Mixed) and high-concentrate (Concentrate) diets.

**Table 5.5.** Blood sampling for methaemoglobin analyses.

**Table 5.6.** Rumen sampling for volatile fatty acid and rumen microbial analyses.

**Table 5.7.** Loin muscle samples ($5^{th}$ to $10^{th}$ rib section).

**Table 5.8.** Point category rating scales used in the assessment of beef by a trained taste panel.

**Table 5.9.** The 15 point EAAP scale for classification of beef carcasses based on conformation and fatness, the EUROP system and those used in the United Kingdom (UK) - derived from Fisher (2007).

**Table 6.1.** Changes in mean and maximum individual blood MetHb concentration (% total Hb) in relation to nitrate intake and long-term nitrate feeding.

**Table 6.2.** Effect of breed (B), basal diet (D) and treatment (T) on growth, feed intake and feed efficiency of Charolais-sired (CHx) and purebred Luing (LU) steers fed either a Mixed- or Concentrate-based diet containing one of three treatments: Control, Nitrate or Lipid.

**Table 6.3.** Effect of breed (B), basal diet (D) and treatment (T) on carcass traits of Charolais-sired (CHx) and purebred Luing (LU) steers fed either a Mixed- or Concentrate-based diet containing one of three treatments: Control, Nitrate or Lipid.

**Table 6.4.** Intakes and $CH_4$ production as measured from the respiration chambers (Means with average SEM).

**Table 6.5.** Hydrogen production as measured from the respiration chambers (Means with average SEM).

**Table 6.6**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained before treatments were introduced to the basal diets.

**Table 6.7**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained 7 days after introduction of dietary treatments.

**Table 6.8**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained at the beginning of the 56 day test period after adaptation to treatments.

**Table 6.9**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained at the mid-point of the 56 day test period after adaptation to treatments.

**Table 6.10**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained at the end of the 56 day test period.

**Table 6.11.** Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained when steers left respiration chambers.

**Table 6.12.** Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained when steers were slaughtered.

**Table 6.13.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in Preliminary samples.

**Table 6.14.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in Adaptation samples.

**Table 6.15.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) at start, middle and end of 56-d period.

**Table 6.16.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in 56-d samples.

**Table 6.17.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in different longitudinal samples.

**Table 6.18.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in all samples.

**Table 6.19.** Microbial species in different samples in Chamber samples.

**Table 6.20a.** Effect of breed on Vitamin E content (ug/g) of loin muscle steaks.

**Table 6.20b.** Effect of basal diet type on Vitamin E content (ug/g) of loin muscle steaks.

**Table 6.20c.** Effect of diet × treatment on Vitamin E content (ug/g) of loin muscle steaks.

**Table 6.21.** Effect of treatment on eating quality of grilled beef steak (using the 8 point category scales).

**Table 6.22**. Effect of breed on eating quality of grilled beef steak (using the 8 point category scales).

**Table 6.23.** Effect of basal diet on eating quality of grilled beef steak (using the 8 point category scales).

**Table 6.24.** Effect of basal diet and treatment on eating quality of grilled beef steak (using the 8 point category scales).

**Table 6.25.** Effect of breed on the sum and ratios of important groups of fatty acids in *M. longissimus thoracis.*

**Table 6.26.** Effect of breed on the amount of individual fatty acids (mg/100g lean) in *M. longissimus thoracis.*

**Table 6.27.** Effect of basal diet on the sum and ratios of important groups of fatty acids in *M. longissimus thoracis.*

**Table 6.28.** Effect of basal diet on the amount of individual fatty acids (mg/100g lean) in *M. longissimus thoracis.*

**Table 6.29.** Effect of treatment on the sum and ratios of important groups of fatty acids in *M. longissimus thoracis.*

**Table 6.30.** Effect of treatment on the amount of individual fatty acids (mg/100g lean) in *M. longissimus thoracis.*

**Table 6.31.** Interactions between breed, basal diet or treatment.

**Table 8.1.** Experimental design of the validation study.

**Table 8.2.** Ingredient composition of experimental diets (dry matter basis; g/kg).

**Table 8.3.** Chemical composition of feed components of the experimental diets*.

**Table 8.4.** Chemical composition of the experimental diets.

**Table 8.5.** Validation study timeline.

**Table 8.6.** Blood sampling for methaemoglobin**.**

**Table 8.7.** Rumen sampling for volatile fatty acid and rumen microbial analyses.

**Table 8.8.** Loin muscle samples ($5^{th}$ to $10^{th}$ rib section)

**Table 9.1.** Methaemoglobin concentrations in blood of steers fed increasing amounts of nitrate.

**Table 9.2.** Effects of longer term inclusion of 100% nitrate in diets on steer methaemoglobin concentrations.

**Table 9.3.** Effect of breed and dietary treatment on growth, fat depth, feed intake and feed efficiency of Aberdeen Angus-sired (AAx) and Limousin-sired (LIMx) steers fed one of four dietary treatments: Control, Nitrate, Lipid or Combined.

**Table 9.4.** Effect of breed and dietary treatment on carcass traits of Aberdeen Angus-sired (AAx) and Limousin-sired (LIMx) steers fed one of four dietary treatments: Control, Nitrate, Lipid or Combined.

**Table 9.5.** Effect of dietary treatment on intakes and GHG production as measured from the respiration chambers.

**Table 9.6.** Effect of breed on intakes and GHG production as measured from the respiration chambers

**Table 9.7.** Main effects of sample time on VFA (mmol / mol total VFA).

**Table 9.8.** Main effects of breed on VFA (mmol / mol total VFA).

**Table 9.9.** Main effects of treatment on VFA (mmol / mol total VFA).

**Table 9.10.** Acetate and propionate molar proportions (mmol / mol total VFA) for different treatments.

**Table 9.11a.** Effect of breed on Vitamin E content (ug/g) of loin muscle steaks.

**Table 9.11b.** Effect of diet treatment on Vitamin E content (ug/g) of loin muscle steaks.

**Table 9.12a.** Effect of diet treatment on eating quality of grilled beef steak (using the 8 point category scales).

**Table 9.12b**. Effect of breed on eating quality of grilled beef steak (using the 8 point category scales).

**Table 9.12c.** Effect of breed × treatment group on eating quality of grilled beef steak (using the 8 point category scales).

**Table 9.13a.** Effect of breed on the weights of important groups of fatty acids in *M. longissimus thoracis.*

**Table 9.13b.** Effect of diet treatment on the weights of important groups of fatty acids in *M. longissimus thoracis.*

**Table 9.13c.** Effect of diet treatment on the proportions and ratios of important groups of fatty acids in *M. longissimus thoracis.*

**Table 9.13d.** Effect of diet treatment on the amount of individual fatty acids (mg/100g lean) in *M. longissimus thoracis.*

**Table 10.1.** Comparison of reductions in $CH_4$ emissions (g/kg DMI) in evaluation and validation trials.

**Table 11.1.** Dry matter intakes (DMI, kg/day), hood (MH) and respiration chamber (RC) measurement phases for Experiment 1.

**Table 11.2.** Dry matter intakes (DMI, kg/day), from the hood (MH) and respiration chamber (RC) measurement phases for Experiment 2.

**Table 11.3.** Average $CH_4$ concentration (MH-MPR, g/day) and $CH_4$ concentration corrected for DMI (MH-Yld, g/kg DMI) from the hood measurement phase, and $CH_4$ output (g/day) and yield (g/kg DMI) measured in chambers for Experiment 1.

**Table 11.4.** Average $CH_4$ concentration (MH-MPR, ppm) and $CH_4$ concentration corrected for DMI (MH-Yld, g/kg DMI) from the hood measurement phase, and $CH_4$ output (g/day) and yield (g/kg DMI) measured in chambers for Experiment 2.

**Table 11.5.** Accuracy of models to predict and validate methane (g/d) from respiration chambers (RC) based on measurements taken during the methane hood (MH) measurement period.

**Table 11.6.** $CH_4$ output (g/day) predicted using Model M4 and measured using chambers for Experiment 1.

**Table 11.7.** $CH_4$ output (g/day) predicted using Model M4 and measured using chambers for Experiment 2.

**Table 12.1.** Sale prices and cattle values along with a "Feeders Margin" calculation for Nitrate and Lipid (rapeseed cake) dietary $CH_4$ mitigation strategies in Charolais crossbreds (CHx) and Luing (LU) finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

**Table 12.2.** Feed inputs and costs along with a "Margin over feed and forage" calculation for Nitrate and Lipid (rapeseed cake) dietary $CH_4$ mitigation strategies in Charolais crossbreds (CHx) and Luing (LU) finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

**Table 12.3.** Bedding and other variable costs along with a "Margin over feed, forage and bedding" calculation for Nitrate and Lipid (rapeseed cake) dietary $CH_4$ mitigation strategies in Charolais crossbreds (CHx) and Luing (LU) finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

**Table 12.4.** Gross and Net Margin calculation for Nitrate and Lipid (rapeseed cake) dietary $CH_4$ mitigation strategies in Charolais crossbreds (CHx) and Luing (LU) finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

**Table 12.5.** Gross and Net Margin calculations for the main breed and dietary $CH_4$ mitigation strategies in finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

**Table 12.6.** Sale prices and cattle values along with a "Feeders Margin" calculation for Nitrate, Lipid (Maize Distillers Dark Grains) and the Combined (Nitrate and Lipid) dietary $CH_4$ mitigation strategies in crossbred Aberdeen Angus (AAx) and crossbred Limousin (LIMx) finishing steers during the validation study (2014).

**Table 12.7.** Feed inputs and costs along with a "Margin over feed and forage" calculation for Nitrate, Lipid (Maize Distillers Dark Grains) and the Combined (Nitrate and Lipid) dietary $CH_4$ mitigation strategies in crossbred Aberdeen Angus (AAx) and crossbred Limousin (LIMx) finishing steers during the validation study (2014).

**Table 12.8.** Bedding and other variable costs along with a "Margin over feed, forage and bedding" calculation for Nitrate, Lipid (Maize Distillers Dark Grains) and the Combined (Nitrate and Lipid) dietary $CH_4$ mitigation strategies in crossbred Aberdeen Angus (AAx) and crossbred Limousin (LIMx) finishing steers during the validation study (2014).

**Table 12.9.** Gross and Net Margin calculations for Nitrate, Lipid (Maize Distillers Dark Grains) and the Combined (Nitrate and Lipid) dietary $CH_4$ mitigation strategies in crossbred Aberdeen Angus (AAx) and crossbred Limousin (LIMx) finishing steers during the validation study (2014).

**Table 12.10.** Gross and Net Margin calculations for the main breed and dietary treatment effects in finishing steers during the validation study (2014).

## LIST OF FIGURES

**Figure 5.1:** Two breed types used in the evaluation study: Charolais-sired steers (shown on the left) and purebred Luing steers (shown on the right).

**Figure 5.2:** Electronic feeders in the study.

**Figure 5.3:** Layout of the pens bedded with wood fibre and sawdust.

**Figure 5.4a:** SRUCs GreenCow training pen facility.

**Figure 5.4b:** SRUCs GreenCow respiration chamber facility.

**Figure 5.5a:** Front view of the inside of the respiration chamber with feed intake recording equipment.

**Figure 5.5b:** View from the rear of the chamber, where the animals are entering the pen.

**Figure 5.6:** The VIA system in use during trial work.

**Figure 5.7.** Loin eye muscle from 5th to 10th rib location.

**Figure 6.1:** Changes in methaemoglobin (MetHb) concentrations (% total blood Hb) when fed 100% dietary nitrate (18 g nitrate/kg DM) for 5 steers with overall smallest and overall greatest mean MetHb concentrations. Solid lines and dashed lines represent the Mixed and Concentrate basal diets, respectively. Samples 1 to 4 refer to sampling days -6, 8, 87 and 101, respectively. Each line represents an individual animal. Sample 4 was not present for 3 animals because they had been already been sent for slaughter before day 101.

**Figure 6.2:** Changes in glucogenic ratio from introduction of Nitrate and Lipid (RSC) to diets until end of 56 day period for steers given the Mixed basal diet.

**Figure 6.3:** Changes in glucogenic ratio from introduction of Nitrate and Lipid (RSC) to diets until end of the 56 day test period for steers given the Concentrate basal diet.

**Figure 6.4:** The effect of diet on the colour chroma of loin steaks displayed in high oxygen modified atmosphere packs. Conc, Concentrate diet; For, Mixed diet; Nit, Nitrate; RSC, Lipid; Cont, Control.

**Figure 6.5:** The relationship between total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids and total fat (mg/100g lean) in beef M. *longissimus thoracis*.

**Figure 6.6:** The relationship between P:S ratio and total fat content (mg/100g lean) in beef M. *longissimus thoracis*.

**Figure 8.1:** Two breed types used in the validation study: Aberdeen Angus-sired steers and Limousin-sired steers.

**Figure 9.1.** Change in MetHb concentrations during adaptation to dietary nitrate.

**Figure 9.2.** Blood MetHb(%) of animals in the lower (25%) and upper (75%) quartiles.

**Figure 9.3:** Change in molar proportions (mmol / mol total VFA) of Acetate throughout the validation study.

**Figure 9.4:** Change in molar proportions (mmol / mol total VFA) of Acetate throughout the validation study.

**Figure 9.5:** Change in molar proportions (mmol / mol total VFA) of Acetate throughout the 2013 evaluation study.

**Figure 9.6.** Change in molar proportions (mmol / mol total VFA) of Acetate throughout the 2014validation study.

**Figure 9.7:** Change in molar proportions (mmol / mol total VFA) of propionate throughout the 2013 evaluation study.

**Figure 9.8:** Change in molar proportions (mmol / mol total VFA) of propionate throughout the 2014 validation study.

**Figure 9.9:** The effect of diet treatment on the colour chroma of loin steaks displayed in high oxygen modified atmosphere packs.

**Figure 11.1**: Schematic of the Hood system used to measure $CH_4$ during feeding.

**Figure 11.2**: Polycarbonate hood positioned above a feed bin.

**Figure 11.3:** Relationship between individual animal predicted $CH_4$ output (Model M6) and $CH_4$ output as measured by the chambers in two experiments (n = 147). Dashed line indicated x = y. Lin's concordance coefficient = 0.86. For individual experiments $R^2$=0.74 for both Experiment 1 and 2. M1 refers to the Mixed diet in Experiment 1; C1, the Concentrate diet in Experiment 1; M2 the Mixed diet in Experiment 2.

## 1. MEMBERS OF THE NUTRIBEEF PROJECT MANAGEMENT GROUP

Dr Mary Vickers (AHDB Beef and Lamb)
Mr Kim Matthews (AHDB Beef and Lamb)
Professor Rainer Roehe (SRUC)
Dr Carol-Anne Duthie (SRUC)
Mr Dave Ross (SRUC)
Dr John Rooke (SRUC)
Dr Shane Troy (SRUC)
Dr Jimmy Hyslop (SRUC)
Dr Tony Waterhouse (SRUC)
Professor Richard Dewhurst (SRUC)
Dr Ian Richardson (University of Bristol)
Professor John Wallace (Rowett Institute, University of Aberdeen)

## 2. EXECUTIVE SUMMARY

### 1. Project Objectives at outset

**Objective 1.** To investigate the effect of different diets in combination with novel candidate feed additives and the interactions between diets and feed additives upon methane emissions, performance and efficiency in different breeds of beef cattle.

**Objective 2.** To assess changes in rumen function by characterising the rumen microbial population differences as a result of different diets and feed additives in different breeds of beef cattle.

**Objective 3.** To assess changes in rumen function by repeated rumen sampling to determine the long-term effect of feed additives on methane emissions, performance and efficiency in different breeds of beef cattle.

**Objective 4.** To investigate the influence of different diets, feed additives and breeds on carcass and meat quality (sensory characteristics, colour and fatty acid profiles) and shelf life.

**Objective 5.** Nutritional and economic modelling to identify the best nutritional strategy for improving overall economic beef production efficiency considering scenarios of mitigation of greenhouse gas emissions and improvement of meat quality.

The project objectives were delivered by two experiments: an Evaluation study in 2013 and a Validation study in 2014. Prior to the experiments, a literature review was performed to identify candidate feed additives and (a) the addition of **Nitrate** to the diet and (b) increasing the concentration of dietary **Lipid** were identified as additives most suitable for use with finishing beef cattle.

## 2. Evaluation study

2.1 The evaluation study was of a two × two × three factorial design consisting of two breeds [crossbred Charolais and purebred Luing cattle], two basal diets [concentrate-straw based (Concentrate, 916 g concentrate / kg diet DM) and silage-based (Mixed, 480 g concentrate / kg diet DM)] and three additive treatments [Control (no additive, 27 g lipid/kg diet DM); **Nitrate** (18 g nitrate / kg DM) and increased **Lipid** (rapeseed cake, 51 g lipid / kg diet DM)]. A total of 72 steers were used (6 per breed × diet × additive combination).

2.2 The effects of **Nitrate** or **Lipid** on methane production depended on the basal diet fed. On the Mixed diet, **Nitrate** reduced methane emissions by 17% and **Lipid** by 7.5%. On the Concentrate diet, neither **Nitrate** nor **Lipid** reduced methane emissions. There was no breed effect on methane emissions.

2.3 Consistently across sample times, addition of **Nitrate** to the diets increased the rumen molar proportions of acetate and decreased those of propionate irrespective of basal diet whilst increasing the **Lipid** content had little effect on rumen volatile fatty acid molar proportions.

2.4 Analysis of the rumen microflora demonstrated that *Selenomonas ruminantium* was the dominant organism capable of reducing nitrate and that the greater numbers of thus bacterium on the high concentrate diet may provide an explanation for the absence of a reduction in methane when **Nitrate** was added to this diet.

2.5 Following an appropriate adaptation period (four weeks), feeding **Nitrate** (18 g nitrate/kg diet DM) with either the Concentrate or Mixed basal diets did not provide measurable adverse effects on animal health.

2.6 Using rapeseed cake to increase dietary **Lipid** from 27 to 51 g /kg diet DM did not suppress feed intake or reduce live-weight gain, suggesting that diets containing 50 g / kg diet DM or less lipid have no adverse effects on animal performance.

2.7 During a 56 day performance test, neither **Nitrate** nor **Lipid** adversely affected animal performance. Crossbred Charolais steers showed superior feed conversion efficiency compared to Luing steers (7.4 *v.* 8.6 kg,kg).

2.8 Carcass quality traits were not influenced by either **Nitrate** or **Lipid**. Crossbred Charolais steers achieved superior conformation (9.9 *v.* 8.1) and lower fat grades (9.5 *v.* 11.0) than Luing steers.

2.9 There was no effect of **Nitrate** or **Lipid** on any sensory attribute of loin meat. Loin muscle steaks from Luing had higher Vitamin E content (2.4 *v.* 2.0 ug/g), superior tenderness, juiciness, flavour and overall liking than steaks from crossbred Charolais cattle. Loin steaks from animals fed the Mixed diet had significant higher Vitamin E content (2.8 *v.* 1.7 ug/g) and longer shelf life (17 *v.* 15 days) than those fed the Concentrate diet. The *M. longissimus thoracis* from Luing had substantially more total fat and thus more saturated, monounsaturated and polyunsaturated fatty acids than that from Charolais crosses. However, the ratio of polyunsaturated and saturated fatty acids was in favour of crossbred Charolais.

2.10 Based on the results of the Evaluation study, it was decided to focus on combinations of methane reducing strategies within the Mixed basal diet in the Validation trial. It was

hypothesised that methane reduction measured using combinations of strategies would be the sum of that observed for individual strategies.

## 3. Validation study

3.1 The validation study was of a two × four factorial design consisting of two breeds [Aberdeen Angus or Limousin sired steers] and four dietary treatments [Control (443 g concentrate and 25 g lipid / kg diet DM); Nitrate (18 g nitrate / kg DM); Lipid (maize distillers dark grains, 37 g lipid / kg diet DM) and Combined (18 g nitrate and 37 g lipid / kg dietary DM)]. A total of 80 steers were used (10 per breed × dietary treatment combination).

**3.2** **Nitrate** reduced methane emissions by 9 % and **Lipid** by 4 %. The magnitude of reductions in methane emissions were less in the Validation study than in the Evaluation study. Aberdeen Angus and Limousin sired cattle showed significantly different methane emissions.

3.3 The effects of **Nitrate** or **Lipid** on methane production when used in combination were the sum of the effects observed when used independently. This indicates that the effects of **Nitrate** and **Lipid** in a mixed forage: concentrate diet were additive.

3.4 Similar to the Validation study and consistent across sample times, addition of **Nitrate** to the diets increased the rumen molar proportions of acetate and decreased those of propionate irrespective of basal diet. Increasing the **Lipid** content had little effect on rumen volatile fatty acid molar proportions.

3.5 As in the Evaluation study, following an appropriate adaptation period (four weeks), feeding **Nitrate** (18 g nitrate/kg diet DM) alone or in combination with lipid did not provide measurable adverse effects on animal health.

3.6 Using maize distillers dark grains to increase dietary **Lipid** from 25 to 37 g /kg diet DM did not suppress feed intake or reduce live-weight gain, confirming the Evaluation study results that diets containing 5% or less lipid have no adverse effects on animal performance.

3.7 Overall, during a 56 day performance study, **Lipid** did not adversely affect animal performance. However, and in contrast to the Evaluation study, addition of **Nitrate** resulted in poorer live-weight gain and reduced feed efficiency compared to diets not containing nitrate. In the performance test, crossbred Aberdeen Angus steers achieved greater average daily gain (1.74 *v.* 1.56 kg), but showed greater fat depth (9.1 *v.* 8.1 mm) and a poorer feed conversion efficiency (higher residual feed intake) compared to Limousin-sired steers.

3.8 Overall, carcass quality traits were not influenced by either **Nitrate** or **Lipid.** Crossbred Aberdeen Angus steers achieved a lower killing out percentage (55.5 *v.* 57.6%) but EUROP conformation and fat scores allocated by visual assessment did not differ between Aberdeen Angus and Limousin sired carcasses.

3.9 In agreement with the Evaluation study, there was no effect of **Nitrate** or **Lipid** on any sensory attribute of loin meat. Loin muscle steaks from Aberdeen Angus sired cattle showed more Vitamin E and superior tenderness, juiciness and overall liking compared to those from Limousin sired cattle. Due to higher total lipid, the *M. longissimus thoracis* from crossbred Aberdeen Angus cattle had more saturated, monounsaturated and polyunsaturated fatty acids

compared to crossbred Limousin cattle. However, the ratio of polyunsaturated to saturated fatty acids was in favour of Limousin-sired carcasses.

## 4. Economic appraisal

4.1 Whilst **Nitrate** feeding has some advantages in terms of reducing methane, in both trials studied here its use was less financially attractive. In addition, to avoid the potential downside in terms of animal toxicity, a careful diet preparation and an appropriate adaptation period has to be considered. Consequently, **Nitrate** feeding cannot be recommended to practical farmers at this stage.

4.2 Feeding high **Lipid** feedstuffs in finishing cattle diets can be recommended provided its use is economically competitive and excessive lipid levels in the total diet are avoided.

4.3 The above conclusions are made in the absence of financial incentives to reduce methane emissions. If this situation changes then these recommendations can be reviewed at any time.

4.4 Breed of cattle can have a significant impact on profitability of finishing enterprises on farm. In the 2013 trial Charolais sired cattle returned better margin figures than purebred Luing, whereas in the 2014 trial Aberdeen Angus sired cattle returned better margin figures than Limousin-sired cattle.

## 3. INTRODUCTION

Livestock systems, in particular ruminant production, are under increasing political pressure to reduce their greenhouse gas (GHG) outputs. Worldwide, beef production systems generate 2.9 Mt of $CO_2$-Equivalent emissions per year. Methane emissions from livestock account for 44% of the global emissions from livestock supply chains. The majority ((39% of the global emissions) of this $CH_4$ comes from enteric emissions, which are primarily affected by feed intake and quality (Gerber *et al.*, 2013). Mitigation strategies for enteric $CH_4$ emissions are required in order to minimize agricultural GHG emissions while still producing the increasing food requirements of the growing population. EBLEX (2010) reported that beef producers who cut their greenhouse gas (GHG) emissions can improve financial margins by up to 50p per kg. The UK Climate Change Act includes commitments by agriculture to reduce emissions by 11% by 2020. The Action Plan also includes a phased reduction of 3 million tonnes $CO_2$e from 2018 to 2022, without further reductions in the cattle and sheep populations. The beef sector contributes substantially to GHG emissions and therefore the development of mitigation strategies is required to meet these demands. The data analysis within the EBLEX (2012) report "Down to Earth" has shown that GHG emissions from commercial beef systems are around 23 kg $CO_2$e per kg dead weight. Any marginal reduction in this figure, achieved through improvements in nutrition and efficiency has the potential to contribute significantly to Action Plan targets in addition to improving the competitiveness of individual producers and the UK production base.

Diet formulation, enterprise and systems management and breeding are all possible strategies to reduce $CH_4$ from cattle (Cottle *et al.*, 2011), with diet formulation representing one of the most practical and promising approaches. Possible dietary GHG mitigation strategies include; (i) changing the nature of fermentable carbohydrate in the rumen (e.g. by replacing forage with concentrates), (ii) introducing substances to provide an alternative pathway for hydrogen utilization within the rumen to compete with methanogens (e.g. through the addition of nitrate), or (iii) the inhibition of the number and activity of methanogens in the rumen (e.g. by increasing the dietary lipid content). Furthermore, combinations of different dietary strategies have the potential to reduce $CH_4$ yield further than through individual strategies alone. Whilst it is important to identify strategies which effectively reduce $CH_4$, it is also important to report their implications on health, overall performance and efficiency, as well as product quality.

Recent interest in the controlled feeding of nitrate has been stimulated because the reduction of nitrate to ammonium in the rumen of adapted animals provides an alternative hydrogen sink to the production of $CH_4$ (van Zijderveld *et al.*, 2010). The reduction of nitrate to nitrite and then to ammonium provides an energetically more favourable route for disposal of metabolic hydrogen produced during fermentation of feed carbohydrates in the rumen than the production of $CH_4$. Although nitrate has been shown in many studies to reduce $CH_4$ emissions from ruminants (Nolan *et al.*, 2010; van Zijderveld *et al.*, 2010; van Zijderveld *et al.*, 2011, Hulshof *et al.*, 2012; Li *et al.*, 2012), the potential for its use has been hindered due to the toxicity of the intermediate product (nitrite). In the rumen, microbes rapidly reduce nitrate to nitrite and then reduce nitrite to ammonia. However, in an animal that has not been previously exposed to nitrate, the rate of reduction of nitrite to ammonia is slower than the reduction of nitrate to nitrite resulting in the accumulation of nitrite in the rumen (van Zijderveld *et al.*, 2010; Jeyanathan *et al.*, 2014). Absorbed nitrite binds to haemoglobin (Hb) in the blood converting it to methaemoglobin (MetHb) which is not capable of transporting oxygen to tissues. High concentrations of MetHb can cause methaemoglobinaemia, in which the functional oxygen carrying capacity of the blood is reduced. Blood MetHb is used as a marker for nitrate poisoning with a value of 30% of total Hb associated with clinical symptoms (Bruning-Fann and Kaneene, 1993). Nitrate toxicity may reduce animal performance (feed intake,

growth, loss of body weight), but in more severe cases may be fatal (Cockburn *et al.*, 2013). Therefore, the use of nitrate in ruminant diets requires careful consideration. The experimental studies within this report provide detailed information about the effect of nitrate on reduction of $CH_4$ and potential consequences on animal health and performance as well as carcass and meat quality.

Increasing the concentration of dietary lipid has been shown to reduce $CH_4$ emissions from ruminants (Martin *et al.*, 2010; Grainger and Beauchemin, 2011; Patra, 2013). This is achieved through various mechanisms: fatty acids are not fermented in the rumen and therefore increasing dietary lipid concentration reduces the proportion of feed which is fermentable within the rumen; lipids can also reduce $CH_4$ production by coating fibre particles, reducing their digestibility, and by reducing the numbers and activity of the rumen methanogens and protozoa responsible for methanogenesis (Johnson and Johnson, 1995; Patra, 2013). Dietary lipid can be increased through the addition of pure fats or oils to the diet or through the use of by-products from distilleries, breweries or plant oil extraction as ingredients in the diet (Brask *et al.*, 2013). Therefore, the effect of dietary lipid on mitigation of $CH_4$ emissions was investigated alongside its potential influence on animal performance, carcass and meat quality.

Methane formation in the rumen depends both on the supply of hydrogen ($H_2$) from acetate- and butyrate-producing bacteria and on the conversion of $H_2$ and carbon dioxide ($CO_2$) to methane by methanogenic archaea. For the first time, Wallace *et al.* (2014) have shown a relationship between the relative abundance of archaea in ruminal digesta and the quantities of $CH_4$ produced by individual animals. A recent review (Yang *et al.*, 2016) identified large gaps in our knowledge of rumen microbial ecology that handicap the further development and safety of nitrate as an additive. Three main bacterial species have been associated historically with ruminal nitrate reduction, namely *Wolinella succinogenes*, *Veillonella parvula* and *Selenomonas ruminantium*, but others almost certainly exist in the largely uncultivated ruminal microbiota. Indications are strong that ciliate protozoa can reduce nitrate, but their role relative to bacteria is not known. Further, although many mitigation strategies are effective *in vitro*, their effectiveness also tends to be transient in nature, because the rumen microbiota adapts around them (Hook *et al.*, 2010; Martin *et al.*, 2010). In this report we investigated the impact of feed additives on the microbial community and how the microbial community changed over time in order to identify potential adaptation of the microbial community to the dietary interventions used.

Compounds affecting methanogenesis may also affect lipolysis and biohydrogenation in the rumen. It is suggested that the higher polyunsaturated fatty acid (PUFA) content in meat and milk of animals grazing 'species rich' grassland relative to improved lowland grass swards may be brought about by either the reduced energy intake or the action of secondary plant metabolites produced in numerous 'weed' species common in 'species-rich' grassland. Such compounds include, polyphenol oxidase but also compounds such as essential oils (Wallace, 2004), saponins (Wallace, 2004; Shi *et al.*, 2004) and catecholamines (Lafontan *et al.*, 2002) all of which inhibit lipases and possess anti-microbial properties. Recently, several commercial companies have developed dietary additives to reduce $CH_4$ emissions in cattle, which may also affect biohydrogenation and hence fatty acid composition. It is therefore essential that meat fatty acid composition, meat quality and shelf life are measured to ensure they are not compromised by nutritional manipulation aimed at mitigating $CH_4$.

When considering mitigation strategies for beef cattle, studies have been mainly focussed on breeds that are managed more intensively, with less focus on breeds suited to extensive systems. The performance characteristics of hill and upland breeds (e.g. Luing), when managed more intensively, may be considerably different to that of intensively managed breeds (e.g. Charolais, Aberdeen Angus, Limousin), although the availability of performance data is limited. For example, baseline

performance data of Luing cattle, a hill and upland breed, is unavailable in the literature, even though their popularity as suckler cows is increasing in the UK and consequently the numbers of Luing calves reaching finishing units for more intensive finishing is also rising. Calf registrations of Luing and crossbred Luing calves in the UK has increased from 6165 in 2011 to 6525 in 2014 and is likely to increase further in 2015 (Agriculture and Horticulture Development Board, UK, 2015, personal communication). The large differences in performance are likely a result of considerable genetic and physiological differences. It is important to consider the effect of mitigation strategies across different breeds, alongside their implications for health and productivity. If this differs across different breeds, the industry and beef producers need to understand this if a real change in $CH_4$ output is to be delivered in commercial practice.

## 4. PROJECT OBJECTIVES

The Nutribeef project addresses three broad scientific objectives that were specified in the tender: (i) how can nutrition meet the challenge of reducing greenhouse gas emissions? (ii) provide an improved understanding of rumen function to improve the performance and efficiency of beef cattle, and (iii) what is the role of cattle nutrition in influencing meat quality? The project has also contributed data to two further broad scientific objectives namely (iv) the lack of up to date nutritional requirements for beef cattle on current farms (finishers) and (v) provision of suitable tools to give clear nutritional advice to farmers and veterinarians. The specific objectives of the Nutribeef project are outlined below:

**Objective 1.** To investigate the effect of different diets in combination with novel candidate feed additives and their interaction with $CH_4$ emissions, performance and efficiency in different breeds of beef cattle.

**Objective 2.** To assess the changes in rumen function by characterising the rumen microbial population differences as a result of different diets and feed additives in different breeds of beef cattle.

**Objective 3.** To assess the changes in rumen function by repeated rumen sampling to determine the long-term effect of feed additives on $CH_4$ emissions, performance and efficiency in different breeds of beef cattle.

**Objective 4.** To investigate the influence of different diets, feed additives and breeds on carcass and meat quality (sensory characteristics, colour and fatty acid profiles) and shelf life.

**Objective 5.** Nutritional and economic modelling to identify the best nutritional strategy for improving overall economic beef production efficiency considering scenarios of mitigation of greenhouse gas emissions and improvement of meat quality.

These objectives were addressed through experimental animal-model studies relating to beef animals in the finishing phase. The studies were conducted at the Beef and Sheep Research Centre, SRUC, UK. The experimental work was approved by the Animal Experiment Committee of SRUC and was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986. To fulfil the objectives two large-scale studies were conducted: (i) Evaluation study (in 2013, project year 1) and (ii) Validation study (in 2014, project year 2).

# 5. EVALUATION STUDY: MATERIAL AND METHODS

## 5.1 Experimental design

The Evaluation study (Table 5.1), conducted between 20th May 2013 and 31st December 2013 (project year 1), was of a two × two × three factorial design with:

- two breeds: (i) crossbred Charolais (CHx) and (ii) purebred Luing (LU)
- two basal diets: (i) concentrate-straw based (Concentrate) and (ii) silage-based (Mixed)
- three treatments (selected for their potential $CH_4$ mitigation effects): (i) Control, (ii) Nitrate, and (iii) Lipid.

**Table 5.1.** Experimental design of the evaluation study.

| Basal Diets | Concentrate | | | Mixed | | |
|---|---|---|---|---|---|---|
| Treatments | Control | Nitrate | Lipid | Control | Nitrate | Lipid |
| No. CHx | 6 | 6 | 6 | 6 | 6 | 6 |
| No. LU | 6 | 6 | 6 | 6 | 6 | 6 |

CHx, crossbred Charolais; LU, purebred Luing.

## 5.2 Breeds

The two breeds studied are shown in Figure 5.1. The breed types were selected to represent two commercially relevant breeds where CHx cattle represent a common continental sired beef breed in the UK well known for fast growth and excellent carcass conformation, whilst the LU breed is typical of a more extensively managed hardy hill and upland breed.

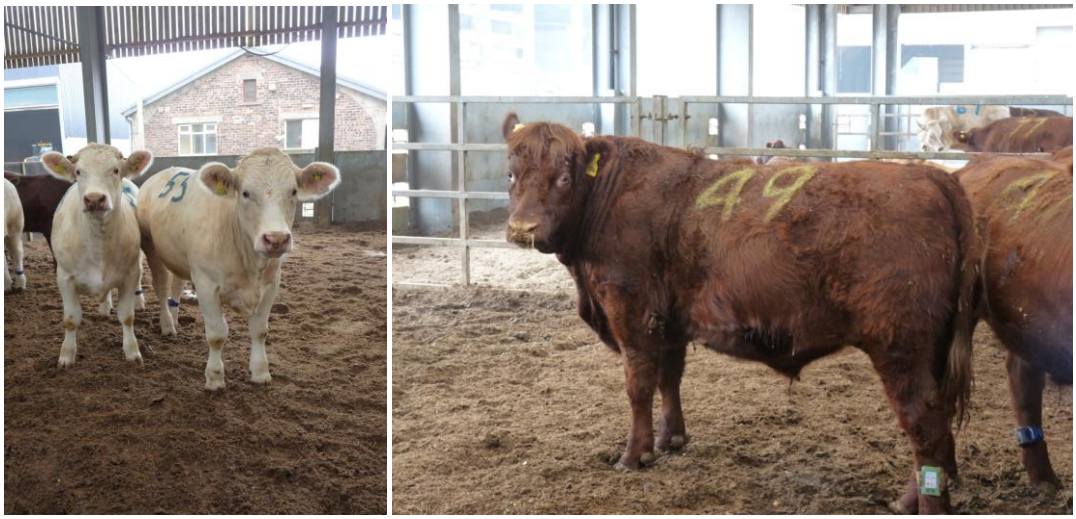

**Figure 5.1:** Two breed types used in the evaluation study: Charolais-sired steers (shown on the left) and purebred Luing steers (shown on the right).

## 5.3 Experimental diets

The steers were fed one of two basal diets (as total mixed rations) using a diet mixing wagon, consisting of (g/kg dry matter (DM)) forage to concentrate ratios of either (i) 520:480 (Mixed) or (ii) 84:916 (Concentrate). Within each basal diet the steers were offered one of three treatments: (i) Control containing rapeseed meal as the main protein source which was replaced with either (ii) Nitrate in the form of calcium nitrate (Calcinit, Yara, Oslo, Norway; 18 g nitrate/kg diet DM) or (iii) an added source of lipid in the form of pelleted rapeseed cake (RSC) which is a by-product from cold-pressing rapeseed which increased acid hydrolysed ether extract (AHEE) of the diet from 27 to 51 g AHEE/kg diet DM. The treatments were chosen in consultation with AHDB Beef and Lamb, the Food Standards Agency (FSA) and the project management team.

The ingredient composition of the experimental diets are given in Table 5.2. The chemical composition of individual components is given in Table 5.3. The chemical composition of experimental diets is given in Table 5.4.

The DM contents of individual components were determined on duplicate samples twice weekly and bulked feed samples (four per component) were analysed. Feed samples were analysed for DM, ash, crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), AHEE, and starch (Ministry of Agriculture Fisheries and Food, 1992) and gross energy (GE) by adiabatic bomb calorimetry. For the Nitrate and Lipid diets, calcium nitrate and RSC were incorporated firstly into a premix which contained the concentrate portion of the diet alongside minerals and molasses. Each batch of premix was mixed using a diet mixing wagon to produce a consistent premix. On a daily basis each premix was then mixed with the forage portion of the diet using the same mixing wagon to generate a consistent total mixed ration. Diets were mixed for a minimum duration of 20 minutes.

**Table 5.2.** Ingredient composition of mixed forage: concentrate (Mixed) and high-concentrate (Concentrate) diets (g/kg DM).

| | **Mixed** | | | **Concentrate** | | |
|---|---|---|---|---|---|---|
| Ingredient | Control | Nitrate | Lipid | Control | Nitrate | Lipid |
| Silage | 189 | 193 | 192 | | | |
| WCBS | 312 | 316 | 315 | | | |
| Straw | | | | 84 | 82 | 80 |
| Bruised barley | 340 | 392 | 296 | 739 | 803 | 700 |
| RSM | 128 | 43 | 7 | 146 | 57 | 10 |
| Calcinit | | 27 | | | 26 | |
| RSC | | | 160 | | | 179 |
| Molasses | 20 | 21 | 20 | 21 | 21 | 21 |
| Minerals* | 10 | 9 | 10 | 10 | 10 | 10 |

Silage, grass silage; WCBS, whole crop barley silage; Straw, barley straw; Barley, barley grain; RSM, rapeseed meal; Calcinit, calcium nitrate; RSC, rapeseed cake.
*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 5.3.** Chemical composition of feed components of the mixed forage: concentrate (Mixed) and high-concentrate (Concentrate) diets*.

| | Silage | WCBS | Straw | Barley | RSM | Calcinit | RSC |
|---|---|---|---|---|---|---|---|
| DM (g/kg) | 267 | 417 | 791 | 865 | 888 | 839 | 894 |
| Ash (g/kg DM) | 78 | 49 | 52 | 23 | 79 | 0 | 73 |
| CP (g/kg DM) | 151 | 102 | 25 | 103 | 366 | 1169 | 317 |
| ADF (g/kg DM) | 345 | 320 | 561 | 74 | 245 | 0 | 201 |
| NDF (g/kg DM) | 480 | 465 | 838 | 156 | 318 | 0 | 211 |
| Starch (g/kg DM) | 0 | 193 | 0 | 578 | 50.8 | 0 | 36.8 |
| AHEE (g/kg DM) | 36 | 16 | 14 | 30 | 28 | 0 | 172 |
| GE (MJ/kg DM) | 19.1 | 17.2 | 16.7 | 18.5 | 19.4 | 0 | 22.4 |
| ME (MJ/kg DM) | 11.4 | 10.5 | 6.3 | 13.1 | 11.0 | 0 | 15.2 |

Straw, barley straw; Silage, grass silage; WCBS, whole crop barley silage; Barley, barley grain; RSM, rapeseed meal; Calcinit, calcium nitrate; RSC, rapeseed cake; DM, dry matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; AHEE, acid hydrolysed ether extract; GE, gross energy; ME, metabolisable energy.
*Molasses contained 617 g DM /kg and Gross Energy 14.7 MJ/kg DM.

**Table 5.4.** Chemical composition of the mixed forage: concentrate (Mixed) and high-concentrate (Concentrate) diets.

| | **Mixed** | | | **Concentrate** | | |
|---|---|---|---|---|---|---|
| | Control | Nitrate | Lipid | Control | Nitrate | Lipid |
| DM (g/kg) | 486 | 481 | 484 | 857 | 855 | 861 |
| Ash (g/kg DM) | 51 | 46 | 52 | 35 | 29 | 37 |
| CP (g/kg DM) | 329 | 314 | 321 | 232 | 210 | 217 |
| ADF (g/kg DM) | 144 | 150 | 146 | 133 | 135 | 136 |
| NDF (g/kg DM) | 221 | 207 | 223 | 137 | 118 | 135 |
| Starch (g/kg DM) | 263 | 289 | 238 | 434 | 466 | 411 |
| AHEE (g/kg DM) | 26 | 25 | 49 | 27 | 27 | 53 |
| GE (MJ/kg DM) | 18 | 17 | 19 | 18 | 18 | 19 |
| ME (MJ/kg DM) | 11.6 | 11.4 | 12.1 | 12.1 | 11.9 | 12.8 |

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

## 5.4 Experimental protocol

In total, 84 steers (42 of each breed) were used. Thus, 14 animals (seven of each breed) were allocated to each of the six basal diet × treatment combinations (shown in Table 5.1). Due to the high risk of ill-health of unadapted animals gaining access to dietary nitrate, and the risks of Mixed-fed animals gaining access to large quantities of concentrate (e.g. acidosis), each diet × treatment combination was allocated to one pen (six pens in total). Treatments were balanced for sire within each breed, farm of origin and body weight (BW) and were balanced across basal diets and treatment groups at the start of the experiment. Fresh water was provided *ad libitum* using a water trough, and

diets were offered *ad libitum* to all steers using 32 electronic feeders (Figure 5.2, HOKO, Insentec, Marknesse, The Netherlands). Electronic feeders allow expression of performance in an environment close to on-farm conditions. All steers were bedded on wood fibre and sawdust to ensure that consumption of bedding did not contribute to nutrient intake. Figure 5.3 shows the layout of the experimental pens.

**Figure 5.2:** Electronic feeders in the study.

**Figure 5.3:** Layout of the pens bedded with wood fibre and sawdust.

**5.4.1 Adaptation:** day -56 to day 0 (20th May 2013 to 14th July 2013)

Steers were adapted to the experimental diets in two stages. In stage one (day -56 to day -29, 20th May 2013 to 16th June 2013), the animals were adapted to the basal diets. All steers were being fed the Mixed diet at the start of the adaptation period. Steers which were allocated the Concentrate diet, were adapted to the full concentrate inclusion over four weeks. This was undertaken at weekly intervals where diets comprising (g/kg DM) forage to concentrate ratios of 38:62, 25:75, 13:87 and 8:92 were offered during weeks one, two, three and four, respectively. During this period, steers were trained to use the electronic feed intake recording equipment. In stage two (day –28 to day 0, 17th June 2013 to 14th July 2013), steers were adapted to the treatments over a second four week period. Treatments (Nitrate and Lipid) were progressively incorporated into the diets at 25%, 50%, 75% and 100% of the required level, on days -28, -21, -14 and -7, respectively.

**5.4.2 Performance test:** day 0 to 56 (15th July 2013 to 8th September 2013*)*

After full adaptation to the experimental diets, performance and feed efficiency were characterised for all steers over a 56 day test period. Steers were maintained under controlled conditions, where group sizes within the pen remained constant. Individual dry matter intake (DMI) (kg/d) was recorded for each animal using the electronic feeding equipment and BW was measured weekly using a calibrated weigh scale. Measurements of BW were obtained before fresh feed was offered. For all steers, ultrasonic fat depth was obtained at the 12th/13th rib at the start (FD0) and end (FD1) of the 56 d test using an industry-standard Aloka 500 machine (BCF technology ltd., Scotland, UK). Images were analysed using Matrox Inspector 8 software (Matrox Video and Imaging Technology Europe Ltd., Middlesex, UK). Hyslop *et al.* (2012) assessed the consequences of alternative test lengths on the precision of average daily gain (ADG) and demonstrated that a 56-day measurement period, with weekly weighing is sufficient for characterising ADG of finishing beef cattle.

**5.4.3 Methane measurements:** day 64 to 148 (11th September to 9th December 2013)

Following the completion of the 56 day performance test described above, steers were successively moved in groups (maximum six steers per group) from the group-housed pens to SRUCs GreenCow respiration chamber facility on the same site to measure their $CH_4$ emissions. The steers remained in the group pens on their allocated diet until they entered the chamber facility. Before entering the chambers, the steers were housed in individual training pens (shown in Figure 5.4a) for a period of six days, to become accustomed to being housed individually.

The steers were allocated to minimise variation in BW (mean BW 696 ± 43 kg) on entry into the chambers. Each steer was allocated to one of six chambers (shown in Figure 5.4b) over a 12 week period, with the 2 × 2 × 3 factorial (breed × basal diet × treatment) experimental design allocated once to each chamber. One chamber malfunctioned during weeks 6 and 7, which resulted in the requirement for a 13th week of chamber analysis. Therefore, emissions from each of 76 steers were measured once.


**Figure 5.4a:** SRUCs GreenCow training pen facility.

**Figure 5.4b:** SRUCs GreenCow respiration chamber facility.

Feed intake and BW were monitored throughout the training pens and chamber phases. Figures 5.5a and 5.5b show the layout inside the chamber where the pen layout was identical to the training pens, and feed intake recording equipment identical to both the training pen and the home pen environment. The $CH_4$ measurements from one steer were discarded as the animal's level of feed intake reduced substantially whilst being housed in the respiration chamber, leaving a total of 75 individual steer $CH_4$ measurements.

The six respiration chambers were ventilated by recirculating fans set at 400 L/s, with exhaust fans set at 50 L/s. Temperature and relative humidity were set at 15°C and 60%, respectively. Exhaust air flow rate and the temperature, pressure and humidity of the exhaust and inlet air were measured. $CH_4$ concentrations were measured for each chamber by a multigas analyser. $CH_4$ production was calculated as the difference between inlet and exhaust gas concentration multiplied by volumetric dry air flow, corrected to standard temperature and pressure (25$^{o}$C and 1013 Mbar). Daily $CH_4$ production was calculated as the average of individual values and converted to a mass basis. The final 48 h of a 72 h measurement period were used to calculate daily $CH_4$ production.

**Figure 5.5a:** Front view of the inside of the respiration chamber with feed intake recording equipment.

**Figure 5.5b:** View from the rear of the chamber, where the animals are entering the pen.

**5.4.4 Slaughter:** day 85 to 148 (15th October 2013 – 10th December 2013)

Steers remained within the same pens and on the same diets from the end of the 56 day test to slaughter. On the day before slaughter, ultrasonic fat depth (FD2) at the 12th/13th rib was measured in all steers as described above. Steers were slaughtered in four batches of 17, 18, 21 and 25 steers on days 85 (15th October 2013), 106 (29th October 2013), 127 (26th November 2013) and 148 (10th December 2013), respectively.

Steers were selected for slaughter based on BW and visual assessment of fatness. 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 specification (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 assessor.

Video Image Analysis (VIA) was used to estimate EUROP carcass classifications (conformation and fat), total lean (kg) and total fat (kg) content of the whole carcass. The VIA systems in use in the EU are automatic machines that perform carcass evaluation based on images of the half carcass. The VBS 2000 system used in this study (Figure 5.6, E+V technology GmbH, Oranienburg, Germany) has been approved by the Department for Environment, Food and Rural Affairs (Defra) for use in the UK since 2010. The system operated at the end of the slaughter line after all necessary dressing and trimming had been completed. A pneumatically operated cradle presented the left half side of each carcass for imaging. The VIA camera took two images of the half carcass, a 2-dimensional image and a pseudo 3-dimensional image using structured light (Craigie *et al.*, 2012). The VBS 2000 required information on the category of the carcass (i.e., steer) and hot carcass weight (kg) and, by combining this information with data automatically captured by the VIA system (i.e., carcass dimensions, angles, areas, colour), predicted EUROP classification and total lean and fat content of the whole carcass.

**Figure 5.6:** The VIA system in use during trial work.

## 5.5 Sample collection and laboratory analyses

### 5.5.1 Blood samples for methaemoglobin

All steers receiving dietary nitrate had blood samples taken weekly throughout the second treatment adaptation phase to monitor blood MetHb concentrations. Blood samples were taken when MetHb was expected to be greatest, i.e., 3 h after fresh feed was offered (van Zijderveld *et al.*, 2010), on the day after dietary nitrate was increased (days -27 (25%), -20 (50%), -13 (75%) and -6 (100%)) and then 15 days after maximum nitrate inclusion was achieved (day 8). To assess the long-term effects of feeding nitrate, blood samples were obtained at day 87 and day 101 (128 days after initial inclusion of nitrate). Blood samples were taken from the caudal vein into an evacuated tube (Vacuette, Griener Bio One Ltd., Gloucestershire, UK) containing heparin. MetHb concentration in blood was measured within 2 h of sampling by co-oximetry (Stat Profile Critical Care Xpress, Nova Biomedical U.K., Cheshire, UK). Table 5.5 provides a summary of blood samples.

**Table 5.5.** Blood sampling for methaemoglobin analyses.

| Sample (day) | Date | Description (% full nitrate inclusion) | Nitrate (g/kg diet DM) |
|---|---|---|---|
| -27 | 18th June 2013 | 25 | 4.5 |
| -20 | 25th June 2013 | 50 | 9 |
| -13 | 2nd July 2013 | 75 | 13.5 |
| -6 | 9th July 2013 | 100 | 18 |
| 8 | 16th July 2013 | 100 | 18 |
| 87 | 10th October 2013 | 100 | 18 |
| 101 | 24th October 2013 | 100 | 18 |

### 5.5.2 Rumen samples

Rumen samples were taken from each animal on seven occasions for volatile fatty acid (VFA) analyses and rumen microbial analyses (Table 5.6).

1. *Preliminary*: After transition to the Mixed and Concentrate diets but before introduction of Nitrate and Lipid treatments.
2. *Adaptation*: Seven days after introduction to Nitrate and Lipid treatments when cattle were being offered 25% of the maximum dose of Nitrate.
3. *Start test*: At the start of the 56 day performance test period.
4. *Mid test*: At the mid-point of the 56 day performance test period.
5. *End test*: At the end of the 56 day performance test period.
6. *Chamber*: After completion of the 56 day performance test period, when each animal left the respiration chambers over a period of 13 weeks (six animals/week).
7. *Slaughter*: At the abattoir, when cattle were slaughtered in four batches.

**Table 5.6.** Rumen sampling for volatile fatty acid and rumen microbial analyses.

| Sample (day) | Date | Description |
|---|---|---|
| -46 | 30th May 2013 | 1. Preliminary |
| -21 | 24th June 2013 | 2. Adaptation |
| -4 | 11th July 2013 | 3. Start test |
| 28 | 12th August 2013 | 4. Mid test |
| 56 | 9th September 2013 | 5. End test |
| 65-143 | 18th September – 5th December 2013 | 6. Chamber |
| 80-149 | 3rd October – 11th December 2013 | 7. Slaughter |

At each sampling approximately 50 mL of rumen liquid were taken by inserting a stomach tube (16 × 2700 mm Equivet Stomach Tube, JørgenKruuse A/S, Langeskov, Denmark) nasally and aspirating manually. This liquid was filtered through two layers of muslin. For VFA analysis a 5 ml sample of the filtered liquid was deproteinised by adding 1 mL metaphosphoric acid (215 g/l) and 0.5 ml methylvaleric acid (10 g/l). For DNA (rumen microbial) analysis, 5 ml strained rumen fluid were mixed with 10 ml phosphate buffered saline containing glycerol (30% v/v). These samples were stored at -20°C between collection and analysis.

Volatile fatty acids concentrations were determined by HPLC (high performance liquid chromatography) for all rumen samples from all the dietary treatments. For rumen microbial analysis DNA extraction was carried out using a method based on repeated bead beating plus column filtration (Rooke *et al.*, 2014). DNA concentrations were determined with a NanoDrop ND 1000 Spectrophotometer and DNA was diluted to 0•5 ng/µl in 5 µg/ml herring sperm DNA for amplification of bacterial 16S RNA genes with universal bacterial primers UniF and UniR and 5 ng/µl in 5 µg/ml herring sperm DNA for amplification of other groups. Quantitative PCR of 16S RNA genes from different bacterial classes was carried out using a BioRad iQ5. Template DNA from *Roseburia hominis* A2-183 was used for bacterial calibration. Amplification of archaeal 16S RNA genes was achieved by using the primers described by Hook *et al.* (2009) and calibrated using DNA extracted from *Methanobrevibacter smithii* PS. Data are reported as copy no / ng DNA.

### 5.5.3 Loin muscle samples

At 48 hours *post-mortem*, samples from the loin eye muscle (Figure 5.7) were obtained from all carcasses for assessment of sensory characteristics, fatty acid profiles and vitamin E content. Further samples (loin) were conditioned and subject to simulated retail display in modified atmosphere packaging, to determine colour shelf life. After collection, all muscle samples were vacuum-packed and delivered, using chilled transport, to the University of Bristol for chemical analysis of fatty acid composition, vitamin E content, colour stability during simulated retail display, and assessment of eating quality by a professional, trained sensory panel. All samples were chilled and aged at 0 to 2$^{o}$C to 10 days post slaughter before samples were cut for packaging and colour stability testing, the rest being frozen and stored at -18°C until analysed. Numbers of loin samples obtained at each slaughter day are given in Table 5.7.

**Figure 5.7:** Loin eye muscle from 5$^{th}$ to 10$^{th}$ rib location.

**Table 5.7.** Loin muscle samples (5$^{th}$ to 10$^{th}$ rib section).

| Slaughter Batch | Number of steers | Number of loins selected | Slaughter day | Slaughter Date |
|---|---|---|---|---|
| 1 | 18 | 18 | 85 | 15$^{th}$ October 2013 |
| 2 | 18 | 18 | 106 | 29$^{th}$ October 2013 |
| 3 | 21 | 21 | 127 | 26$^{th}$ November 2013 |
| 4 | 25 | 25 | 148 | 10$^{th}$ December 2013 |

*Sensory taste panel assessment*

Sensory analysis was carried out by a 10-person trained taste panel (BSI, 1993). The samples were defrosted overnight at 4 °C and then cut into steaks 20 mm thick. Steaks were grilled to an internal temperature of 74 °C in the geometric centre of the steak (measured by a thermocouple probe), after which, all fat and connective tissue were trimmed and the muscle was cut into blocks of 2 cm$^3$. The blocks were wrapped in pre-labelled foil, placed in a heated incubator and then given to the assessors in random order chosen by a random number generator. Assessors are asked to rate the samples on eight point category scales (Table 5.8) for texture, juiciness, flavour intensity (higher values denote more favourable responses), abnormal flavour intensity (lower values denote more favourable responses). Two additional hedonic questions relating to flavour liking and overall liking are also used.

**Table 5.8.** Point category rating scales used in the assessment of beef by a trained taste panel.

| Rating | Texture | Juiciness | Flavour intensity |
|---|---|---|---|
| 8 | Extremely Tender | Extremely Juicy | Extremely Strong |
| 7 | Very Tender | Very Juicy | Very Strong |
| 6 | Moderately Tender | Moderately Juicy | Moderately Strong |
| 5 | Slightly Tender | Slightly Juicy | Slightly Strong |
| 4 | Slightly Tough | Slightly Dry | Slightly Weak |
| 3 | Moderately Tough | Moderately Dry | Moderately Weak |
| 2 | Very Tough | Very dry | Very Weak |
| 1 | Extremely Tough | Extremely Dry | Extremely Weak |

| | Abnormal flavour intensity | Flavour liking | Hedonic<br>Overall liking |
|---|---|---|---|
| 8 | Extremely Strong | Like Extremely | Like Extremely |
| 7 | Very Strong | Like Very Much | Like Very Much |
| 6 | Moderately Strong | Like Moderately | Like Moderately |
| 5 | Slightly Strong | Like Slightly | Like Slightly |
| 4 | Slightly Weak | Dislike Slightly | Dislike Slightly |
| 3 | Moderately Weak | Dislike Moderately | Dislike Moderately |
| 2 | Very Weak | Dislike Very Much | Dislike Very Much |
| 1 | Extremely Weak | Dislike Extremely | Dislike Extremely |

*Fatty acid analyses*

Total fatty acid (FA) analysis was carried out by direct saponification as described in detail by Teye *et al*. (2006). Muscle samples, trimmed of outer fat and connective tissue, were blended thoroughly, hydrolysed with 6M KOH in water:methanol (1:1) for 2h at 60°C then acidified using 5M $H_2SO_4$ and the free fatty acids (FFA) extracted into petroleum spirit. The free FA were methylated using methanolic hydrogen chloride (freshly made by adding 10% acetyl chloride dropwise to cold anhydrous methanol) and leaving for1h at 50°C. The fatty acid methyl ester (FAME) mixture was shaken well with water and n-hexane and after centrifuging were extracted into the n-hexane, dried with anhydrous sodium sulphate and analysed by gas liquid chromatography. Samples were injected in the split mode, 50:1, onto a CP Sil 88, 50 m x 0.25 mm FAME column (Agilent Technologies) with helium as the carrier gas. Linearity and identification of individual FAME was tested using methyl ester quantitative standards (Thames Restek UK Ltd, Windsor, UK). Muscle fatty acids are reported as mg of fatty acid per 100 g wet tissue, quantified by reference to the internal standard (C21:0), and also as percentage of total fatty acids.

*Vitamin E analyses*

Muscle vitamin E (α-tocopherol) was determined using a modification of the procedure described by Liu et al. (1996) scaled up for 1 g of tissue. Homogenised lean tissue was saponified with ethanolic KOH using BHT and L-ascorbic acid as antioxidants. Ras-5,7-dimethyl-tocol solution was used as internal standard. The vitamin E was extracted into hexane, dried, dissolved in hexane and injected onto an HPLC column with mobile phase 4% dioxane and 96% hexane. The quantification was based on the comparison between peaks of a known α-tocopherol external standard and the peak resulted from the sample, adjusted by recovery percentage in the process, calculated with the DMT internal standard.

*Colour shelf life*

At the end of the conditioning period, two steaks were cut and packed in modified atmosphere (75:25, $O_2$:$CO_2$) and subjected to simulated retail display under lights at 4ºC (700lux, 16h on: 8h off) until the chroma values dropped below 18. Colour was measured through the pack lid daily using a Minolta CR400 chromameter (Konica-Minolta Measuring Instruments, Basildon, Essex, UK) to measure Lightness (L*), a* and b* co-ordinates. Chroma was calculated as $C= \sqrt{(a^{*2} + b^{*2})}$.

*Nitrate/Nitrite analyses*

Frozen samples for nitrate/nitrite analysis were sent to Eurofins Food Testing UK Limited (Wolverhampton, UK) and analysed for Nitrate ($NO_3$) and Nitrite ($NO_2$) by Colorimetry (Eurofins analytical code UD02L). Representative samples were later sent to International Laboratory Services (Shardlow, Derbyshire, UK) for repeat analysis.

## 5.6 Calculations and statistical analysis

### 5.6.1 Blood methaemoglobin response to dietary nitrate

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

### 5.6.2 Performance and slaughter traits

Data from three steers were unavailable as the animals were removed from the trial during the 56 day test period for health reasons unconnected to the diets and treatments imposed. Growth was modelled by linear regression of BW against test date, to obtain ADG, mid-test BW (mid-BW) and mid-test metabolic BW (mid-MBW = $BW^{0.75}$). Mean DMI over the 56 day period was expressed as kg per day or as a proportion of mid-BW and mid-MBW. Feed conversion ratio (FCR) was calculated as average DMI per day (kg/d)/ADG. Residual feed intake (RFI) was calculated as deviation of actual DMI (kg/d) from DMI predicted based on linear regression of actual DMI on ADG, mid-MBW and FD1 (Basarab *et al.*, 2003). Cold carcass weight (CCW) was calculated as a percentage of slaughter BW (SBW) to determine killing out percentage (KO). To allow for statistical comparison, the EUROP carcass classification values were expressed on the equivalent 15 point scale (Table 5.9, Kempster *et al.*, 1986). Statistical analyses of performance and carcass data were conducted using the mixed procedure of SAS software with the fixed effects of breed, basal diet and treatment, and the random effect of pen (and slaughter batch for carcass traits). In addition, in the analysis of FD1 and FD2 the deviation from the breed mean of FD0 was included as a covariable. The interaction effects of breed × basal diet, basal diet × treatment, breed × treatment and breed × basal diet × treatment were included in the model when these effects proved significant ($P<0.05$). Data are reported as means with their SEM. Differences between means were tested using a protected least square means comparison test. Probability values were deemed significant where $P<0.05$ and indicated a tendency when probability values were between $P=0.05$ and $P=0.1$.

**Table 5.9.** The 15 point EAAP scale for classification of beef carcasses based on conformation and fatness, the EUROP system and those used in the United Kingdom (UK) - derived from Fisher (2007).

| **Conformation**[1] | Poor → | | | | | | | | | | Excellent | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 Point Scale | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| EUROP | –P | P | P+ | –O | O | O+ | –R | R | R+ | –U | U | U+ | –E | E | E+ |
| UK | –P | | P+ | –O | | O+ | | R | | –U | | U+ | | E | |
| **Fatness**[1] | Low → | | | | | | | | | | Excessive | | | | |
| 15 Point Scale | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| EUROP | –1 | 1 | 1+ | –2 | 2 | 2+ | –3 | 3 | 3+ | –4 | 4 | 4+ | –5 | 5 | 5+ |
| UK | | 1 | | | 2 | | | 3 | | 4L | | 4H | 5L | | 5H |

[1]Note that the terms "Poor", "Excellent", "Low" and "Excessive" are for illustration only, in application the classification scheme uses the EUROP symbols only.

### 5.6.3 Methane and hydrogen emissions

The respiration chamber measurements from one steer were discarded as the animal's level of feed intake decreased substantially (> 30%) whilst being housed in the respiration chamber, leaving data from a total of 75 individual steers. Respiration chamber $CH_4$ and hydrogen data were analysed using the Statistical Analyses System (SAS 9.3, SAS Inst. Inc., North Carolina) using linear mixed models. The fixed effects were breed, diet and treatment, while the random effects were week and chamber. The effect of the diet × treatment, breed × treatment and breed × diet interactions were included in the model when these proved significant ($P<0.05$). Data are reported as means with their SEM unless indicated otherwise. Differences between means were tested using a protected least squared means test with probability values of $P<0.05$ deemed to be significant, while probability values between $P=0.05$ and $P=0.1$ were deemed to indicate a tendency.

### 5.6.4 Rumen microbial populations and VFA

As there were no effects of breed or interactions between breed and either diet or treatment, effects of diet and treatment at each sampling point were analysed as a 2 × 3 factorial design within Genstat. Changes in VFA over time were assessed by calculation of glucogenic ratio (GR = ((acetate + butyrate) / propionate)) and analysed using a split plot analysis of variance within Genstat where the main plot effects were the effects of diet and treatment (as above) and the split plot was sample. Interactions between sample and main plot effects (diet and additive) were also assessed. GR in Preliminary sample was included as a covariate. Microbial populations were analysed in a similar manner, except that microbial populations in Preliminary samples were included as a covariate throughout.

### 5.6.5 Meat quality

Data was analysed by analysis of variance using diet, treatment and breed as factors (IBM SPSS v21)

# 6. EVALUATION STUDY: RESULTS

## 6.1 Blood methaemoglobin response to dietary nitrate

During the adaptation period (Table 6.1), blood MetHb concentrations were similar when feed contained up to 75% of total nitrate (up to -13 d) but increased when nitrate was included at the 100% level (18 g nitrate/kg diet DM) on both basal diets. During adaptation there was no difference (P>0.05) in MetHb between the basal diets but blood MetHb concentrations of steers offered the Concentrate diet were consistently greater (day × basal diet interaction, P<0.001) than those offered the Mixed diet from day 8 onwards.

There was a consistent individual animal response across sampling days in MetHb concentrations when animals were offered the maximum dietary nitrate (100%, day -6 to 101). Of 28 steers, six always had MetHb concentrations less than the median MetHb for each sampling day whilst nine steers consistently had MetHb concentrations greater than the upper quartile. Figure 6.1 shows individual values for five steers with the smallest mean MetHb concentration and the five steers with the greatest mean concentrations and demonstrates consistency of steer response across time (from day -6 onwards, when 100% nitrate was offered). Maximum values for blood MetHb concentration (Table 6.1) were always less than 30% of total Hb. The greatest individual MetHb concentration value was 15.4% total Hb. There was no significant effect of breed on blood MetHb concentrations (P>0.05).

**Table 6.1.** Changes in mean and maximum individual blood MetHb concentration (% total Hb) in relation to nitrate intake and long-term nitrate feeding.

| Day[1] | -27 | -20 | -13 | -6 | 8 | 87 | 101 | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Nitrate (%)[2] | 25 | 50 | 75 | 100 | 100 | 100 | 100 | SEM | Day | Diet | Day× Diet |
| Mixed | 0.26$^{a}$ | 0.78$^{ab}$ | 0.80$^{ab}$ | 3.50$^{c}$ | 2.16$^{bc}$ | 1.29$^{ab}$ | 3.60$^{c}$ | 0.615 | *** | * | *** |
| Concentrate | 0.32$^{a}$ | 0.62$^{a}$ | 0.98$^{a}$ | 2.80$^{b}$ | 4.53$^{bc}$ | 6.46$^{d}$ | 4.61$^{c}$ | | | | |
| Maximum | 0.60 | 2.00 | 3.20 | 9.50 | 11.60 | 15.40 | 10.30 | | | | |

Number of steers = 28.
[1]Day relative to start of 56 day performance period.
[2]Nitrate as percentage of maximum level of intake (100% = 18 g/kg DM).
Within a row, means without a common superscript differ (P<0.05).
*P<0.05; ***P<0.001.

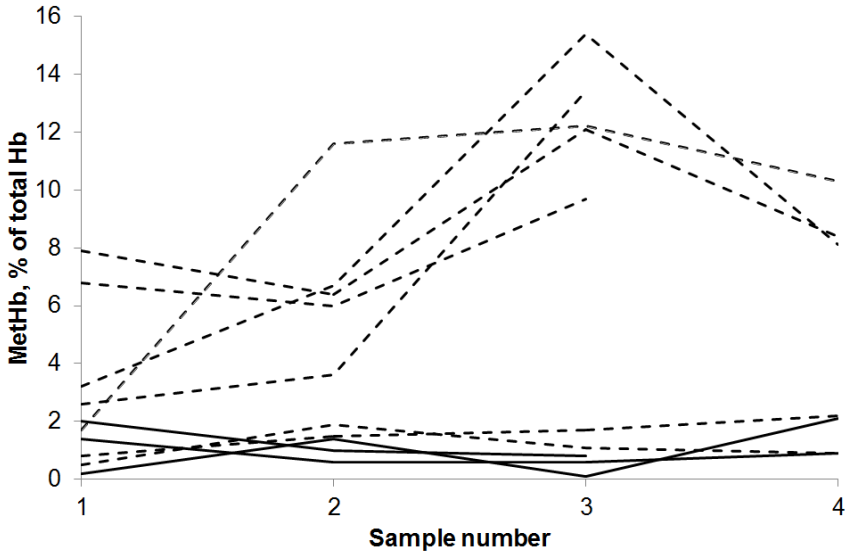


**Figure 6.1** Changes in methaemoglobin (MetHb) concentrations (% total blood Hb) when fed 100% dietary nitrate (18 g nitrate/kg DM) for 5 steers with overall smallest and overall greatest mean MetHb concentrations. Solid lines and dashed lines represent the Mixed and Concentrate basal diets, respectively. Samples 1 to 4 refer to sampling days -6, 8, 87 and 101, respectively. Each line represents an individual animal. Sample 4 was not present for 3 animals because they had been already been sent for slaughter before day 101.

## 6.2 Performance test

Neither age at the start (AgeST) nor Mid-BW differed between basal diets (P>0.05; Table 6.2). Although not significant, the greater ADG in Mixed-fed steers (1.54 *v.* 1.41 kg/d; P>0.05) was associated with greater daily DMI than Concentrate-fed steers (12.0 *v.* 11.0 kg/day; P<0.001). Basal diet did not affect DMI per kg BW (P>0.05). Concentrate-fed steers were more efficient (lower RFI) than Mixed-fed steers (-0.24 *v.* 0.22 kg; P<0.01) due to lower daily DMI. Basal diet did not affect FD1 (P>0.05).

Mid-BW, ADG, DMI and FD1 (kg/day or g/kg BW) did not differ across treatments (P>0.05). An interaction between basal diet and treatment was identified for FCR and RFI (P<0.05). For Concentrate-fed steers, FCR did not differ between RSC and Control treatments (P>0.05). There was, however, a tendency for steers offered Nitrate to have improved (lower) FCR values compared to steers offered the Control (7.40 *v.* 8.17 kg,kg; P=0.07). Similarly Nitrate-fed steers achieved lower RFI values than steers offered the Control treatment but this was not significant (P>0.05). When offered the Mixed basal diet, neither Nitrate nor RSC treatments differed to the Control for FCR or RFI (P>0.05*)*.

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

### 6.3 Carcass characteristics

Carcass traits were not affected by basal diet (Table 6.3), except for fat score (determined by VIA) where Concentrate-fed steers had lower fat scores than Mixed-fed steers (8.02 *v.* 9.08; P<0.001).

There was no difference between treatments for any carcass quality trait other than for FD2, where steers offered the Lipid treatment had greater FD2 compared to the Control treatment (10.07 *v.* 8.48 mm; P<0.05).

Compared to LU steers, CHx steers had lower FD2 (6.99 *v.* 10.79 mm; P<0.001), greater SBW (723 *v.* 701 kg; P=0.051), greater CCW (415 *v.* 369 kg; P<0.001) and greater KO (57.5 *v.* 52.8%; P<0.001). LU steers offered the Concentrate diet had lower CCW than those offered the Mixed diet (357 *v.* 379 kg; P<0.05). For visually assigned EUROP classifications, CHx steers achieved greater conformation grades (9.90 *v.* 8.05; P<0.001) and lower fat grades (9.50 *v.* 11.03; P<0.001) compared to the LU steers which are in agreement with the VIA data. LU steers had greater total fat content (51.4 *v.* 40.4 kg; P<0.01) and lower total meat content (258.8 *v.* 305.7 kg; P<0.001) determined by VIA than CHx steers. There were neither any treatment nor breed × treatment interaction effects for any performance or carcass-related trait (P>0.05).

**Table 6.2.** Effect of breed (B), basal diet (D) and treatment (T) on growth, feed intake and feed efficiency of Charolais-sired (CHx) and purebred Luing (LU) steers fed either a Mixed- or Concentrate-based diet containing one of three treatments: Control, Nitrate or Lipid.

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

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 end of the 56 d test; FCR, feed conversion ratio; RFI, residual feed intake; FD1, fat depth at the 12/13$^{th}$ rib at the end of the 56 d test.

[1]Breed × Diet and Breed × Treatment interaction effects were not significant for all variables (P>0.05).

[2]Diet × Treatment interaction (P<0.05): Concentrate-Nitrate different to Concentrate-RSC (P<0.05); Concentrate-Control different to Concentrate-Nitrate (P=0.07).

[3]Diet × Treatment interaction (P<0.05): Mixed-Nitrate different to Mixed-RSC (P<0.01).

[4]Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable.

NS, not significant; **, P<0.01; ***, P<0.001.

**Table 6.3.** Effect of breed (B), basal diet (D) and treatment (T) on carcass traits of Charolais-sired (CHx) and purebred Luing (LU) steers fed either a Mixed- or Concentrate-based diet containing one of three treatments: Control, Nitrate or Lipid.

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

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

[1]Breed × Treatment and Basal Diet × Treatment interaction effects were not significant for all variables (P>0.05).

[2]Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable.

[3]Breed × Diet interaction (P<0.05): CHx-Concentrate different from LU-Concentrate and LU-Mixed (P<0.001); CHx-Mixed different from LU-Concentrate and LU-Mixed (P<0.001); LU-Mixed different from LU-Concentrate (P<0.01). NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

### 6.4 Methane and hydrogen emissions

During the chamber measurement period, steers offered the Mixed diet tended to have a higher daily DMI than those offered the Concentrate diet (P=0.051, Table 6.4). However, when intake was expressed per kg BW there was no difference between diets (P=0.41). Daily DMI was not affected by breed (P=0.25). However, the LU steers had a higher DMI per kg BW (P<0.05) than the CHx steers. Addition of Nitrate or Lipid to either basal diet did not affect DMI (kg/d, P=0.56 or g/kg BW, P=0.62).

There was no difference in $CH_4$ production between CHx and LU steers, regardless of how $CH_4$ production was expressed (g/d, P=0.73; g/kg DMI, P=0.41; kJ/MJ gross energy intake (GEI), P=0.40, Table 6.4). However, CHx steers produced more $H_2$ than LU steers regardless of how the $H_2$ production was expressed (P<0.001, Table 6.5).

Whether expressed as g/d, g/kg DMI or kJ/MJ GEI, steers fed the Concentrate diet produced less $CH_4$ (P<0.001; Table 6.4) and $H_2$ (P<0.001; Table 6.5) than steers fed the Mixed diet. When $H_2$ production was expressed as a proportion of $CH_4$ production (mol $H_2$/mol $CH_4$), steers fed the Concentrate diet produced a lower proportion of $H_2$ to $CH_4$ than those fed the Mixed diet (P<0.001). Plasma nitrate plus nitrite concentrations in the steers receiving the Control treatments were low and did not differ between the Mixed and Concentrate basal diets (6.0 ± 0.63 and 5.8 ± 0.73 µmol/l, respectively).

The steers receiving the Nitrate treatments produced less daily $CH_4$ than those receiving the Control and Lipid treatments (P<0.05). When expressed as g/kg DMI, there was a diet × treatment interaction (P<0.05); the addition of nitrate to the Mixed basal diet reduced $CH_4$ yield (g/kg DMI) by 17% when compared with the Control treatment (P<0.01); however, the addition of Nitrate to the Concentrate basal diet did not reduce $CH_4$ yield (P=0.65). Nitrate addition to the Mixed basal diet also increased $H_2$ production compared with the Control, whether expressed as g/d, g/kg DMI or kJ/MJ GEI (P<0.001). Again there was a significant diet × treatment interaction (P<0.05), where the addition of nitrate to the Concentrate basal diet did not change $H_2$ production compared with the Control treatment (g/d P=0.40, g/kg DMI P=0.29 and kJ/MJ GEI P=0.26). The addition of nitrate increased the proportion of $H_2$ to $CH_4$ in the Mixed diet, when compared with the Control (P<0.001), however, nitrate addition to the Concentrate diet did not affect the proportion of $H_2$ to $CH_4$ (P=0.23). The addition of nitrate to both diets increased nitrate plus nitrite concentrations in the blood plasma of those steers, and concentrations were greater (P<0.001) in the blood of the steers receiving the Concentrate basal diet (168 ± 24.8 µM) compared with those receiving the Mixed basal diet (58 ± 13.4 µM). Plasma nitrite accounted for only a small proportion of total nitrate plus nitrite in the Nitrate-fed animals which did not differ between Concentrate (1.51 %) and Mixed basal diets (1.56 %).

The addition of RSC to the Mixed diet resulted in a non-significant 7.5 % reduction in $CH_4$ yield when compared with the Control treatment (P=0.18). However, for the Concentrate diet there was no difference in daily $CH_4$ emissions (P=0.84) or $CH_4$ production per kg DMI (P=0.6) between the Lipid and Control treatments. The addition of RSC did not alter the daily $H_2$ production from steers fed the Mixed (P=0.37) or Concentrate diets (P=0.70), nor was the ratio of $H_2$ to $CH_4$ affected when RSC was added to the diets (P=0.70).

**Table 6.4.** Intakes and $CH_4$ production as measured from the respiration chambers (Means with average SEM).

| Basal Diet | Mixed | | | | | | Concentrate | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | | Nitrate | | Lipid | | Control | | Nitrate | | Lipid | | | Significance | | | |
| Breed | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | SEM | Breed | Diet | Treatment | D x T |
| DMI | | | | | | | | | | | | | | | | | |
| kg/d | 10.3 | 9.4 | 9.7 | 11.1 | 10.7 | 10.3 | 9.8 | 10.3 | 8.6 | 9.5 | 9.4 | 10.0 | 0.67 | NS | NS | NS | NS |
| g/kg BW | 14.1 | 13.7 | 13.9 | 15.5 | 15.3 | 14.3 | 14.3 | 15.3 | 12.3 | 14.4 | 13.0 | 15.1 | 0.81 | * | NS | NS | NS |
| $CH_4$ | | | | | | | | | | | | | | | | | |
| g/day[1] | 252 | 232 | 209 | 216 | 246 | 238 | 134 | 164 | 138 | 135 | 158 | 140 | 14.2 | NS | *** | * | NS |
| g/kg DMI[2] | 24.9 | 25.2 | 21.7 | 19.5 | 23.0 | 23.3 | 13.5 | 15.8 | 16.1 | 14.7 | 17.2 | 14.2 | 1.32 | NS | *** | NS | * |
| kJ/MJ GEI | 75.2 | 76.1 | 67.7 | 60.8 | 67.8 | 68.6 | 41.2 | 47.9 | 50.6 | 46.1 | 51.3 | 41.9 | 4.04 | NS | *** | NS | NS |

No. of animals = 75. CHx, Charolais-cross; LU, Luing; D x T, diet x treatment; DMI, dry matter intake; GEI, Gross Energy intake.

[1]Treatment - Nitrate different from Control ($P<0.05$) and Rapeseed Cake ($P<0.05$); [2]D x T - NitMix different from CtrlMix ($P<0.01$).

NS, not significant; *, $P<0.05$;***, $P<0.001$.

**Table 6.5.** Hydrogen production as measured from the respiration chambers (Means with average SEM).

| Basal Diet | Mixed | | | | | | Concentrate | | | | | | | Significance | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | | Nitrate | | Lipid | | Control | | Nitrate | | Lipid | | | | | | |
| Breed | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | SEM | Breed | Diet | Treatment | D x T |
| Hydrogen | | | | | | | | | | | | | | | | | |
| g/day[1] | 0.49 | 0.44 | 1.35 | 1.14 | 0.88 | 0.32 | 0.17 | 0.23 | 0.51 | 0.16 | 0.11 | 0.20 | 0.121 | NS | *** | *** | ** |
| g/kg DMI[2] | 0.048 | 0.047 | 0.145 | 0.101 | 0.085 | 0.031 | 0.016 | 0.022 | 0.060 | 0.015 | 0.012 | 0.020 | 0.013 | * | *** | *** | * |
| kJ/MJ GEI[3] | 0.37 | 0.37 | 1.17 | 0.81 | 0.65 | 0.24 | 0.13 | 0.18 | 0.49 | 0.12 | 0.09 | 0.15 | 0.104 | * | *** | *** | * |
| $H_2$:$CH_4$[4] | 0.016 | 0.016 | 0.054 | 0.041 | 0.030 | 0.011 | 0.008 | 0.011 | 0.027 | 0.009 | 0.006 | 0.012 | 0.005 | NS | *** | *** | * |

CHx, Charolais-cross; LU, Luing; D x T, diet x treatment; GEI, Gross Energy intake; $H_2$:$CH_4$, molar ratio.

[1, 2, 3, 4] Treatment – Nitrate different from Control and Rapeseed Cake, D x T – NitMix different from CtrlMix and RscMix.

NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

## 6.5 Volatile fatty acids

Results are shown for acetic, propionic and butyric acids, i.e. the major constituents of total VFA and are given as VFA molar proportions (moles / mole total VFA), as absolute concentrations were not appropriate because of variable contamination of samples by other fluids during sampling. The glucogenic ratio (GR = (acetate+butyrate)/propionate) was calculated as a summary index of changes in VFA proportions. There were no differences between breeds and, therefore results are presented for the effects of basal diet and treatment.

Before supplementation with Nitrate or Lipid treatments (Table 6.6), and as expected, Concentrate-fed animals had lower proportions of acetate and greater proportions of propionate and hence a lower GR

**Table 6.6**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained before treatments were introduced to the basal diets.

| VFA | Mixed | Concentrate | SED | Significance |
|---|---|---|---|---|
| Acetate | 655 | 494 | 9.4 | *** |
| Propionate | 185 | 352 | 9.1 | *** |
| Butyrate | 118 | 100 | 5.1 | *** |
| GR | 4.0 | 1.8 | 0.13 | *** |

***, P<0.001. GR, Glucogenic ratio.

Seven days after introduction of nitrate and RSC there were differences in VFA proportions (Table 6.7). For the Mixed diet, there was little difference between the Control and Lipid treatments but the GR was higher for the Nitrate treatment than Control. For the Concentrate diet, GR was also higher when nitrate was fed but this was also true for the Lipid compared to Control treatment.

**Table 6.7**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained 7 days after introduction of dietary treatments.

| VFA | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SED | Diet | T | D×T |
| Acetate | 599 | 633 | 613 | 517 | 576 | 599 | 9.8 | *** | *** | ** |
| Propionate | 220 | 193 | 216 | 335 | 266 | 249 | 9.6 | *** | *** | *** |
| Butyrate | 130 | 131 | 124 | 101 | 94 | 85 | 5.3 | *** | NS | NS |
| GR | 3.5 | 4.3 | 3.6 | 2.1 | 2.7 | 2.9 | 0.17 | *** | *** | *** |

T, treatment; D×T, diet × treatment interaction. GR, Glucogenic ratio.
NS, not significant; **, P<0.01; ***, P<0.001.

At the start of the 56-d performance test period, differences between Mixed and Concentrate basal diets were maintained (Table 6.8). Adding Nitrate to the diets had little effect on VFA whereas adding Lipid (as RSC) increased the molar proportion of propionate but reduced the molar proportion of acetate and therefore reduced GR.

**Table 6.8**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained at the beginning of the 56 day test period after adaptation to treatments.

| VFA | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SED | Diet | T | D×T |
| Acetate | 698 | 666 | 651 | 508 | 541 | 474 | 11.1 | *** | *** | * |
| Propionate | 161 | 164 | 200 | 355 | 319 | 405 | 15.5 | *** | *** | NS |
| Butyrate | 100 | 129 | 113 | 95 | 98 | 81 | 7.1 | *** | * | ** |
| GR | 4.6 | 4.4 | 3.5 | 2.5 | 2.8 | 1.8 | 0.29 | *** | *** | NS |

T, treatment; D×T, diet × treatment interaction. GR, Glucogenic ratio.
NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

At the mid-point of the 56-d performance test period, differences between treatments were much less marked and non-significant (Table 6.9).

**Table 6.9**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained at the mid-point of the 56 day test period after adaptation to treatments.

| VFA | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SED | Diet | T | D×T |
| Acetate | 650 | 671 | 641 | 513 | 532 | 493 | 14.4 | *** | NS | NS |
| Propionate | 221 | 202 | 232 | 302 | 336 | 340 | 18.6 | *** | NS | NS |
| Butyrate | 120 | 116 | 116 | 97 | 83 | 92 | 6.1 | *** | NS | NS |
| GR | 3.6 | 4.1 | 3.3 | 2.7 | 2.4 | 2.4 | 0.27 | ** | NS | NS |

T, treatment; D×T, diet × treatment interaction. GR, Glucogenic ratio.
NS, not significant; **, P<0.01; ***, P<0.001.

At the end of the 56 day period (Table 6.10), rumen samples from Mixed-fed animals receiving Nitrate contained higher acetate and lower propionate molar proportions than Control samples and therefore a higher GR. The same was true for the Concentrate diet but differences were smaller.

**Table 6.10**. Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained at the end of the 56 day test period.

| VFA | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SED | Diet | T | D×T |
| Acetate | 527 | 650 | 520 | 474 | 523 | 473 | 12.4 | *** | *** | ** |
| Propionate | 288 | 175 | 298 | 399 | 333 | 374 | 18.7 | *** | *** | NS |
| Butyrate | 132 | 136 | 132 | 88 | 93 | 103 | 11.1 | *** | NS | NS |
| GR | 2.5 | 4.7 | 2.3 | 1.5 | 2.0 | 1.7 | 0.24 | *** | *** | *** |

T, treatment; D×T, diet × treatment interaction. GR, Glucogenic ratio.
NS, not significant; **P<0.01; ***P<0.001.

Rumen samples taken after steers left the respiration chambers (Table 6.11), exhibited greater acetate molar proportions and lower propionate proportions when Nitrate was fed but the differences were less marked than those measured in samples at the end of the 56 day test period (Table 6.10).

**Table 6.11.** Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained when steers left respiration chambers.

| VFA | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SED | Diet | T | D×T |
| Acetate | 638 | 669 | 646 | 536 | 597 | 552 | 12.4 | *** | *** | NS |
| Propionate | 208 | 168 | 196 | 325 | 253 | 295 | 19.0 | *** | * | NS |
| Butyrate | 109 | 123 | 111 | 90 | 105 | 89 | 9.1 | NS | NS | NS |
| GR | 4.0 | 5.0 | 4.0 | 2.2 | 3.3 | 2.4 | 0.37 | *** | ** | NS |

T, treatment; D×T, diet × treatment interaction. GR, Glucogenic ratio.
NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

Finally, Table 6.12 gives VFA proportions in rumen samples taken at slaughter. GR of steers receiving Nitrate was greater than for Control steers with Lipid having little effect.

**Table 6.12.** Volatile fatty acid (VFA) molar proportions in rumen samples (mmol / mol) obtained when steers were slaughtered.

| VFA | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SED | Diet | T | D×T |
| Acetate | 661 | 656 | 652 | 542 | 558 | 547 | 13.5 | *** | NS | NS |
| Propionate | 190 | 185 | 198 | 301 | 264 | 319 | 15.6 | *** | NS | NS |
| Butyrate | 103 | 121 | 107 | 96 | 119 | 69 | 7.2 | ** | *** | * |
| GR | 4.1 | 4.3 | 4.0 | 2.2 | 2.8 | 2.2 | 0.21 | *** | * | NS |

T, treatment; D×T, diet × treatment interaction. GR, Glucogenic ratio.
NS, not significant; *, P<0.05; **, P<0.01; ***, P< 0.001.

The objective of taking repeated rumen samples was to investigate how rumen fermentation changed with time. To test if any changes resulting from imposing the treatments were dependant on the rumen fermentation established in individual animals prior to this, GR in rumen samples taken prior to imposing treatments was tested as a covariate. From seven days (P<0.001) after introducing Nitrate and Lipid to the mid-point of the 56 day test period (P<0.01), pre-treatment GR was a significant covariate and thereafter became non-significant.

Changes in GR over the period from 7 days after introducing Nitrate and Lipid until the end of the 56 day test periods are shown in Figures 6.2 and 6.3. There were significant changes in GR with time (P<0.001) and the response was dependant on both basal diet (interaction, P<0.001) and treatment (interaction, P=0.004). On the Mixed basal diet, Control and Lipid GR were similar across sampling times and in particular were lower at the end of the 56-d test period; GR for Nitrate-fed animals was consistently greater than Controls and did not decline at the end of the 56-d test period. On the

Concentrate basal diets, overall, there was less variation between diets and sampling times than for the Mixed diet.

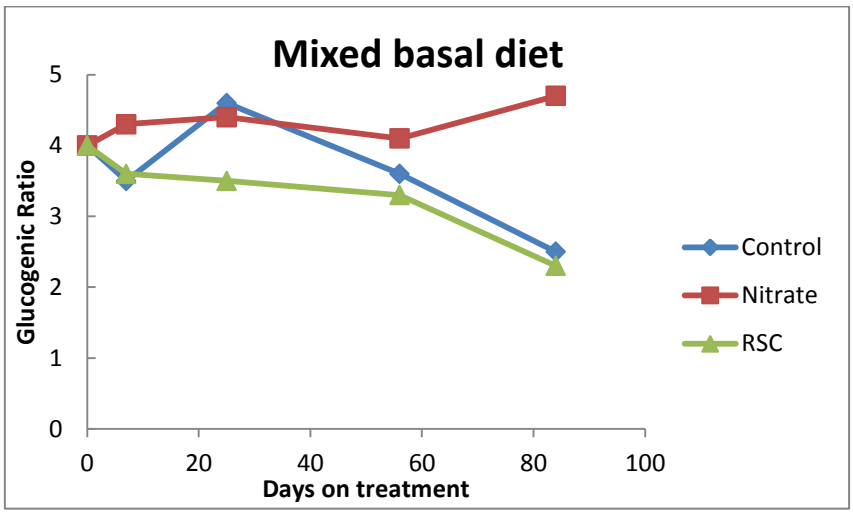


**Figure 6.2:** Changes in glucogenic ratio from introduction of Nitrate and Lipid (RSC) to diets until end of 56 day period for steers given the Mixed basal diet.

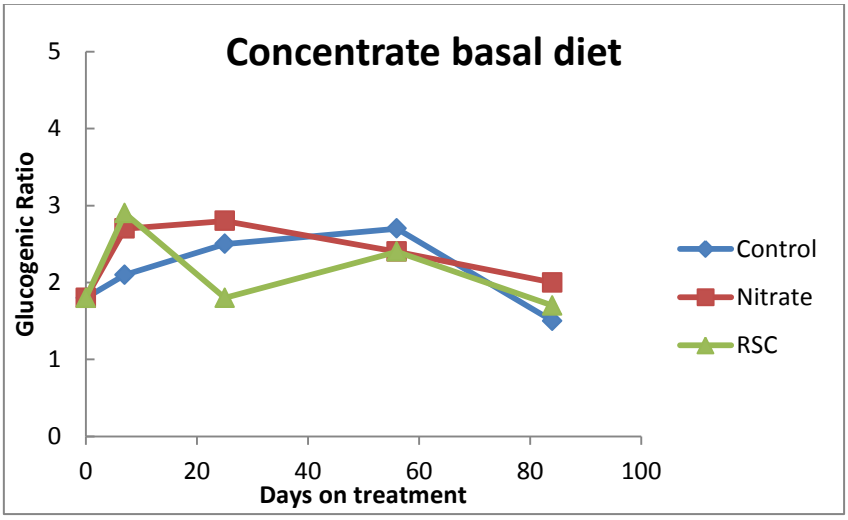


**Figure 6.3:** Changes in glucogenic ratio from introduction of Nitrate and Lipid (RSC) to diets until end of the 56 day test period for steers given the Concentrate basal diet.

Because rumen samples from the chamber period were taken weekly over a 13 week period and from slaughtered animals on four separate occasions, the effect of sample week and of slaughter date were included in statistical analysis; neither sample week (chamber animals) or slaughter date significantly affected VFA proportions suggesting that rumen fermentation was stable over the 13 week period after completion of the 56 day test period. Furthermore, there were no differences between VFA proportions or GR between samples taken when animals left chambers or were obtained at slaughter. Differences between 56 d test period and the chamber period were assessed by comparing mean values for the start, mid and end 56 d samples with chamber measurements. GR in the 56-d period was significantly ($P<0.001$) lower (2.9) than that from chamber samples (3.4) because acetate molar proportions were less (561 *v.* 603, $P<0.001$) and propionate proportions greater (275 *v.* 245, $P=0.003$) during the 56-d period.

### 6.6. Rumen microbial analysis

Initial plans were to measure the abundance of archaea and total bacteria in all rumen samples taken (from 72 animals at seven sample points). Initial analyses were completed on 32 animals receiving the Control and Nitrate treatments for both Concentrate and Mixed diets for all sample points. Analysis and conclusions from these data are given in section 6.6.1. At this point, it was decided that with resources available it would be more informative to investigate why there were differences in the effectiveness of nitrate in reducing $CH_4$ on the Concentrate and Mixed basal diets. Analysis and conclusions from these data are given in section 6.6.2.

#### 6.6.1. Changes in microbial populations with time.

No differences were noted between breeds of cattle at any sample time.

There were no differences between the Concentrate and Mixed diets in numbers of archaea (Table 6.13). However, bacteria numbers were greater and therefore the ratio of archaea to bacteria smaller for the Concentrate diet. As expected, since treatments had not been included in the diet at this time, there were no differences as a result of Nitrate addition.

**Table 6.13.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in Preliminary samples.

| Diet | Mixed | | Concentrate | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Control | Nitrate | SED | Diet | Nitrate | D × N |
| Archaea | 10.4 | 12.0 | 11.9 | 10.1 | 1.87 | NS | NS | NS |
| Bacteria | 575 | 660 | 1113 | 1385 | 128 | *** | NS | NS |
| A: B | 20.1 | 19.5 | 10.4 | 8.7 | 2.51 | ** | NS | NS |

NS, not significant P>0.05; **, P<0.01; ***, P<0.001.
D × N, Diet × Nitrate interaction.

The populations of archaea and bacteria in the Preliminary samples were tested as a covariate in analysis of subsequent samples and as covariates were significant in most cases. Thus these data were included as covariates in all subsequent analyses.

After 7 days of feeding, samples from Nitrate-fed cattle had greater bacteria numbers than Control animals (Table 6.14). There were no differences in numbers of archaea.

**Table 6.14.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in Adaptation samples.

| Diet | Mixed | | Concentrate | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Control | Nitrate | SED | Diet | Nitrate | D × N |
| Archaea | 11.3 | 10.1 | 8.5 | 10.8 | 1.68 | NS | NS | NS |
| Bacteria | 843 | 1037 | 1104 | 1251 | 128 | * | * | NS |
| A: B | 15.1 | 11.0 | 9.5 | 10.3 | 2.47 | NS | NS | NS |

NS, not significant, P>0.05; * P<0.05.
D × N, Diet × Nitrate interaction.
Preliminary samples were significant covariates: archaea, P=0.015; bacteria, P<0.001; A:B, P<0.001.

The numbers of archaea decreased (Table 6.15), bacteria increased and thus the ratio of archaea to bacteria decreased over the 56 d test period. Therefore microbial populations were not stable over the 56-d test period.

**Table 6.15.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) at start, middle and end of 56-d period.

| Sample | Start | Mid | End | SED | Significance |
|---|---|---|---|---|---|
| Archaea | 11.1 | 10.6 | 7.6 | 1.26 | * |
| Bacteria | 966 | 1019 | 1239 | 150.2 | ** |
| A:B | 15.1 | 12.0 | 8.1 | 1.29 | *** |

* P < 0.05, **, P < 0.01; ***, P < 0.001.

Overall, during the 56 d test period, there were no differences in numbers of archaea as a result of basal diet or addition of nitrate (Table 6.16). However, numbers of total bacteria were greater when the Concentrate diet was fed.

**Table 6.16.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in 56-d samples.

| Diet | Mixed | | Concentrate | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Control | Nitrate | SED | Diet | Nitrate | D × N |
| Archaea | 10.4 | 8.7 | 12.4 | 7.7 | 1.99 | NS | NS | NS |
| Bacteria | 920 | 758 | 1262 | 1358 | 150 | *** | NS | NS |
| A: B | 14.4 | 12.2 | 12.2 | 8.1 | 2.13 | NS | NS | NS |

NS, not significant P>0.05; ***, P<0.001.
D × N, Diet × Nitrate interaction.
Preliminary samples significant covariate: archaea, P=0.014; bacteria, NS; A:B, P<0.001.

Because either the diet (Adaptation *v.* other samples), the time of day at which animals were sampled (Adaptation and 56-d performance test *v.* Chamber and Slaughter samples) and method of sampling (Slaughter *v.* other samples) differed between samples, differences between sample types compared mean values from 56-d performance test samples with individual values for Adaptation, Chamber and Slaughter samples.

There were no interactions between sample type and either diet or treatment. Therefore results in Tables 6.17 (longitudinal sample type) and 6.18 (diet and treatment) are presented as main effects.

**Table 6.17.** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in different longitudinal samples.

| Sample | Prelim | 56-d | Chamber | Slaughter | SED | Significance |
|---|---|---|---|---|---|---|
| Archaea | 10.2 | 10.1 | 13.5 | 26.1 | 1.61 | *** |
| Bacteria | 1047 | 1085 | 860 | 1077 | 57.9 | *** |
| A:B | 11.5 | 11.9 | 19.5 | 28.2 | 2.22 | *** |

***, P<0.001.

The main difference between the samples was that the numbers of archaea in the Chamber and in particular the Slaughter samples were greater than in the Prelim or 56-d samples between which there was little difference. As a result a similar pattern was seen in the A:B ratios.

**Table 6.18** Archaea and total bacteria (copy number / ng DNA x $10^{-3}$) and ratio of archaea to bacteria (A:B, archaea x 1000 / bacteria) in all samples.

| Diet | Mixed | | Concentrate | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Control | Nitrate | SED | Diet | Nitrate | D × N |
| Archaea | 16.2 | 14.0 | 16.3 | 13.4 | 2.12 | NS | NS | NS |
| Bacteria | 887 | 837 | 1105 | 1239 | 112 | *** | NS | NS |
| A: B | 22.3 | 19.4 | 17.1 | 12.3 | 3.61 | NS | NS | NS |

NS, not significant P > 0.05; ***, P < 0.001.
D × N, Diet × Nitrate interaction.
Preliminary samples significant covariate: archaea, P = 0.07; bacteria, P = 0.02; A:B, NS.

Overall, the main difference as a result of treatments imposed was that total bacteria numbers were greater in Concentrate-fed animals. Numerically, archaea and A:B ratio were lower in Nitrate-fed animals and A:B ratio greater in Mixed-fed animals.

### 6.6.2. Quantification of microbial species implicated in nitrate reduction in the rumen

The main finding from the Evaluation study was that while Nitrate added to the Mixed diet reduced $CH_4$ emissions by approximately 20%, on the Concentrate diet Nitrate did not reduce $CH_4$ emissions. A literature review identified that the main bacterial species known to reduce nitrate within the rumen are: *Selenomonas ruminantium, Veillonella parvula* and *Wolinella succinogenes.* There is also evidence that protozoa may have the ability to reduce nitrate. Therefore the populations of these groups were quantified by qPCR in rumen samples taken when cattle left the respiration chambers (Chamber samples) for the Control and Nitrate treatments on both Concentrate and Mixed diets.

The copy numbers for *Wolinella* and *Veillonella* were below the limits of detection of the assay. The numbers of archaea and protozoa were greater while the numbers of *Selenomonas* were less on the Mixed diet (Table 6.19). There were also changes in microbial species potentially relevant to nitrate reduction when nitrate was added to the diets. The numbers of protozoa (copy number basis) significantly increased and those of *Selenomonas* tended to decrease (ratio to total bacteria) when nitrate was added to the diet.

**Table 6.19.** Microbial species in different samples in Chamber samples.

| Diet | Mixed | | Concentrate | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Control | Nitrate | SEM | Diet | Nitrate | D × N |
| Copy number / ng DNA x $10^{-3}$ | | | | | | | | |
| Archaea | 16.3 | 13.4 | 9.4 | 12.2 | 2.3 | 0.09 | NS | NS |
| Protozoa | 50.3 | 85.3 | 12.2 | 33.4 | 10.3 | *** | ** | NS |
| Total Bacteria | 631 | 574 | 585 | 1201 | 98 | ** | ** | ** |
| *Selenomonas* | 47 | 25 | 74 | 107 | 14.6 | *** | NS | NS |
| *Wolinella* | ND | ND | ND | ND | | | | |
| *Veillonella* | ND | ND | ND | ND | | | | |
| Ratio (/total bacteria) | | | | | | | | |
| Archaea | 0.032 | 0.024 | 0.021 | 0.011 | 0.005 | * | NS | NS |
| Protozoa | 0.100 | 0.170 | 0.032 | 0.042 | 0.231 | *** | NS | NS |
| *Selenomonas* | 0.077 | 0.040 | 0.115 | 0.090 | 0.017 | * | NS | NS |

NS, not significant P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001.
ND, below limits of detection.

For the Concentrate and Mixed diets, the changes in microbial numbers are consistent with the lower $CH_4$ emissions on the Concentrate diet. Thus, the numbers of protozoa ($H_2$ producers) and archaea ($CH_4$ producers) were lower on the Concentrate diet.

In respect of microbial numbers, the differences between the Concentrate and Mixed diets in the ability of Nitrate to reduce $CH_4$ are more difficult to interpret. Organisms known to be able to reduce nitrate were most abundant (protozoa and *Selenomonas*) on the Concentrate-Nitrate treatment (where no reduction in $CH_4$ production was observed) compared to the Concentrate-Control diet. This is opposite to what might have been expected if the ability to reduce nitrate was the factor limiting the reduction in $CH_4$ on this treatment. Measurement of nitrate reductase gene copy numbers in these samples (in progress) may help to resolve this contradiction. However, *Selenomonas* uses NADH rather than $H_2$ as the main electron donor. Therefore any nitrate reduced by *Selenomonas* will not result in a concomitant reduction in $CH_4$. The net effect of the increase in *Selenomonas* numbers on the Concentrate diet may therefore be a reduction in the amount of nitrate available for $H_2$ producers and therefore the potential for $CH_4$ reduction. This may in part explain the lack of effectiveness of nitrate in reducing $CH_4$ on the Concentrate diet.

## 6.7 Meat quality

### 6.7.1 Vitamin E content

Tables 6.20a to 6.20c show the Vitamin E content of loin muscle steaks: a) by breed; b) by basal diet type and c) by individual diets. Loin steaks from LU steers had significantly (P=0.012) more Vitamin E than those from CHx steers (Table 6.20a). This is probably because the steaks from LU cattle had greater fat content, in which Vitamin E is soluble. In such steaks, the concentration of fat to lean tissue is greater by a factor of about 5:1.

**Table 6.20a.** Effect of breed on Vitamin E content (ug/g) of loin muscle steaks.

| Breed | Count | Mean | SD |
|---|---|---|---|
| CHx | 38 | 1.99 | 0.649 |
| LU | 38 | 2.44 | 0.862 |
| Significance | | * | |

CHx, crossbred Charolais; LU, Luing; *, P<0.05.

Loin steaks from Mixed-fed animals had greater vitamin E than those from the Concentrate-fed animals because green forage has greater natural vitamin E than cereal grains (Table 6.20b). This was also apparent for all the individual diets where all Mixed-based diets produced more vitamin E in the loin steaks than the Concentrate-based diets (Table 6.20c).

**Table 6.20b.** Effect of basal diet type on Vitamin E content (ug/g) of loin muscle steaks.

| Diet | Count | Mean | SD |
|---|---|---|---|
| Mixed | 37 | 2.78 | 0.655 |
| Concentrate | 39 | 1.68 | 0.469 |
| Significance | | *** | |

***, P<0.001.

**Table 6.20c.** Effect of diet × treatment on Vitamin E content (ug/g) of loin muscle steaks.

| Basal Diet | Treatment | Count | Mean | SD |
|---|---|---|---|---|
| Mixed | Control | 13 | 2.65$^{c}$ | 0.543 |
| | Nitrate | 13 | 2.59$^{c}$ | 0.493 |
| | Lipid | 13 | 3.13$^{d}$ | 0.803 |
| Concentrate | Control | 13 | 1.35$^{a}$ | 0.308 |
| | Nitrate | 13 | 1.67$^{ab}$ | 0.446 |
| | Lipid | 13 | 2.03$^{b}$ | 0.391 |
| | Significance | | | *** |

***, P<0.001; means without a common superscript differ.

From comparing the Mixed-fed steers, those supplemented with RSC produced a significantly greater (P<0.001) concentration of Vitamin E in the loin steaks compared to those receiving the Control or Nitrate treatments. For the Concentrate-fed steers, there was only a statistically significant difference (P<0.001) in Vitamin E between the Lipid and Control treatments, with the Nitrate treatment being intermediate (Table 6.20c). It has been suggested that Vitamin E as an antioxidant modulates microbial lipid metabolism in the rumen (Bauchart *et al.*, 2005). But in turn rapeseed oil may also increase the stability of ingested Vitamin E in the rumen.

### 6.7.2 Colour shelf life

Figure 6.4 shows the effect of diet treatment on the colour chroma of loin steaks.

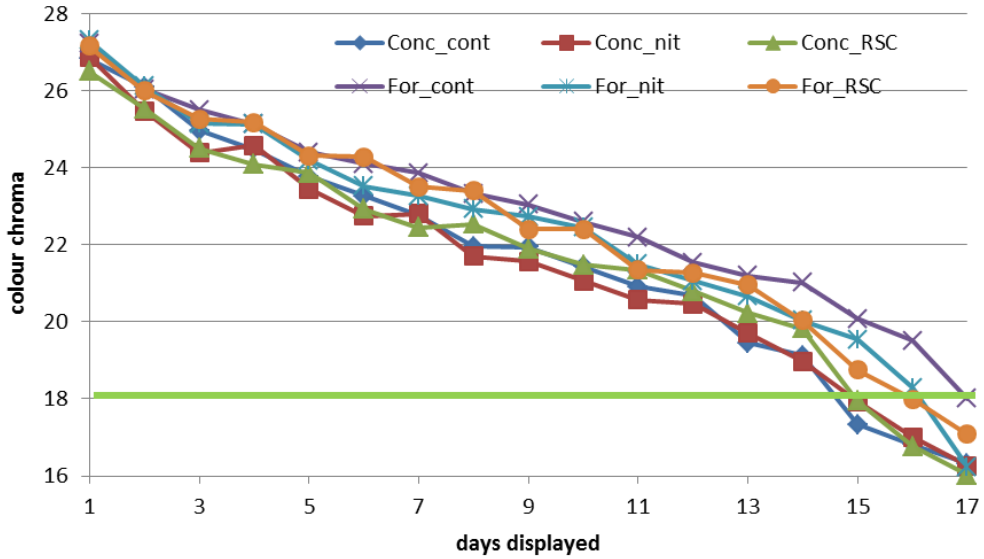


**Figure 6.4:** The effect of diet on the colour chroma of loin steaks displayed in high oxygen modified atmosphere packs. Conc, Concentrate diet; For, Mixed diet; Nit, Nitrate; RSC, Lipid; Cont, Control.

Whilst the Mixed-Control fed animals produced loin steaks that had a 17 day colour shelf life, those fed Mixed-Nitrate or Mixed-Lipid had a colour shelf life of 16 days (Figure 6.4). All those fed Concentrate-based diets had a colour shelf life of 15 days. This colour stability is in line with the Vitamin E values, except that they do not differentiate the higher concentration in the animals supplemented with RSC.

### 6.7.3 Nitrate and nitrite levels in the meat

Initial results for nitrate analysis revealed inconsistent and lower than expected results for quality control samples submitted for analysis and therefore these results were considered unreliable. On re-test by a different laboratory, more consistent results were obtained but samples submitted were too few to make any conclusions concerning effects of nitrate. Overall mean nitrate concentration (n=8) was 32 (SD 2.3) mg nitrate / kg sample.

### 6.7.4 Sensory taste panel assessment

Tables 6.21 and 6.22 show the effect of treatment and breed, respectively, on eating quality parameters. There was no significant effect of treatment on sensory attributes of loin muscle. The LU breed produced loin meat which was more tender, juicy and of higher flavour than loin meat from the CHx steers. Consequently the flavour of the LU meat was preferred and they were more liked overall.

**Table 6.21.** Effect of treatment on eating quality of grilled beef steak (using the 8 point category scales).

| Treatment<br>Attributes | Control | Nitrate | Lipid | P | Significance |
|---|---|---|---|---|---|
| Tenderness | 5.52 | 5.41 | 5.6 | 0.136 | NS |
| Juiciness | 5.31 | 5.27 | 5.38 | 0.251 | NS |
| Beef Flavour | 4.44 | 4.44 | 4.49 | 0.706 | NS |
| Abnormal Flavour | 2.17 | 2.20 | 2.26 | 0.559 | NS |
| Hedonic | | | | | |
| Flavour Liking | 5.48 | 5.49 | 5.46 | 0.932 | NS |
| Overall Liking | 5.36 | 5.31 | 5.32 | 0.838 | NS |

NS, not significant P>0.05.

**Table 6.22**. Effect of breed on eating quality of grilled beef steak (using the 8 point category scales).

| Treatment<br>Attributes | CHx | LU | P | Sig. | SED |
|---|---|---|---|---|---|
| Tenderness | 5.10 | 5.92 | <0.001 | *** | 0.069 |
| Juiciness | 5.19 | 5.45 | <0.001 | *** | 0.056 |
| Beef Flavour | 4.36 | 4.56 | <0.001 | *** | 0.052 |
| Abnormal Flavour | 2.34 | 2.08 | <0.001 | *** | 0.061 |
| Hedonic | | | | | |
| Flavour Liking | 5.22 | 5.73 | <0.001 | *** | 0.060 |
| Overall Liking | 5.00 | 5.65 | <0.001 | *** | 0.061 |

***, P<0.001.

The effects of basal diet type (Concentrate or Mixed) and treatment, respectively, on eating quality parameters are presented in Tables 6.23 and 6.24. The Mixed-fed animals were less tender than the Concentrate-fed animals and although the flavour was not significantly different, the panel preferred the flavour of the Mixed-fed animals and liked them best overall. Therefore, flavour out-weighed tenderness, or they were both sufficiently tender, flavour predominated.

**Table 6.23.** Effect of basal diet on eating quality of grilled beef steak (using the 8 point category scales).

| Treatment | Mixed | Concentrate | P | Sig. | SED |
|---|---|---|---|---|---|
| Attributes | | | | | |
| Tenderness | 5.36 | 5.66 | <0.001 | *** | 0.078 |
| Juiciness | 5.35 | 5.29 | 0.262 | NS | |
| Beef Flavour | 4.50 | 4.42 | 0.097 | NS | |
| Abnormal Flavour | 2.14 | 2.28 | 0.028 | * | |
| Hedonic | | | | | |
| Flavour Liking | 5.57 | 5.38 | 0.004 | ** | 0.064 |
| Overall Liking | 5.40 | 5.26 | 0.048 | * | 0.067 |

NS, not significant P > 0.05; * P < 0.05, **, P < 0.01; ***, P < 0.001.

**Table 6.24.** Effect of basal diet and treatment on eating quality of grilled beef steak (using the 8 point category scales).

| | Mixed | | | Concentrate | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Control | Nitrate | Lipid | P | Sig. | SED |
| Attributes | | | | | | | | | |
| Tenderness | 5.49$^{ab}$ | 5.25$^{b}$ | 5.34$^{b}$ | 5.56$^{ab}$ | 5.57$^{ab}$ | 5.85$^{a}$ | <0.001 | *** | 0.135 |
| Juiciness | 5.35 | 5.34 | 5.36 | 5.26 | 5.19 | 5.41 | 0.307 | NS | |
| Beef Flavour | 4.51 | 4.43 | 4.57 | 4.38 | 4.46 | 4.41 | 0.278 | NS | |
| Abnormal Flavour | 2.11 | 2.20 | 2.11 | 2.24 | 2.19 | 2.40 | 0.073 | NS | |
| Hedonic | | | | | | | | | |
| Flavour Liking | 5.62 | 5.50 | 5.58 | 5.34 | 5.47 | 5.33 | 0.047 | NS | |
| Overall liking | 5.48 | 5.34 | 5.36 | 5.23 | 5.28 | 5.28 | 0.343 | NS | |

$^{ab}$ values with different superscripts are significantly different P<0.05.
NS, not significant; ***, P<0.001.

### 6.7.5 Fatty acids

Table 6.25 shows that the M. *longissimus thoracis* (LT) from the LU had significantly more fat than the LT from the CHx, which was reflected in the sum of individual groupings, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Whilst the LU had higher proportions of SFA and MUFA than CHx, this was reversed for PUFA, where CHx had higher proportions of PUFA than LU (though less total amount). The reason for this is illustrated in Figure 6.5. As animals get fatter it is mainly through the deposition of SFA and MUFA in intramuscular fat (IMF).

Important ratios such as P:S and n-6 to n-3 fatty acids were also significantly different between breed. Whilst some 18:2n-6 is deposited in IMF, more 18:3n-3 is deposited in phospholipids than IMF. Hence the fatter LU have poorer P:S and n-6:n-3 ratios than the CHx. A desirable P:S ratio is > 0.4 and neither CHx or LU meet this criteria. Figure 6.6 shows how, as animals get fatter, then the P:S ratio declines.

**Table 6.25.** Effect of breed on the sum and ratios of important groups of fatty acids in *M. longissimus thoracis.*

| Fatty acid | Breed | Mean | SEM | Significance |
|---|---|---|---|---|
| mg/100g lean | | | | |
| Total FA | CHx | 3205 | 273.1 | *** |
| | LU | 5832 | | |
| SFA | CHx | 1353 | 127.2 | *** |
| | LU | 2556 | | |
| MUFA | CHx | 1373 | 126.9 | *** |
| | LU | 2599 | | |
| PUFA | CHx | 193 | 4.7 | ** |
| | LU | 216 | | |
| n-6 PUFA | CHx | 163 | 4.1 | ** |
| | LU | 179 | | |
| n-3 PUFA | CHx | 29.0 | 0.81 | *** |
| | LU | 37.0 | | |
| | | | | |
| Proportions (g/100g fatty acids) | | | | |
| SFA % | CHx | 41.7 | 0.44 | ** |
| | LU | 43.5 | | |
| MUFA % | CHx | 42.5 | 0.38 | ** |
| | LU | 44.5 | | |
| PUFA % | CHx | 6.61 | 0.270 | *** |
| | LU | 3.98 | | |
| n-6 PUFA % | CHx | 5.62 | 0.232 | *** |
| | LU | 3.31 | | |
| n-3 PUFA % | CHx | 0.99 | 0.040 | *** |
| | LU | 0.67 | | |
| | | | | |
| Ratios | | | | |
| P:S ratio | CHx | 0.10 | 0.005 | *** |
| | LU | 0.06 | | |
| C18:2n-6 to 18:3n-3 | CHx | 11.01 | 0.206 | *** |
| | LU | 7.80 | | |
| n-6:n-6 ratio | CHx | 5.83 | 0.102 | *** |
| | LU | 5.05 | | |

CHx, crossbred Charolais; LU, Luing; **, P<0.01; ***, P<0.001

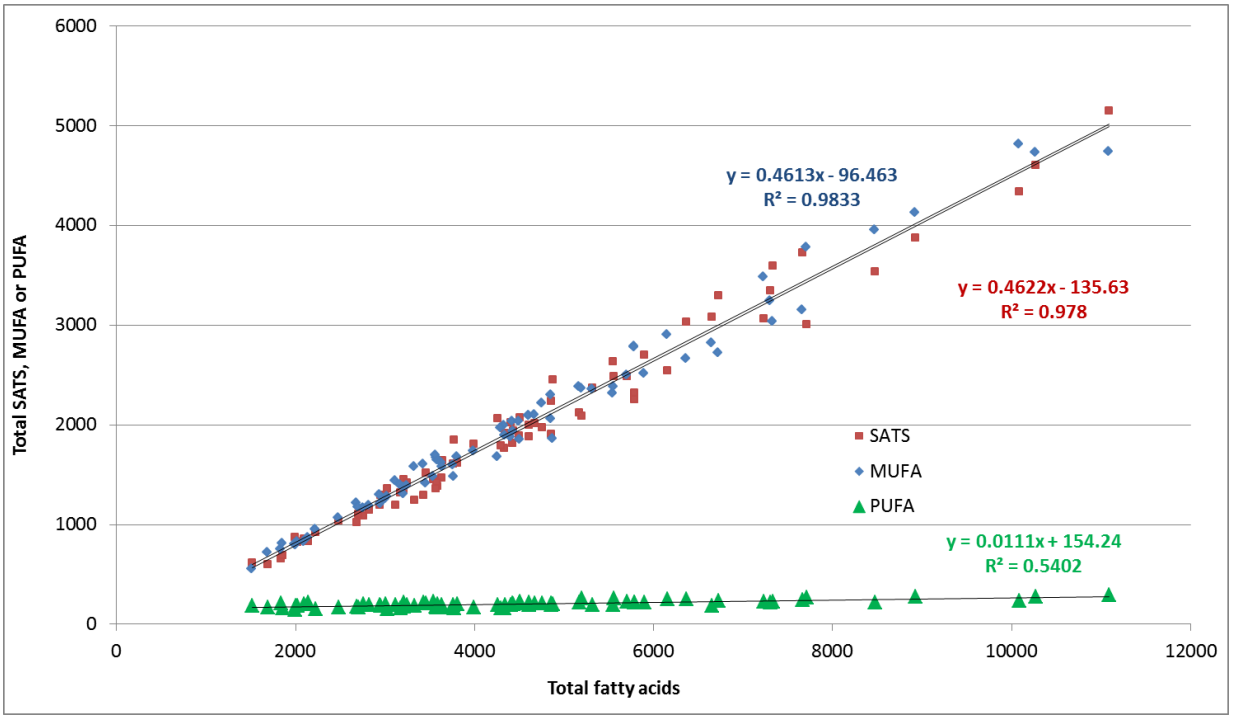


**Figure 6.5:** The relationship between total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids and total fat (mg/100g lean) in beef M. *longissimus thoracis*.

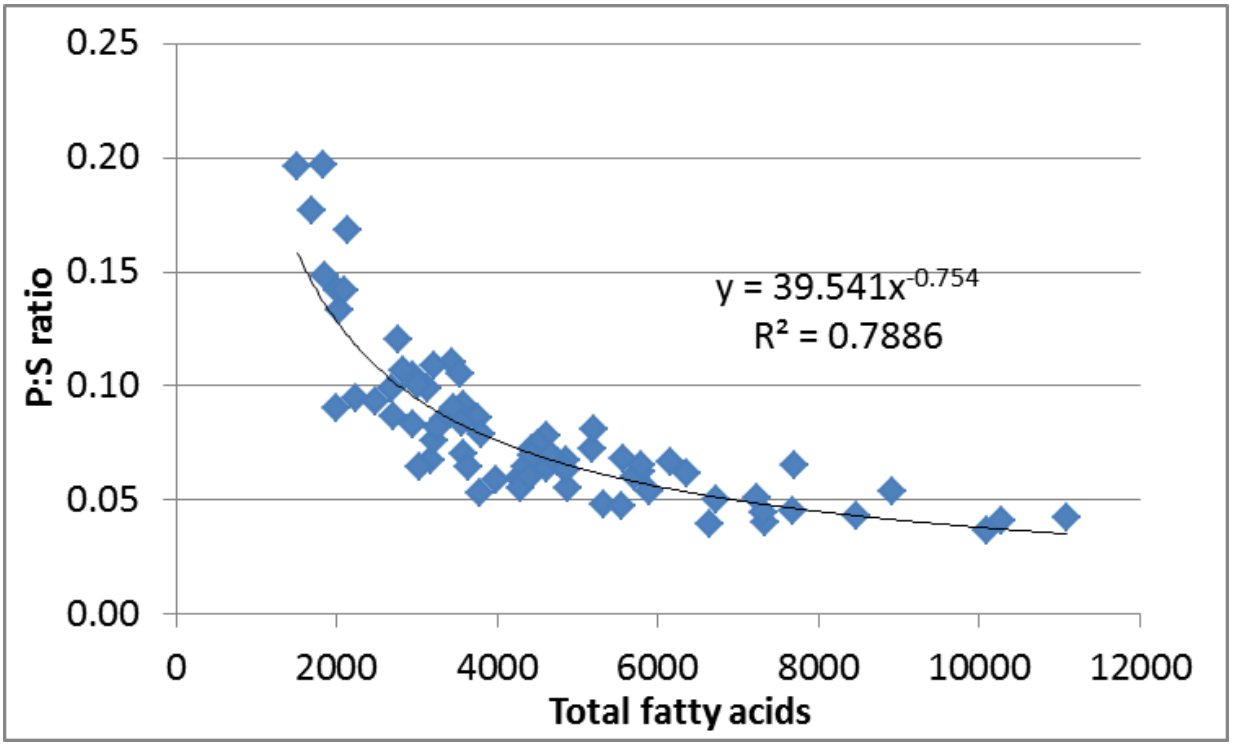


**Figure 6.6:** The relationship between P:S ratio and total fat content (mg/100g lean) in beef M. *longissimus thoracis*.

Table 6.26 shows the effect of breed on individual fatty acids with many being significantly higher in concentration in the LU due to the total greater fatty acid concentration.

**Table 6.26.** Effect of breed on the amount of individual fatty acids (mg/100g lean) in *M. longissimus thoracis.*

| Fatty acid | Breed | Mean | SEM | Significance |
|---|---|---|---|---|
| 14:00 | CHx | 89.9 | 10.22 | *** |
| | LU | 177.3 | | |
| 15:00 | CHx | 11.9 | 1.35 | *** |
| | LU | 22.8 | | |
| 16:00 | CHx | 805 | 76.7 | *** |
| | LU | 1547 | | |
| 16DMA ald | CHx | 35.0 | 0.68 | NS |
| | LU | 35.7 | | |
| 16:01 | CHx | 120 | 12.0 | *** |
| | LU | 233 | | |
| 17:00 | CHx | 35.0 | 3.63 | *** |
| | LU | 64.2 | | |
| 18:00 | CHx | 454 | 42.0 | *** |
| | LU | 827 | | |
| 18DMA ald | CHx | 20.8 | 0.39 | * |
| | LU | 22.1 | | |
| tr18:1n-7 | CHx | 49.5 | 4.72 | *** |
| | LU | 88.7 | | |
| 18:1n-9 | CHx | 1145 | 107.4 | *** |
| | LU | 2183 | | |
| 18:1n-7 | CHx | 52.5 | 3.57 | *** |
| | LU | 83.6 | | |
| 18:2n-6 | CHx | 109.3 | 3.59 | *** |
| | LU | 127.3 | | |
| 20:1n-9 | CHx | 5.21 | 0.608 | *** |
| | LU | 9.98 | | |
| 18:3n-3 | CHx | 10.9 | 0.69 | *** |
| | LU | 17.8 | | |
| 9c11tCLA | CHx | 8.1 | 1.08 | *** |
| | LU | 18.8 | | |
| 20:3n-6 | CHx | 11.46 | 0.308 | NS |
| | LU | 11.78 | | |
| 20:4n-6 | CHx | 36.83 | 0.589 | *** |
| | LU | 33.74 | | |
| 20:4n-3 | CHx | 1.06 | 0.057 | *** |
| | LU | 1.33 | | |
| 20:5n-3 | CHx | 4.18 | 0.118 | NS |
| | LU | 3.97 | | |
| 22:4n-6 | CHx | 5.92 | 0.163 | * |
| | LU | 6.41 | | |
| 22:5n-3 | CHx | 11.57 | 0.156 | *** |
| | LU | 12.67 | | |
| 22:6n-3 | CHx | 1.35 | 0.049 | NS |
| | LU | 1.29 | | |

CHx, crossbred Charolais; LU, purebred Luing; NS, not significant, *, P<0.05, ***, P<0.001.

Table 6.27 shows the effect of the basal diet on the groups of fatty acids. Only the total amount of n-3 fatty acids was significantly different, being higher in the Mixed-fed animals than the Concentrate-fed animals. The most obvious difference is the ratio of the two main PUFA to each other or the sum of n-6 and n-3 PUFA ratios. Mixed-fed animals have a higher proportion of n-3 fatty acids than n-6 fatty acids compared to Concentrate-fed animals as is to be expected due to the higher 18:3 n-3 in the grass silage. However, as this ratio is not below 4.0, the desirable ratio is symptomatic of these being mixed rations with the silage based diets containing concentrates. A typical ratio in grass-grazed animals would be 1.

**Table 6.27.** Effect of basal diet on the sum and ratios of important groups of fatty acids in *M. longissimus thoracis.*

| Fatty acid | Basal Diet | Mean | SEM | Significance |
|---|---|---|---|---|
| mg/100g lean | | | | |
| Total FA | Concentrate | 4303 | 269.6 | NS |
| | Mixed | 4733 | 276.4 | |
| SFA | Concentrate | 1795 | 125.6 | NS |
| | Mixed | 2114 | 128.8 | |
| MUFA | Concentrate | 1916 | 125.3 | NS |
| | Mixed | 2057 | 128.5 | |
| PUFA | Concentrate | 203 | 4.7 | NS |
| | Mixed | 206 | 4.8 | |
| n-6 PUFA | Concentrate | 175 | 4.1 | NS |
| | Mixed | 168 | 4.2 | |
| n-3 PUFA | Concentrate | 28.2 | 0.8 | *** |
| | Mixed | 37.9 | 0.82 | |
| Proportions (g/100g fatty acid) | | | | |
| SFA % | Concentrate | 41 | 0.44 | *** |
| | Mixed | 44.2 | 0.45 | |
| MUFA % | Concentrate | 44.2 | 0.37 | ** |
| | Mixed | 42.7 | 0.38 | |
| PUFA % | Concentrate | 5.44 | 0.266 | NS |
| | Mixed | 5.15 | 0.273 | |
| n-6 PUFA % | Concentrate | 4.7 | 0.229 | NS |
| | Mixed | 4.23 | 0.235 | |
| n-3 PUFA % | Concentrate | 0.74 | 0.039 | ** |
| | Mixed | 0.92 | 0.04 | |
| Ratios | | | | |
| P:S ratio | Concentrate | 0.09 | 0.005 | NS |
| | Mixed | 0.07 | 0.005 | |
| C18:2n-6 to 18:3n-3 | Concentrate | 11.6 | 0.204 | *** |
| | Mixed | 7.21 | 0.209 | |
| n-6:n-3 ratio | Concentrate | 6.36 | 0.101 | *** |
| | Mixed | 4.51 | 0.104 | |

NS, not significant; **, P<0.01; ***, P<0.001

Table 6.28 shows the amount of individual fatty acid of different diets. The main statistically significant differences being found in the individual PUFA, though interestingly the 18:2n-6 is not different between Concentrate- and Mixed-fed animals.

**Table 6.28.** Effect of basal diet on the amount of individual fatty acids (mg/100g lean) in *M. longissimus thoracis.*

| Fatty acid | Basal Diet | Mean | SEM | Significance |
|---|---|---|---|---|
| 14:00 | Concentrate | 126.3 | 10.1 | NS |
| | Mixed | 140.9 | 10.35 | |
| 15:00 | Concentrate | 20 | 1.33 | ** |
| | Mixed | 14.7 | 1.36 | |
| 16:00 | Concentrate | 1083.5 | 75.73 | NS |
| | Mixed | 1269.7 | 77.64 | |
| 16DMA ald | Concentrate | 37.2 | 0.67 | *** |
| | Mixed | 33.5 | 0.69 | |
| 16:01 | Concentrate | 171.7 | 11.85 | NS |
| | Mixed | 182 | 12.15 | |
| 17:00 | Concentrate | 57.7 | 3.58 | ** |
| | Mixed | 41.5 | 3.67 | |
| 18:00 | Concentrate | 581.9 | 41.50 | * |
| | Mixed | 700.5 | 42.56 | |
| 18DMA ald | Concentrate | 20.7 | 0.38 | ** |
| | Mixed | 22.2 | 0.39 | |
| tr18:1n-7 | Concentrate | 80.5 | 4.66 | ** |
| | Mixed | 57.7 | 4.77 | |
| 18:1n-9 | Concentrate | 1581.3 | 106.04 | NS |
| | Mixed | 1747.9 | 108.72 | |
| 18:1n-7 | Concentrate | 75 | 3.53 | ** |
| | Mixed | 61.2 | 3.62 | |
| 18:2n-6 | Concentrate | 120 | 3.54 | NS |
| | Mixed | 116.6 | 3.63 | |
| 20:1n-9 | Concentrate | 7.5 | 0.60 | NS |
| | Mixed | 7.7 | 0.62 | |
| 18:3n-3 | Concentrate | 11.4 | 0.68 | *** |
| | Mixed | 17.2 | 0.70 | |
| 9c11tCLA | Concentrate | 13.2 | 1.07 | NS |
| | Mixed | 13.8 | 1.09 | |
| 20:3n-6 | Concentrate | 11.1 | 0.30 | * |
| | Mixed | 12.1 | 0.31 | |
| 20:4n-6 | Concentrate | 37.2 | 0.58 | *** |
| | Mixed | 33.3 | 0.60 | |
| 20:4n-3 | Concentrate | 0.79 | 0.056 | *** |
| | Mixed | 1.6 | 0.057 | |
| 20:5n-3 | Concentrate | 3.87 | 0.117 | * |
| | Mixed | 4.28 | 0.120 | |
| 22:4n-6 | Concentrate | 6.25 | 0.161 | NS |
| | Mixed | 6.08 | 0.165 | |
| 22:5n-3 | Concentrate | 10.87 | 0.154 | *** |
| | Mixed | 13.37 | 0.158 | |
| 22:6n-3 | Concentrate | 1.27 | 0.049 | NS |
| | Mixed | 1.37 | 0.050 | |

NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

Table 6.29 shows the effect of the treatments on groups of fatty acids and ratios. A significant difference was obtained for total n-3 PUFA, which were increased by Lipid and decreased by Nitrate compared to the Control, also reflected in the n-6 to n-3 ratio.

**Table 6.29.** Effect of treatment on the sum and ratios of important groups of fatty acids in *M. longissimus thoracis.*

| Fatty acid | Treatment | Mean | SEM | Significance |
|---|---|---|---|---|
| mg/100g fat | | | | |
| Total FA | Control | 4277 | 330.2 | NS |
| | Nitrate | 4411 | 336.5 | |
| | Lipid | 4867 | 336.5 | |
| SUM SFA | Control | 1815 | 153.9 | NS |
| | Nitrate | 1988 | 156.8 | |
| | Lipid | 2060 | 156.8 | |
| SUM MUFA | Control | 1891 | 153.5 | NS |
| | Nitrate | 1874 | 156.4 | |
| | Lipid | 2194 | 156.4 | |
| SUM PUFA | Control | 208 | 5.7 | NS |
| | Nitrate | 196 | 5.8 | |
| | Lipid | 209 | 5.8 | |
| SUM n-6 PUFA | Control | 175 | 5.0 | NS |
| | Nitrate | 166 | 5.1 | |
| | Lipid | 173 | 5.1 | |
| SUM n-3 PUFA | Control | 32.6 | 0.98 | *** |
| | Nitrate | 29.7 | 0.99 | |
| | Lipid | 36.8 | 0.99 | |
| Proportions (mg /100g fatty acid) | | | | |
| SUM SFA % | Control | 41.7 | 0.54 | * |
| | Nitrate | 43.9 | 0.55 | |
| | Lipid | 42.1 | 0.55 | |
| SUM MUFA % | Control | 43.6 | 0.46 | ** |
| | Nitrate | 42.2 | 0.47 | |
| | Lipid | 44.6 | 0.47 | |
| SUM PUFA % | Control | 5.72 | 0.326 | NS |
| | Nitrate | 5.38 | 0.332 | |
| | Lipid | 4.78 | 0.332 | |
| SUM n-6 PUFA % | Control | 4.84 | 0.281 | NS |
| | Nitrate | 4.60 | 0.286 | |
| | Lipid | 3.95 | 0.286 | |
| SUM n-3 PUFA % | Control | 0.880 | 0.05 | NS |
| | Nitrate | 0.777 | 0.05 | |
| | Lipid | 0.830 | 0.05 | |
| Ratios | | | | |
| P:S ratio | Control | 0.088 | 0.01 | NS |
| | Nitrate | 0.081 | 0.01 | |
| | Lipid | 0.073 | 0.01 | |
| C18;2n-6 to 18:3n-3 | Control | 9.69 | 0.2 | *** |
| | Nitrate | 10.66 | 0.3 | |
| | Lipid | 7.86 | 0.3 | |
| n-6:n-3 ratio | Control | 5.53 | 0.1 | *** |
| | Nitrate | 5.96 | 0.1 | |
| | Lipid | 4.82 | 0.1 | |

NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

For individual fatty acids (Table 6.30), transvaccenic acid (TVA, tr18:1n-7) was significantly (P=0.001) increased by the presence of RSC in the diet. TVA is formed in the rumen as an intermediate in biohydrogenation. It is converted into CLA in the tissues. Though not statistically different, RSC-fed animals had numerically more CLA than Nitrate or Controls. Rapeseed contains more of the fatty acids which are converted into TVA and CLA and so it is not possible to say from this data whether the RSC had contributed more substrate or had reduced biohydrogenation of PUFA.

The presence of nitrate decreased the concentration of the longer chain PUFA, 20:5n-3, 22:5n-3 and 22:6n-3 compared to the Lipid and Control diets. It is interesting that RSC did not increase these fatty acids above the level of the Control when their precursor 18:3n-3 was increased by this treatment.

**Table 6.30.** Effect of treatment on the amount of individual fatty acids (mg/100g lean) in *M. longissimus thoracis.*

| Fatty acid | Treatment | Mean | SEM | Significance |
|---|---|---|---|---|
| 14:00 | Control | 123.3 | 12.36 | NS |
| | Nitrate | 140.9 | 12.60 | |
| | Lipid | 136.6 | 12.60 | |
| 15:00 | Control | 16.9 | 1.63 | NS |
| | Nitrate | 17.0 | 1.66 | |
| | Lipid | 18.2 | 1.66 | |
| 16:00 | Control | 1097.8 | 92.74 | NS |
| | Nitrate | 1220.6 | 94.51 | |
| | Lipid | 1211.5 | 94.51 | |
| 16DMA ald | Control | 37.1 | 0.82 | NS |
| | Nitrate | 35.2 | 0.84 | |
| | Lipid | 33.8 | 0.84 | |
| 16:01 | Control | 169.9 | 14.52 | NS |
| | Nitrate | 182.7 | 14.79 | |
| | Lipid | 178.0 | 14.79 | |
| 17:00 | Control | 49.6 | 4.38 | NS |
| | Nitrate | 46.3 | 4.47 | |
| | Lipid | 52.8 | 4.47 | |
| 18:00 | Control | 590.9 | 50.83 | NS |
| | Nitrate | 623.8 | 51.80 | |
| | Lipid | 709.0 | 51.80 | |
| 18DMA ald | Control | 20.9 | 0.47 | *** |
| | Nitrate | 20.2 | 0.48 | |
| | Lipid | 23.3 | 0.48 | |
| tr18:1n-7 | Control | 60.8 | 5.70 | ** |
| | Nitrate | 58.9 | 5.81 | |
| | Lipid | 87.6 | 5.81 | |
| 18:1n-9 | Control | 1585.3 | 129.87 | NS |
| | Nitrate | 1561.9 | 132.34 | |
| | Lipid | 1846.6 | 132.34 | |
| 18:1n-7 | Control | 67.5 | 4.32 | NS |
| | Nitrate | 64.4 | 4.40 | |
| | Lipid | 72.3 | 4.40 | |
| 18:2n-6 | Control | 120.9 | 4.34 | NS |
| | Nitrate | 113.3 | 4.42 | |
| | Lipid | 120.7 | 4.42 | |

**Table 6.30.** Cont.

| Fatty acid | Treatment | Mean | SEM | Significance |
|---|---|---|---|---|
| 20:1n-9 | Control | 7.25 | 0.735 | * |
| | Nitrate | 6.14 | 0.749 | |
| | Lipid | 9.40 | 0.749 | |
| 18:3n-3 | Control | 13.73 | 0.830 | |
| | Nitrate | 12.54 | 0.846 | |
| | Lipid | 16.73 | 0.846 | |
| 9c11tCLA | Control | 12.82 | 1.307 | NS |
| | Nitrate | 12.31 | 1.332 | |
| | Lipid | 15.29 | 1.332 | |
| 20:3n-6 | Control | 11.83 | 0.373 | NS |
| | Nitrate | 11.51 | 0.380 | |
| | Lipid | 11.51 | 0.380 | |
| 20:4n-6 | Control | 36.52 | 0.713 | NS |
| | Nitrate | 34.74 | 0.726 | |
| | Lipid | 34.60 | 0.726 | |
| 20:4n-3 | Control | 1.11 | 0.069 | *** |
| | Nitrate | 1.05 | 0.070 | |
| | Lipid | 1.43 | 0.070 | |
| 20:5n-3 | Control | 4.24 | 0.143 | ** |
| | Nitrate | 3.60 | 0.146 | |
| | Lipid | 4.38 | 0.146 | |
| 22:4n-6 | Control | 6.25 | 0.197 | |
| | Nitrate | 6.57 | 0.200 | |
| | Lipid | 5.67 | 0.200 | |
| 22:5n-3 | Control | 12.23 | 0.189 | *** |
| | Nitrate | 11.34 | 0.192 | |
| | Lipid | 12.78 | 0.192 | |
| 22:6n-3 | Control | 1.30 | 0.060 | |
| | Nitrate | 1.19 | 0.061 | |
| | Lipid | 1.46 | 0.061 | |

NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 6.31 shows the few statistical interactions that were found in the data. There was no breed × basal diet × treatment interaction.

The breed × basal diet interactions indicated that Mixed-fed LU steers produce more 22:5n-3 than the CHx steers in comparison to the Concentrate-fed animals, which is then reflected in the total n-3PUFA. There is a greater difference between the 18:2n-6 to 18:3n-3 ratio for CHx between Concentrate and Mixed feeding than for LU.

There are greater differences due to treatment in CHx than LU and for treatment between Concentrate and Mixed-fed animals for 18:2n-6 to 18:3n-3 and n-6 to n-3 ratios.

Whilst the LU animals were fatter and this may have contributed to a better sensory flavour it was at the expense of their fat composition being more saturated than CHx.

**Table 6.31.** Interactions between breed, basal diet or treatment.

| Interaction | Fatty acid | Breed | Diet | Mean | Significance |
|---|---|---|---|---|---|
| Breed × Basal Diet | | | | | |
| | Weight 22:5n-3 | | | | |
| | | CHx | Concentrate | 10.8 | ** |
| | | | Mixed | 12.41 | |
| | | LU | Concentrate | 10.98 | |
| | | | Mixed | 14.3 | |
| | SUM n-3 PUFAW | | | | |
| | | CHx | Concentrate | 25.8 | * |
| | | | Mixed | 32.5 | |
| | | LU | Concentrate | 30.7 | |
| | | | Mixed | 43 | |
| | C18:2n6 to 18:3n-3 | | | | |
| | | CHx | Concentrate | 13.6 | ** |
| | | | Mixed | 8.3 | |
| | | LU | Concentrate | 9.5 | |
| | | | Mixed | 6.2 | |
| Breed × Treatment | | | | | |
| | C18:2n6 to 18:3n-3 | | | | |
| | | CHx | Control | 11.3 | ** |
| | | | Nitrate | 13 | |
| | | | Lipid | 9 | |
| | | LU | Control | 8.1 | |
| | | | Nitrate | 8.4 | |
| | | | Lipid | 6.9 | |
| | n-6 to n-3 ratio | | | | |
| | | CHx | Control | 5.8 | * |
| | | | Nitrate | 6.3 | |
| | | | Lipid | 5.1 | |
| | | LU | Control | 5.2 | |
| | | | Nitrate | 5.4 | |
| | | | Lipid | 5.1 | |
| Basal Diet × Treatment | | | | | |
| | C18:2n6 to 18:3n-3 | | | | |
| | | Concentrate | Control | 12.1 | ** |
| | | | Nitrate | 13.2 | |
| | | | Lipid | 9.5 | |
| | | Mixed | Control | 7.4 | |
| | | | Nitrate | 7.8 | |
| | | | Lipid | 6.3 | |
| | n-6 to n-3 ratio | | | | |
| | | Concentrate | Control | 6.5 | * |
| | | | Nitrate | 7.1 | |
| | | | Lipid | 5.5 | |
| | | Mixed | Control | 4.6 | |
| | | | Nitrate | 4.8 | |
| | | | Lipid | 4.5 | |

*, $P < 0.05$; **, $P < 0.01$.

## 7. EVALUATION STUDY: MAIN CONCLUSIONS

This study demonstrated that (i) the addition of nitrate to the diet or (ii) increasing the level of dietary lipid through the use of cold-pressed RSC, does not adversely affect either the performance or feed efficiency of finishing beef steers when used within either a Mixed forage/concentrate diet or a high Concentrate diet. The use of nitrate in the diet of ruminants has been limited to date due to the potential toxicity of the intermediate product (nitrite) which, at high levels, can severely impact animal health and productivity. The present study demonstrated that, following an appropriate adaptation period (four weeks), feeding of nitrate at the level considered here (18 g nitrate/kg diet DM) together with the basal diet types studied did not provide measureable adverse effects, in terms of blood MetHb response (where the maximum level reached was 15% of total Hb), animal performance and carcass characteristics. This study demonstrated that the use of RSC to increase the level of dietary lipid from 27 to 51 g AHEE/kg diet DM did not suppress DMI or ADG.

This study found the reduction in $CH_4$ yield from the implementation of two dietary strategies to steers fed mixed forage and concentrate diets (50:50 DM basis) were broadly in line with previous literature studies; the addition of 18 g nitrate/kg DM reduced the $CH_4$ yield of finishing steers by 17%, while increasing the lipid content of the same diet from 27 to 51 g AHEE/kg DM reduced the $CH_4$ yield by 7.5%. However, neither of these strategies reduced $CH_4$ emissions from a high concentrate diet (920 g/kg DM). Therefore, it was concluded that while both addition of nitrate and increased dietary lipid concentration are appropriate strategies to mitigate $CH_4$ emissions from mixed forage and concentrate diets, their use with high concentrate diets is not advisable. It is important to investigate combinations of different diet strategies for $CH_4$ mitigation. In doing so $CH_4$ yield may be reduced further than through one strategy alone. For example, combining the use of nitrate with increased lipid may yield further advantages.

Overall and consistently across sample times, addition of nitrate to the diets increased the molar proportion of acetate and decreased those of propionate irrespective of basal diet whilst increasing the lipid content had little effect on VFA molar proportions. This contrasts with the observation that nitrate did not reduce $CH_4$ on the high concentrate diet and suggests that nitrate did change the rumen fermentation on the high concentrate diet, but by mechanisms that did not reduce $CH_4$.

Although, there were consistently greater numbers of bacteria in the Concentrate than Mixed diet, neither addition of nitrate nor increasing dietary lipid content had any significant effect on archaea and total bacteria copy numbers in the rumen. It is noteworthy, that for both VFA and archaea and total bacteria populations, the data obtained prior to introduction of treatments significantly influenced measurements at subsequent times and therefore the rumen microbial population present at the start of the experiment for each individual animal was an important factor in determining that animal's response. Archaea numbers differed between samples and these differences depending on sampling time during the day and the method of sampling (stomach tube or at slaughter). More detailed analysis of the rumen microflora demonstrated that *Selenomonas ruminantium* was the dominant organism capable of reducing nitrate and that the greater numbers of thus bacterium on the high concentrate diet may provide an explanation for the absence of a reduction in $CH_4$ when nitrate was added to this diet.

A number of meat quality parameters were compared between treatments in this study. Vitamin E concentrations were higher in the Lipid treatment compared to the Control treatment, a hypothesis being that rapeseed oil may increase the stability of ingested Vitamin E in the rumen. Shelf life colour stability ranged between 15-17 days of acceptable shelf life, in line with Vitamin E levels. Notably, Nitrate and Lipid treatments had one less day on the Mixed basal diet at 16 days, but all

Concentrate diets were equal across treatments at 15 days. Although there were significant breed differences, there was no treatment effect on any of the sensory attributes of loin meat, suggesting there are no antagonistic effects of either dietary $CH_4$ mitigation strategy. Again no differences across treatment were found for saturated, mono-unsaturated, or poly-unsaturated fatty acids, apart from total n-3 fatty acids, which were higher in Lipid and lower in Nitrate treatments. Individual fatty acid, transvaccenic acid (TVA, tr18:1n-7) was significantly increased in the Lipid treatment, which may have related to increased content fatty acids in the Lipid treatment. The Nitrate treatment did decrease the concentration of the longer chain PUFA, 20:5n-3, 22:5n-3 and 22:6n-3 compared to the Lipid and Control diets.

The results of project year 1 (Evaluation study) were used to inform on experimental design of the Validation study in project year 2. Based on the results of the Evaluation study, and the industry relevance of the Mixed basal diet type, the project management team decided in year 2 to focus on combinations of strategies within the Mixed basal diet type. In doing so $CH_4$ yield may be reduced further than through one strategy alone. Thus, by combining the use of nitrate with a high lipid diet may yield further advantages. Alternative dietary components which are naturally high in lipid and more readily available were investigated (maize dark grains).

# 8. VALIDATION STUDY: MATERIAL AND METHODS

## 8.1 Experimental design

The validation study (Table 8.1), conducted between 24th March 2014 and 4th November 2014 (project year 2), was of a two × four design with:

- two breeds: (i) crossbred Aberdeen Angus (AAx) and (ii) crossbred Limousin (LIMx)
- four experimental diets (2 × 2 arrangement of nitrate × lipid): (i) Control, (ii) Nitrate, (iii) Lipid, (iv) Combined (Nitrate + Lipid).

**Table 8.1.** Experimental design of the validation study.

| Treatments | Control | Nitrate | Lipid | Combined[1] |
|---|---|---|---|---|
| No. AAx | 10 | 10 | 10 | 10 |
| No. LIMx | 10 | 10 | 10 | 10 |

[1]Combined = Nitrate + Lipid.
AAx, crossbred Aberdeen Angus; LIMx, crossbred Limousin.

## 8.2 Breeds

The two breeds utilised in this study are shown in Figure 8.1. The breeds were selected to represent two commercially relevant breed types and steers were selected from those available at the Beef Research Centre, SRUC. The steers were either Aberdeen Angus (AA) or Limousin (LIM) sired and bred from a 2-breed reciprocal-crossing program. In this program, AAx cows are always mated to a LIM sire and LIMx cows are always mated to an AA sire. After three generations of this breeding policy, the proportion of each breed type is 62.5:37.5 within the makeup of every respective individual animal.

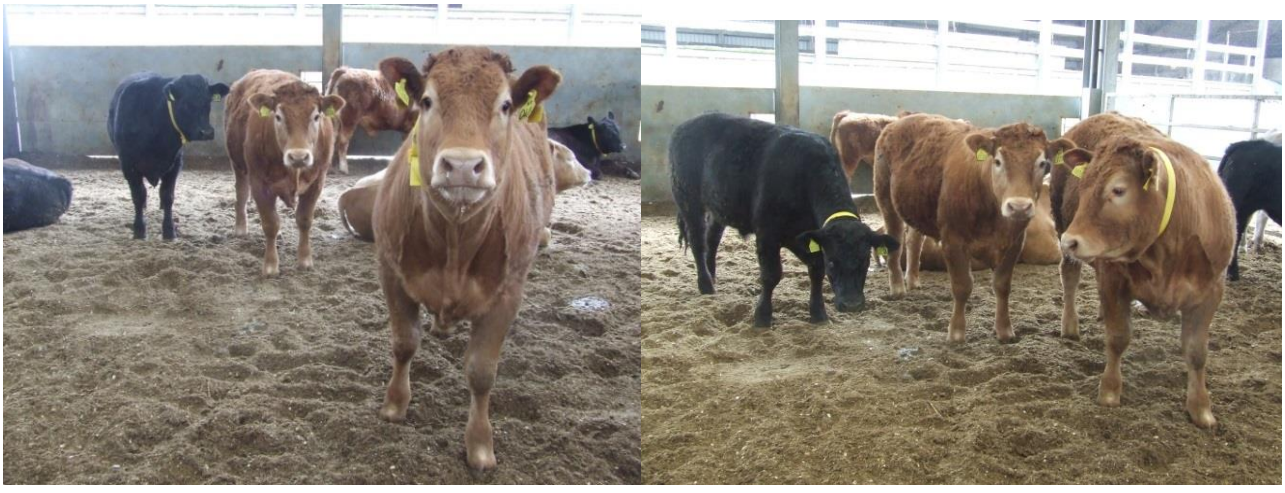

**Figure 8.1:** Two breed types used in the validation study: Aberdeen Angus-sired steers and Limousin-sired steers.

## 8.3 Experimental diets

The steers were fed one basal diets (as a total mixed rations) using a diet mixing wagon, consisting of (g/kg dry matter (DM)) forage to concentrate ratios of 557:443 (Mixed). Within the Mixed basal diet the steers were offered one of four treatments: (i) Control containing rapeseed meal as the main protein source which was replaced with either (ii) Nitrate in the form of calcium nitrate (Calcinit, Yara, Oslo, Norway; 18 g nitrate/kg diet DM) or (iii) an added source of lipid in the form of maize dark grains (MDG) which is a by-product of the distilling industry (acid hydrolysed ether extract (AHEE) increased from 25 to 37 g AHEE/kg diet DM). The treatments were chosen in consultation with AHDB Beef and Lamb, the Food Standards Agency (FSA) and the project management team.

The ingredient composition of the experimental diets is given in Table 8.2. The chemical composition of individual components is given in Table 8.3. The chemical composition of experimental diets is given in Table 8.4.

The DM contents of individual components were determined on duplicate samples twice weekly and bulked feed samples (four per component) were analysed. Feed samples were analysed for DM, ash, crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), AHEE, and starch (Ministry of Agriculture Fisheries and Food, 1992) and gross energy (GE) by adiabatic bomb calorimetry. For the Nitrate and Lipid diets, calcium nitrate and MDG were incorporated firstly into a premix which contained the concentrate portion of the diet alongside minerals and molasses. Each batch of premix was mixed using a diet mixing wagon to produce a consistent premix. On a daily basis each premix was then mixed with the forage portion of the diet using the same mixing wagon to generate a consistent total mixed ration. Diets were mixed for a minimum duration of 20 minutes.

**Table 8.2.** Ingredient composition of experimental diets (dry matter basis; g/kg).

| Ingredient | Control | Nitrate | Lipid | Combined |
|---|---|---|---|---|
| Silage | 210 | 211 | 209 | 210 |
| WCBS | 347 | 347 | 346 | 346 |
| Bruised barley | 336 | 388 | 289 | 263 |
| RSM | 79 | 0 | 0 | 0 |
| Calcinit | 0 | 25 | 0 | 25 |
| MDG | 0 | 0 | 128 | 127 |
| Molasses | 19 | 20 | 19 | 19 |
| Minerals* | 9 | 9 | 9 | 9 |

Silage, grass silage; WCBS, whole crop barley silage; Barley, barley grain; RSM, rapeseed meal; MDG, maize dark grains; Calcinit, calcium nitrate.
*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 8.3.** Chemical composition of feed components of the experimental diets*.

| | GS | WCBS | Barley | RSM | Calcinit | MDG | Molasses | Minerals |
|---|---|---|---|---|---|---|---|---|
| DM (g/kg) | 360 | 443 | 864 | 894 | 855 | 880 | 785 | 969 |
| Ash (g/kg DM) | 82.0 | 54.9 | 20.0 | 78.0 | 0.0 | 48.4 | 172.4 | 0.0 |
| CP (g/kg DM) | 137.8 | 105.2 | 113.0 | 386.8 | 1169.0 | 286.6 | 95.1 | 0.0 |
| ADF (g/kg DM) | 303.2 | 237.0 | 50.6 | 260.8 | 0.0 | 185.4 | 0.0 | 0.0 |
| NDF (g/kg DM) | 452.4 | 405.0 | 151.0 | 270.2 | 0.0 | 300.4 | 0.0 | 0.0 |
| Starch (g/kg DM) | 7.3 | 265.0 | 554.0 | 13.4 | 0.0 | 84.6 | 0.0 | 0.0 |
| AHEE (g/kg DM) | 32.4 | 15.4 | 28.9 | 39.3 | 0.0 | 126.8 | 0.3 | 0.0 |
| GE (MJ/kg DM) | 19.2 | 18.2 | 17.8 | 19.1 | 0.0 | 21.2 | 15.2 | 0.0 |
| ME (MJ/kg DM) | 11.5 | 10.7 | 13.3 | 11.7 | 0.0 | 14.3 | 11.6 | 0.0 |

GS, grass silage; WCBS, whole crop barley silage; Barley, barley grain; RSM, rapeseed meal; MDG, maize dark grains; Calcinit, calcium nitrate; DM, dry matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; AHEE, acid hydrolysed ether extract; GE, gross energy; ME, metabolisable energy.
*Molasses contained 785 g DM /kg and Gross Energy 15.2 MJ/kg DM.

**Table 8.4.** Chemical composition of the experimental diets.

| | Control | Nitrate | Lipid | Combined |
|---|---|---|---|---|
| DM (g/kg) | 533 | 531 | 533 | 533 |
| Ash (g/kg DM) | 52 | 48 | 51 | 51 |
| CP (g/kg DM) | 135 | 141 | 136 | 162 |
| ADF (g/kg DM) | 184 | 166 | 184 | 183 |
| NDF (g/kg DM) | 308 | 295 | 317 | 313 |
| Starch (g/kg DM) | 281 | 308 | 264 | 295 |
| AHEE (g/kg DM) | 25.0 | 23.4 | 36.7 | 35.9 |
| GE (MJ/kg DM) | 18.1 | 17.6 | 18.5 | 18.0 |
| ME (MJ/kg DM) | 11.7 | 11.5 | 12.0 | 11.7 |

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

## 8.4 Experimental protocol

In total, 80 steers (40 of each breed) were used. Thus 20 animals (10 of each breed) were allocated to each experimental diet (shown in Table 8.1). In the same way as the evaluation study, due to the high risk of ill-health of unadapted animals gaining access to dietary nitrate, each experimental diet was allocated to one pen (four pens in total). Experimental diets were balanced for sire within each breed, farm of origin and BW and were balanced across treatment groups at the start of the experiment. The experimental protocol consisted of the same phases as described in Chapter 5 (evaluation study). A summary of the validation study timeline is provided in Table 8.5.

**Table 8.5.** Validation study timeline.

| | Start Day | End Day | Start Date | End Date |
|---|---|---|---|---|
| Adaptation Stage 1 | -63 | -36 | 24th March 2014 | 20th April 2014 |
| Adaptation Stage 2* | -35 | -1 | 21st April 2014 | 25th May 2014 |
| 56 d performance test | 0 | 55 | 26th May 2014 | 20th July 2014 |
| $CH_4$ measurements | 64 | 157 | 29th July 2014 | 30th October 2014 |
| Slaughter Batch 1 | 99 | - | 2nd September 2014 | - |
| Slaughter Batch 2 | 120 | - | 23rd September 2014 | - |
| Slaughter Batch 3 | 141 | - | 14th October 2014 | - |
| Slaughter Batch 4 | 162 | - | 4th November 2014 | - |

*Adaptation stage 2 was 5 weeks instead of 4 (as in evaluation study) due to electronic feeder maintenance.

## 8.5. Sample collection and laboratory analyses

Samples were collected and analysed in the same way as described in the Evaluation study. For full details of samples collected, storage and laboratory analyses see Section 5.5. A tabular summary of samples obtained during the Validation study are provided below (Tables 8.6 through to 8.8):

**Table 8.6.** Blood sampling for methaemoglobin**.**

| Sample (day) | Date | Description (% full nitrate inclusion) | Nitrate (g/kg diet DM) |
|---|---|---|---|
| -34 | 22nd April 2014 | 25 | 4.5 |
| -27 | 29th April 2014 | 50 | 9 |
| -13 | 13th May 2014 | 100 | 18 |
| -6 | 20th May 2014 | 100 | 18 |
| 1 | 27th May 2014 | 100 | 18 |

**Table 8.7.** Rumen sampling for volatile fatty acid and rumen microbial analyses.

| Sample (day) | Date | Description |
|---|---|---|
| -42 | 14th April 2014 | 1. Preliminary |
| -28 | 28th April 2014 | 2. Adaptation |
| -11 | 15th May 2014 | 3. Start test |
| 56 | 21st July 2014 | 4. End test |
| 72-157 | 6th August – 30th October 2014 | 5. Chamber |
| 99-161 | 2nd September – 3rd November 2014 | 6. Slaughter |

**Table 8.8.** Loin muscle samples (5th to 10th rib section).

| Slaughter Batch | Number of steers | Number of loins selected | Slaughter day | Slaughter Date |
|---|---|---|---|---|
| 1 | 18 | 18 | 99 | 2nd September 2014 |
| 2 | 17 | 17 | 120 | 23rd September 2014 |
| 3 | 20 | 18 | 141 | 14th October 2014 |
| 4 | 25 | 19 | 162 | 4th November 2014 |

.

In addition to measuring changes in archaea and bacteria across all sampling points during the Evaluation study, detailed microbial analyses were also conducted to provide an explanation for the absence of a reduction in CH4 when nitrate was added to the high Concentrate diet. Therefore there was insufficient time and resources to undertake microbial analysis of samples from the Validation study. However, as part of an aligned PhD project, this analysis is underway and results on the microbial populations based on 16S microbiome analysis will be communicated to sponsors when available.

## 8.6 Calculations and statistical analysis

### 8.6.1 Blood methaemoglobin response to dietary nitrate

Two analyses were carried out to test: (a) The effects of increasing nitrate inclusion rate (samples 1, 2 and 3) on MetHb (% total Hb), Total Hb (g/100 ml) and blood haematocrit (%); and (b) The effect of longer term exposure to the full inclusion rate of nitrate (samples 3, 4 and 5). Analyses were performed as a repeated measures analysis with REML in Genstat and the model included fixed effect of breed, sample time and the interaction between breed and sample time.

### 8.6.2 Performance and slaughter traits

Data from one steer from the 56 d test period was discarded as the steer was removed from the trial for health reasons unconnected to the diets and treatments imposed, leaving n=79 available for analyses. Growth was modelled by linear regression of BW against test date, to obtain ADG, mid-test BW (mid-BW) and mid-test metabolic BW (mid-MBW = $BW^{0.75}$). Mean DMI over the 56 day period was expressed as kg per day or as a proportion of mid-BW and mid-MBW. Feed conversion ratio (FCR) was calculated as average DMI per day (kg/d)/ADG. Residual feed intake (RFI) was calculated as deviation of actual DMI (kg/d) from DMI predicted based on linear regression of actual DMI on ADG, mid-MBW and FD1 (Basarab *et al.*, 2003). Cold carcass weight (CCW) was calculated as a percentage of slaughter BW (SBW) to determine killing out percentage (KO). To allow for statistical comparison, the EUROP carcass classification values were expressed on the equivalent 15 point scale (see Table 5.9, Kempster *et al.*, 1986). Statistical analyses of performance and carcass data were conducted using the mixed procedure of SAS software with the fixed effects of breed, nitrate and lipid, and the random effect of pen (and slaughter batch for carcass traits). In addition, in the analysis of FD1 and FD2 the deviation from the breed mean of FD0 was included as a covariable. The interaction effects of breed × nitrate, nitrate × lipid, breed × lipid and breed ×

nitrate × lipid were included in the model when these effects proved significant ($P<0.05$). Data are reported as means with their SEM. Differences between means were tested using a protected least squared means test. Probability values were deemed significant where $P<0.05$ and indicated a tendency when probability values were between $P=0.05$ and $P=0.1$.

### 8.6.3 Methane and hydrogen emissions

The steers were allocated to minimise variation in LW (mean LW 659 ± 37 kg) on entry into the respiration chambers. Each steer was allocated to one of six respiration chambers (shown in Figure 4.4b) over a 12-week period, with each treatment allocated 3 times to each respiration chamber. One chamber malfunctioned during weeks 1 to 6, and another on week 2 which resulted in the requirement for a $13^{th}$ week of chamber analysis. Therefore, emissions from each of 71 steers were measured once. Respiration chamber $CH_4$ and hydrogen data were analysed using the Statistical Analyses System (SAS 9.3, SAS Inst. Inc., North Carolina) using linear mixed models. The fixed effects were breed, added nitrate and increased fat content, while the random effects were week and chamber. The effects of breed x nitrate, breed x lipid and nitrate x lipid were included in the model when these proved significant ($P<0.05$). Data are reported as means with their SEM unless indicated otherwise. Differences between means were tested using a protected least squared means test with probability values of $P<0.05$ deemed to be significant, while probability values between $P=0.05$ and $P=0.1$ were deemed to indicate a tendency.

### 8.6.4 Rumen volatile fatty acids

A split plot analysis of variance was carried out to estimate the differences between samples (time) using repeated measures analysis within REML in Genstat. As VFA concentrations in samples taken before the mitigation treatments were introduced (Preliminary samples) were found to include significant “treatment” effects, these data were included as covariates in the split plot analysis to account for differences between animals when allocated to treatments. The model included fixed effects of breed and diet (2 × 2 factorial arrangement of nitrate and lipid) and assessed the effects of sample time and interactions between fixed effects and sample time.

### 8.6.5 Meat quality

Data was analysed by analysis of variance using diet and breed as factors (IBM SPSS v21)

## 9. VALIDATION STUDY: RESULTS

### 9.1 Blood methaemoglobin response to dietary nitrate

During the adaptation period (weeks 1 to 4, Table 9.1) there were no consistent effects of diet or breed or any interactions between sample and either diet and breed.

As in 2013, although MetHb increased when nitrate inclusion increased from 25 to 50% of the maximum nitrate inclusion, MetHb concentrations overall were low (maximum individual values of 0.7 and 1.1% for 25 and 50 % inclusion respectively). Adding 100% nitrate increased MetHb further with the largest individual value being 13% total Hb. Haematocrit increased as nitrate inclusion rate was increased and there were differences between samples in total Hb concentration.

**Table 9.1.** Methaemoglobin concentrations in blood of steers fed increasing amounts of nitrate.

| Day[1] | -34 | -27 | -13 | | |
|---|---|---|---|---|---|
| Week | 1 | 2 | 4 | | |
| Nitrate (%) | 25 | 50 | 100 | SED | Sig. |
| MetHb (% total Hb) | 0.29 | 0.46 | 2.83 | 0.312 | *** |
| Total Hb (g/100ml) | 12.9 | 12.2 | 12.8 | 0.123 | *** |
| Haematocrit (%) | 30.8 | 31.1 | 31.3 | 0.18 | * |

[1]Day relative to start of the 56 day performance test period.
[2]Nitrate as percentage of maximum level of intake (100% = 18 g/kg DM).
*P<0.05; ***P<0.001.

During longer term exposure to 100% nitrate (weeks 4 to 6, Table 9.2) there were no effects of diet or interactions between diet and sample time. However, there was a significant breed and sample by breed interaction for MetHb.

MetHb concentrations increased from week 4 to week 5 and then declined again in week 6 but at all times were greater than 0. There was a breed by sample time interaction such that MetHb concentrations for LIMx steers were greater than for AAx steers in weeks 5 and 6. Maximum individual values for MetHb in weeks 4, 5 and 6 were 13.0, 20.5 and 7.6 % total Hb respectively.

Both total Hb and haematocrit increased as length of exposure to nitrate increased, possibly indicating an adaptation to reduced oxygen carrying capacity as a result of MetHb formation.

**Table 9.2.** Effects of longer term inclusion of 100% nitrate in diets on steer methaemoglobin concentrations.

| Day[1] | -13 | | -6 | | 1 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Week | 4 | | 5 | | 6 | | | Significance | | |
| Breed | AAx | LIMx | AAx | LIMx | AAx | LIMx | SED | Day | Breed | D × B |
| MetHb (% total Hb) | 3.3 | 2.4 | 7.0 | 9.8 | 1.4 | 3.1 | 1.22 | *** | ** | *** |
| Total Hb (g/100ml) | 12.7 | 12.8 | 13.4 | 13.7 | 13.7 | 14.0 | 0.30 | *** | NS | NS |
| Haematocrit (%) | 31.3 | 31.3 | 32.5 | 32.6 | 31.9 | 32.0 | 0.42 | *** | NS | NS |

[1]Day relative to start of the 56 day performance test period. D × B, Sample Day × Breed interaction
NS, not significant; **, P<0.01; ***, P<0.001.

To investigate whether individual animals were consistent in their response to nitrate inclusion (as measured by MetHb concentration), animals were ranked by MetHb concentration within each sample day and overall mean rank for each animal calculated. The animals were then grouped into quartiles (10 steers per quartile). Figures 9.1 and 9.2 below show the MetHb concentrations for individual animals in the lowest (0-25%) and highest quartiles (75-100%).

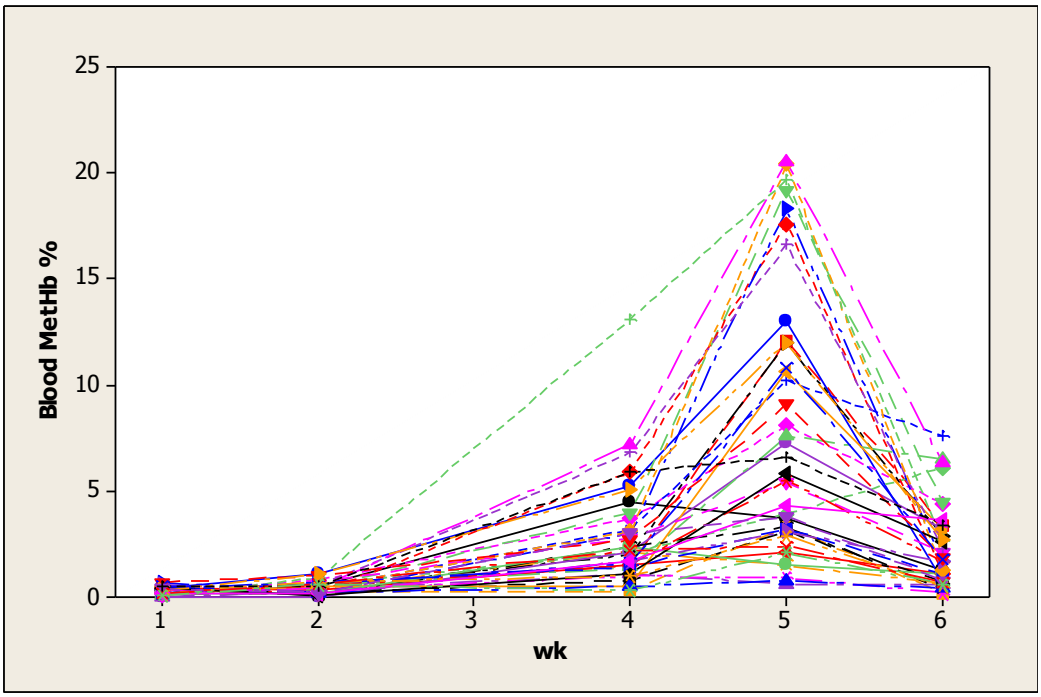


**Figure 9.1:** Change in MetHb concentrations during adaptation to dietary nitrate.

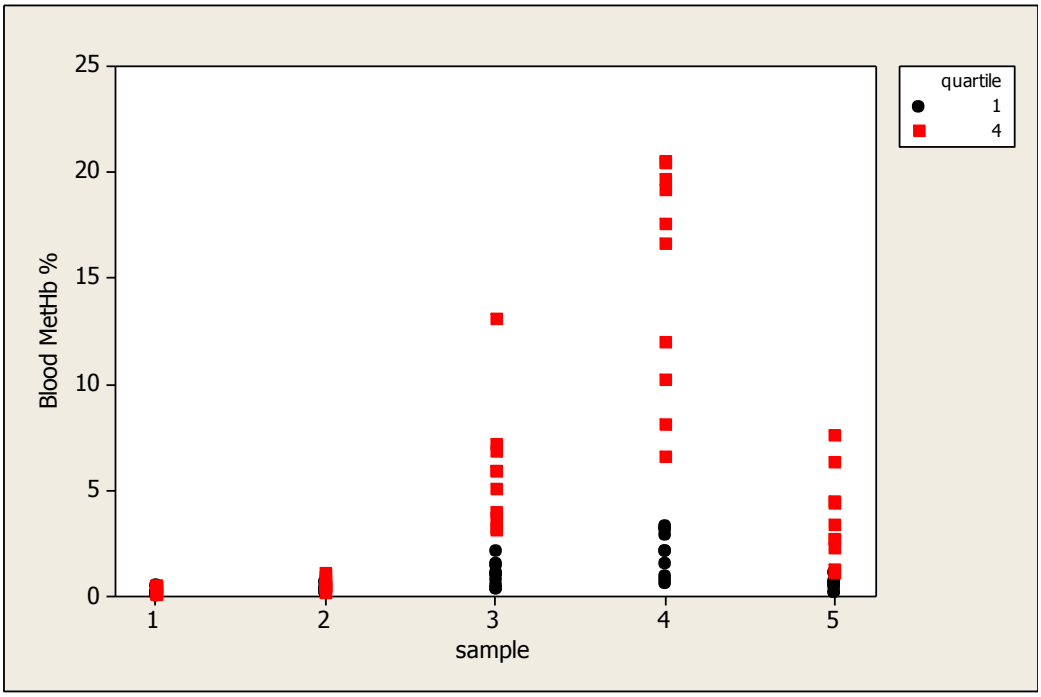


**Figure 9.2:** Blood MetHb(%) of animals in the lower (25%) and upper (75%) quartiles.

Figure 9.2 shows clear separation between the 25% and 75% quartiles and Figure 9.1 shows the consistent responses of individual animals. That is, during weeks 3, 4 and 5, the same steers in the 75% quartile had the highest concentrations of MetHb.

## 9.2 Performance test

At the start of the experiment the treatments were balanced for age and BW. Thus, age at the start of the test period (AgeST) and Mid-BW did not differ across dietary treatments (P>0.05, Table 9.3). DMI was not affected by the inclusion of nitrate or lipid (P>0.05). However, in contrast to Year 1 data steers receiving dietary nitrate achieved poorer ADG throughout the 56-day test (P<0.01). The inclusion of nitrate or lipid did not affect fat depth at the end of the 56-d test (FD1) (P>0.05). In contrast to the evaluation study, steers receiving dietary nitrate were less efficient (greater FCR; P<0.05) than the Control steers. Although the results of RFI were not significant, the same trend was observed with Nitrate-fed steers achieving greater RFI (less efficient) than the Control steers. Dietary lipid did not affect feed efficiency (P>0.05). There was no significant effect of nitrate × lipid interaction on any performance trait.

AgeST and Mid-BW did not differ between breeds (P>0.05). AAx steers achieved greater ADG compared to LIMx steers (1.74 *v.* 1.56 kg; P<0.01). DMI was greater in AAx steers compared to LIMx steers, whether expressed daily (12.15 *v.* 11.07 kg/d; P<0.001); or as a proportion of BW (22.44 *v.* 20.59 g/kg BW; P<0.001). FD1 was greater in AAx compared to LIMx steers (9.13 *v.* 8.05 mm; P<0.05). Due to the higher levels of DMI and FD1, AAx steers were less efficient with greater RFI scores than LIMx steers (0.24 *v.* -0.24 kg; P<0.01).

### 9.3 Carcass characteristics

The inclusion of either nitrate or lipid was not shown to adversely affect any trait measured at slaughter, except for a tendency for poorer KO proportion in Nitrate-fed steers (P=0.07, Table 9.4).

AAx steers achieved greater SBW than LIMx steers (689.2 *v.* 667.4 kg; P>0.05). However CCW did not differ between breeds (P>0.05), thus KO was lower in AAx than LIMx steers (555.3 *v.* 576.6; P<0.001). At slaughter, AAx steers had greater fat depth (FD2) (10.9 *v.* 8.7 mm; P<0.001) than LIMx steers. EUROP conformation and fat score allocated by visual assessor did not differ between breeds (P>0.05). Fat scores and total fat weight determined by the VIA system did not differ between breeds (P>0.05). However, conformation and total muscle weight determined by the VIA system were greater for the LIMx compared to AAx steers (CONF (VIA): 9.8 *v.* 9.1, P<0.01; TOTMeat: 273.2 *v.* 264.7, P<0.05; for LIMx and AAx respectively).

**Table 9.3.** Effect of breed and dietary treatment on growth, fat depth, feed intake and feed efficiency of Aberdeen Angus-sired (AAx) and Limousin-sired (LIMx) steers fed one of four dietary treatments: Control, Nitrate, Lipid or Combined.

| Breed | AAx | | | | LIMx | | | | | Significance[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Combined | Control | Nitrate | Lipid | Combined | SEM | Breed | Nitrate | Lipid |
| AgeST (days) | 416 | 419 | 416 | 416 | 411 | 409 | 410 | 413 | 5.3 | NS | NS | NS |
| Mid-BW (kg) | 546.8 | 549.0 | 534.0 | 537.3 | 545.8 | 536.1 | 541.6 | 530.9 | 17.50 | NS | NS | NS |
| Mid-MBW (kg) | 113.0 | 113.2 | 111.1 | 111.5 | 112.9 | 111.3 | 112.2 | 110.5 | 2.73 | NS | NS | NS |
| ADG (kg/day) | 1.78 | 1.66 | 1.78 | 1.77 | 1.69 | 1.41 | 1.66 | 1.49 | 0.076 | ** | ** | NS |
| DMI (kg/day) | 12.07 | 12.35 | 12.19 | 11.98 | 11.48 | 10.51 | 11.32 | 10.95 | 0.425 | *** | NS | NS |
| DMI/BW(g/kg) | 22.13 | 22.47 | 22.86 | 22.28 | 21.06 | 19.68 | 20.93 | 20.66 | 0.483 | *** | NS | NS |
| DMI/MBW(g/kg) | 106.83 | 108.56 | 109.77 | 107.21 | 101.74 | 94.52 | 100.89 | 99.06 | 2.314 | *** | NS | NS |
| FCR (kg, kg) | 6.79 | 7.53 | 6.92 | 6.82 | 6.91 | 7.50 | 6.87 | 7.54 | 0.269 | NS | * | NS |
| RFI (kg) | 0.04 | 0.51 | 0.33 | 0.06 | -0.20 | -0.39 | -0.36 | 0.01 | 0.231 | ** | NS | NS |
| FD1 (mm)[2] | 8.86 | 9.65 | 8.81 | 9.22 | 7.94 | 8.07 | 8.81 | 7.39 | 0.663 | ** | NS | NS |

Number of animals = 80; AgeST, Age at start of test; Mid-BW, mid-test BW; Mid-MBW, mid-test metabolic BW; ADG, average daily gain at the end of the 56 d test; FCR, feed conversion ratio; RFI, residual feed intake; FD1, fat depth at the 12/13$^{th}$ rib at the end of the 56 d test.

[1]Breed × Nitrate and Breed × Lipid, Nitrate × Lipid interaction effects were not significant for all variables (P>0.05).

[2]Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable.

NS, not significant; *P<0.05; **P<0.01; ***P<0.001.

**Table 9.4.** Effect of breed and dietary treatment on carcass traits of Aberdeen Angus-sired (AAx) and Limousin-sired (LIMx) steers fed one of four dietary treatments: Control, Nitrate, Lipid or Combined.

| Breed | AAx | | | | LIMx | | | | | Significance[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Combined | Control | Nitrate | Lipid | Combined | SEM | Breed | Nitrate | Lipid |
| FD2 (mm)[2] | 12.05 | 10.09 | 10.26 | 11.11 | 9.09 | 7.61 | 8.88 | 9.22 | 0.904 | *** | NS | NS |
| CCW (kg) | 384.9 | 381.1 | 379.2 | 384.4 | 379.2 | 379.3 | 385.9 | 376.5 | 7.39 | NS | NS | NS |
| KO (%) | 55.7 | 55.5 | 56.0 | 54.8 | 58.4 | 57.3 | 57.4 | 57.5 | 0.45 | *** | NS | NS |
| SBW (kg) | 691.4 | 687.8 | 676.8 | 700.6 | 680.4 | 661.6 | 672.6 | 655.0 | 13.91 | * | NS | NS |
| CONF | 10.0 | 9.2 | 9.4 | 9.2 | 10.2 | 9.2 | 9.8 | 9.4 | 0.34 | NS | NS | NS |
| FAT | 10.4 | 10.6 | 10.2 | 10.8 | 10.6 | 9.4 | 10.6 | 10.6 | 0.31 | NS | NS | NS |
| CONF (VIA)[3] | 9.1 | 9.0 | 9.3 | 8.8 | 10.4 | 9.5 | 9.4 | 9.9 | 0.37 | ** | NS | NS |
| FAT (VIA)[3] | 8.9 | 9.0 | 8.7 | 9.5 | 8.8 | 8.2 | 9.4 | 8.6 | 0.40 | NS | NS | NS |
| TOTFat(kg)[4] | 46.4 | 44.2 | 43.1 | 49.2 | 45.1 | 38.5 | 44.4 | 41.0 | 3.21 | NS | NS | NS |
| TOTMeat(kg)[4] | 265.3 | 266.7 | 264.4 | 262.3 | 280.7 | 272.7 | 270.0 | 268.1 | 5.83 | * | NS | NS |

Number of animals = 80; FD2, pre-slaughter fat depth at the 12/13$^{th}$ rib; CCW, cold carcass weight; KO, killing out %; SBW, slaughter BW; CONF, EUROP conformation (15 pt scale) assigned by visual assessor; FAT, EUROP fatness (15pt scale) assigned by visual assessor; CONF (VIA), conformation grade (15pt scale) assigned by VIA; FAT (VIA), fatness grade (15pt scale) assigned by VIA; TOTFat; total fat content predicted by VIA; TOTMeat, total meat content predicted by VIA.

[1]Breed × Nitrate and Breed × Lipid, Nitrate × Lipid interaction effects were not significant for all variables (P>0.05).

[2]Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable.

[3]data unavailable for 1 steer; [4]data unavailable for 6 steers.

NS, not significant; *P<0.05; ***P<0.001.

## 9.4 Methane and hydrogen emissions

There were no interactions between breed of steer and nutritional treatments and therefore the effects of diet and breed on emissions are shown in Tables 9.5 and 9.6 respectively.

The steers receiving the treatments which included Nitrate produced less $CH_4$ (Table 9.5) than those receiving treatments without added nitrate, when expressed on both a daily and g/kg DMI basis ($P<0.001$). Animals fed treatments including nitrate produced more $H_2$ measured both on a daily and intake basis ($P<0.001$) than diets that did not include nitrate. Increasing the lipid content of the diets reduced $CH_4$ emissions on both a daily ($P=0.34$) or g/kg DMI basis ($P=0.11$) but these reductions were not significant; increasing lipid had no effect on $H_2$ emissions ($P=0.99$). There were no significant interactions between nitrate and lipid on $CH_4$ or $H_2$ emissions; thus the effects of adding nitrate and increasing lipid were independent of each other.

Animals receiving the treatments including Nitrate produced 2.1 g/kg DMI less $CH_4$ than those animals that did not receive nitrate. Given the amount of nitrate added, the Nitrate-including treatments had the theoretical potential to reduce $CH_4$ yield by 4.7 g/kg DMI. However, only 45% of this potential was achieved, compared with 80% potential reduction achieved in the Evaluation study.

**Table 9.5.** Effect of dietary treatment on intakes and GHG production as measured from the respiration chambers.

| | Treatment | | | | Significance | | |
|---|---|---|---|---|---|---|---|
| | Control | Nitrate | Lipid | Combined | Nitrate | Lipid | Nitrate×Lipid |
| $CH_4$ (g/day) | 245.5 | 218.6 | 238.2 | 209.9 | *** | NS | NS |
| $CH_4$ (g/kg DMI) | 23.98 | 22.09 | 23.38 | 20.89 | *** | NS | NS |
| $H_2$ (g/day) | 0.457 | 0.989 | 0.401 | 1.046 | *** | NS | NS |
| $H_2$ (g/kg DMI) | 0.044 | 0.100 | 0.039 | 0.103 | *** | NS | NS |
| DMI (kg/day) | 10.35 | 9.82 | 10.23 | 10.21 | NS | NS | NS |
| BW (kg) | 674.0 | 655.8 | 652.8 | 652.4 | NS | NS | * |

Number of animals = 71.
NS, not significant; *, $P<0.05$; ***, $P<0.001$.

Table 9.6 shows the effect of breed on emissions of $CH_4$ and $H_2$ from the finishing steers. AAx steers were heavier than the LIMx steers ($P<0.01$) and had a higher DMI during the chamber period ($P<0.001$). Therefore, they produced more $CH_4$ on a daily basis ($P<0.001$). However, the LIMx steers produced more $CH_4$ when corrected for DMI ($P<0.05$). Breed had no effect on $H_2$ emissions on both a daily and intake corrected basis

**Table 9.6.** Effect of breed on intakes and GHG production as measured from the respiration chambers.

| | Breed | | Significance | | |
|---|---|---|---|---|---|
| | AAx | LIMx | Breed | Breed×Nitrate | Breed×Lipid |
| $CH_4$ (g/day) | 241.5 | 214.5 | *** | NS | NS |
| $CH_4$ (g/kg DMI) | 21.97 | 23.23 | * | NS | NS |
| $H_2$ (g/day) | 0.769 | 0.668 | NS | NS | NS |
| $H_2$ (g/kg DMI) | 0.069 | 0.072 | NS | NS | NS |
| DMI (kg/day) | 11.00 | 9.33 | *** | NS | NS |
| BW (kg) | 668.9 | 648.4 | *** | NS | NS |

Number of animals= 71.
NS, not significant; *, P<0.05; ***, P<0.001.

## 9.5 Volatile fatty acids

There were significant sample time × treatment interactions. These were complex, so before dealing with these interactions, the main effects of breed and nutritional treatment are presented.

There were significant differences between breeds, and the effect of pre-additive VFA molar proportions (covariate effect) was significant for acetate (P<0.01); propionate (P<0.01), butyrate (P<0.05), valerate (P<0.01) and branched chain VFA (P<0.05).

Overall, molar proportions of acetate increased from adaptation to slaughter and those of propionate and to a lesser extent butyrate and valerate decreased (Table 9.7).

**Table 9.7.** Main effects of sample time on VFA (mmol / mol total VFA).

| Sample | Adapt | Start | End | Chamb | Slaught | SED | Significance |
|---|---|---|---|---|---|---|---|
| Acetate | 573 | 630 | 672 | 682 | 666 | 3.5 | *** |
| Propionate | 246 | 186 | 166 | 160 | 173 | 4.1 | *** |
| Butyrate | 138 | 143 | 129 | 124 | 125 | 3.5 | *** |
| Valerate | 24 | 17 | 12 | 12 | 12 | 0.8 | *** |
| Branched-chain | 19 | 24 | 21 | 23 | 24 | 0.8 | *** |

***, P <0.001.

Samples from AAx steers contained less acetate and more propionate and valerate than samples from LIMx steers (Table 9.8).

**Table 9.8.** Main effects of breed on VFA (mmol / mol total VFA).

| Breed | AAx | LIMx | SED | Significance |
|---|---|---|---|---|
| Acetate | 640 | 649 | 3.7 | ** |
| Propionate | 191 | 181 | 4.2 | * |
| Butyrate | 130 | 134 | 3.2 | NS |
| Valerate | 16 | 14 | 0.6 | * |
| Branched-chain | 23 | 22 | 0.7 | NS |

NS, not significant; *P, < 0.05; **, P <0.01.

The effects of nitrate and lipid on VFA proportions were independent (Table 9.9). Nitrate inclusion increased acetate and butyrate and decreased propionate, valerate and branched chain VFA molar proportions. Inclusion of lipid (dark grains) increased acetate and decreased propionate molar proportions.

**Table 9.9.** Main effects of treatment on VFA (mmol / mol total VFA).

| Nitrate | Absent | | Present | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Lipid | Absent | Present | Absent | Present | SED | N | L | N × L |
| Acetate | 634 | 648 | 645 | 651 | 4.7 | * | ** | NS |
| Propionate | 199 | 190 | 182 | 174 | 5.6 | *** | * | NS |
| Butyrate | 128 | 123 | 135 | 133 | 4.6 | *** | NS | NS |
| Valerate | 16 | 17 | 14 | 14 | 0.7 | *** | NS | NS |
| Branched-chain | 25 | 22 | 22 | 21 | 1.0 | * | NS | NS |

N. nitrate; L, lipid (maize dark grains); N × L, interaction.
NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

Table 9.10 and the Figures 9.3 and 9.4 show that the increases in acetate and decreases in propionate noted above as time on the treatments increased were consistent across treatments.

**Table 9.10.** Acetate and propionate molar proportions (mmol / mol total VFA) for different treatments.

| Nitrate | Absent | | Present | | | | |
|---|---|---|---|---|---|---|---|
| Lipid | Absent | Present | Absent | Present | SED | Significance | |
| *Acetate* | | | | | | Nitrate | * |
| Adapt | 583 | 556 | 569 | 585 | 7.8 | Lipid | ** |
| Start | 619 | 614 | 645 | 645 | | Nit x Lip | NS |
| End | 640 | 713 | 671 | 664 | | Sample | *** |
| Chamb | 665 | 678 | 684 | 702 | | S x Nit | *** |
| Slaught | 665 | 678 | 658 | 661 | | S x Lip | *** |
| | | | | | | | |
| *Propionate* | | | | | | | |
| Adapt | 248 | 268 | 251 | 219 | 9.2 | Nitrate | *** |
| Start | 212 | 190 | 170 | 172 | | Lipid | * |
| End | 179 | 158 | 168 | 160 | | Nit x Lip | NS |
| Chamb | 176 | 160 | 155 | 146 | | Sample | *** |
| Slaught | 178 | 175 | 166 | 174 | | S x Nit | ** |
| | | | | | | S x Lip | NS |

Preliminary samples (all cattle fed same diet) included in analysis as covariate; acetate (610 mmol / mol total VFA, SEM, 6.7) **; propionate (194 mmol / mol total VFA, SEM 6.3) **.

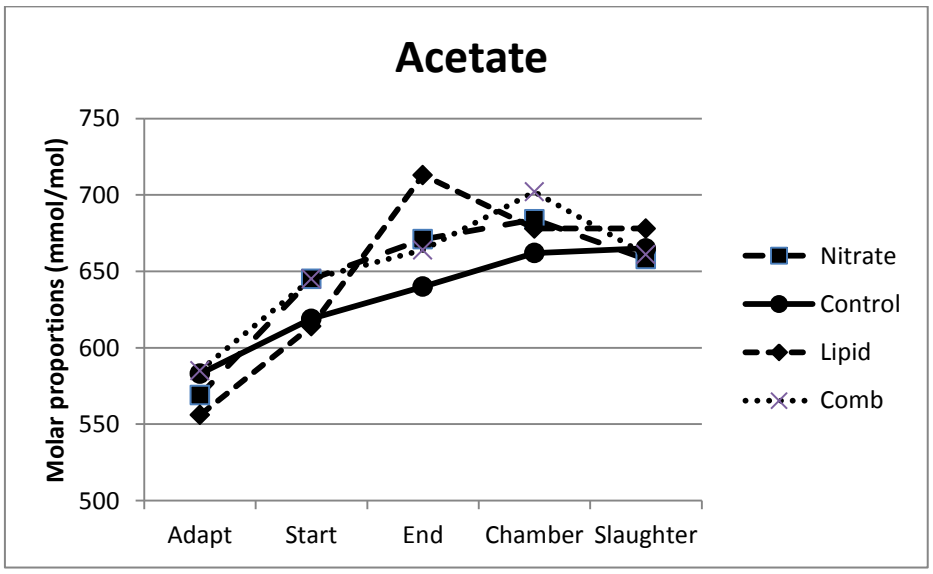


**Figure 9.3:** Change in molar proportions (mmol / mol total VFA) of Acetate throughout the validation study.

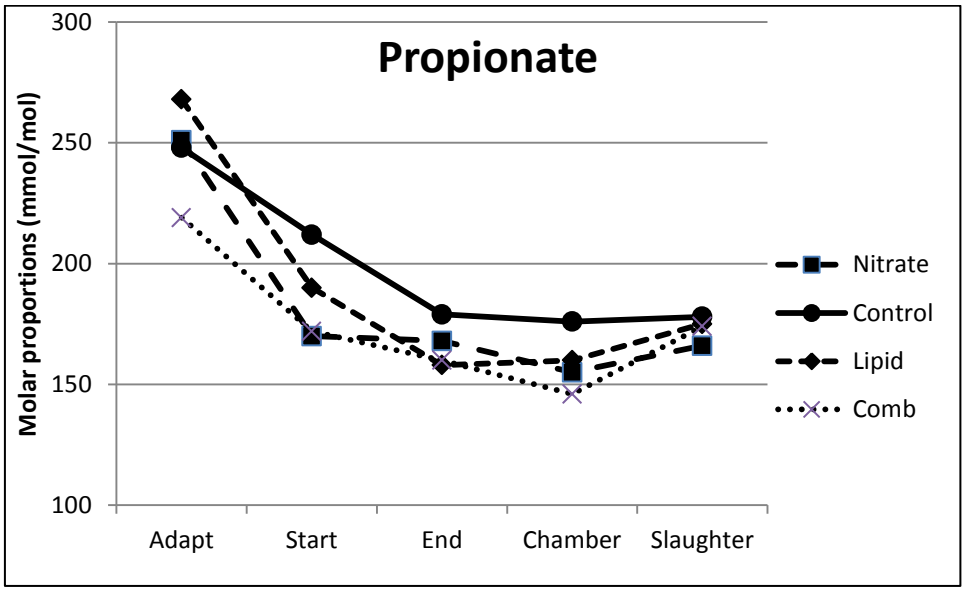


**Figure 9.4:** Change in molar proportions (mmol / mol total VFA) of Acetate throughout the validation study.

Figure 9.5 to 9.8 below showing the differences in acetate and propionate molar proportions between samples obtained in 2013 and 2014 emphasise that apart from the End of 56-d sample for Control and Lipid treatments in 2013, the proportions of acetate and propionate changed little in 2013 while, in 2014, acetate increased and propionate decreased from the first sample (Adapt) until samples were taken from animals as they left the respiration chambers.

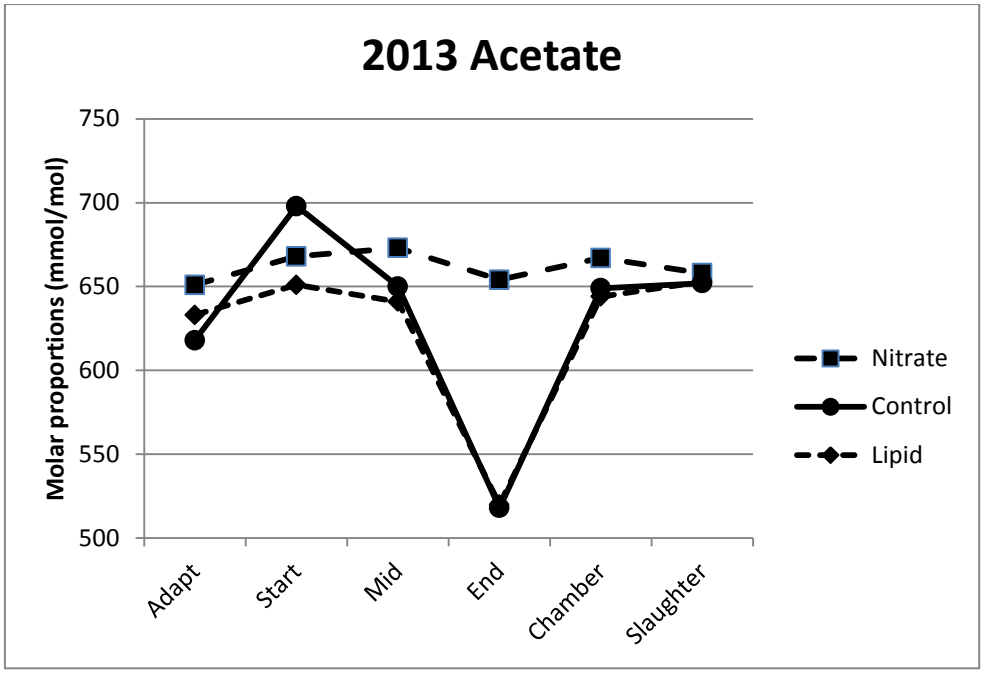


**Figure 9.5:** Change in molar proportions (mmol / mol total VFA) of Acetate throughout the 2013 evaluation study.

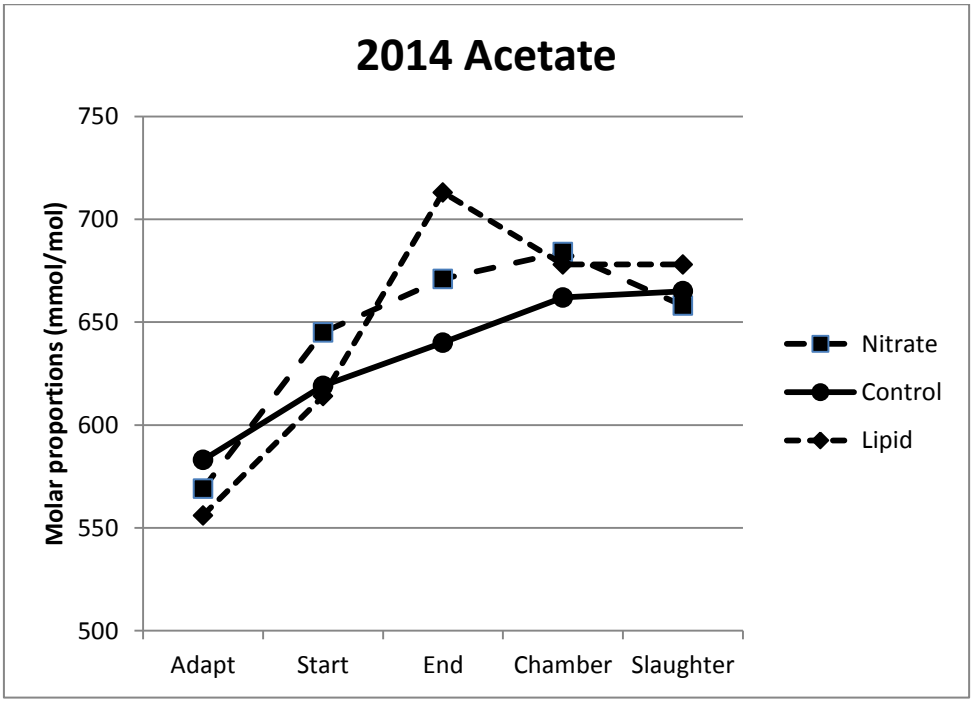


**Figure 9.6.** Change in molar proportions (mmol / mol total VFA) of Acetate throughout the 2014validation study.

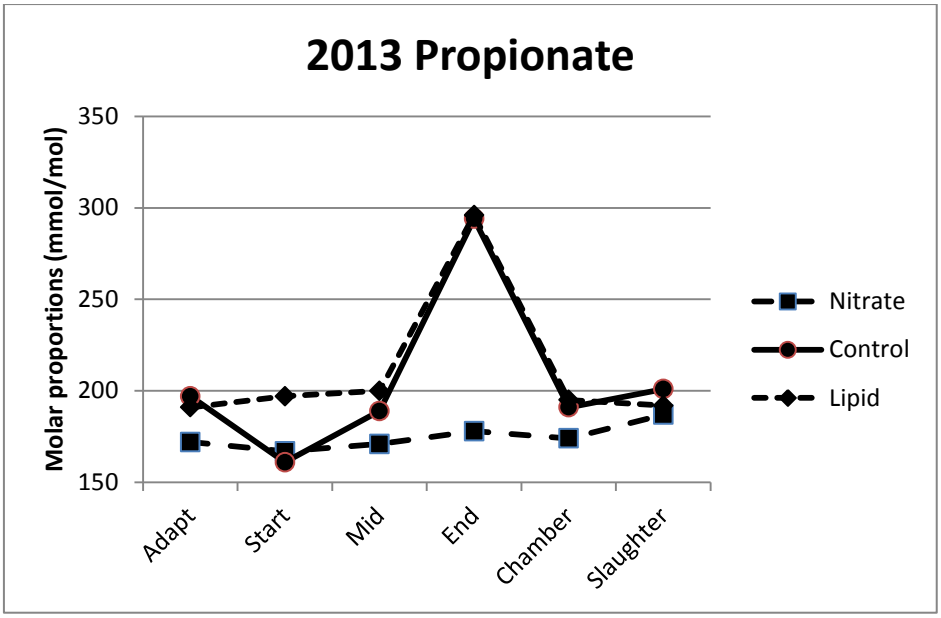


**Figure 9.7:** Change in molar proportions (mmol / mol total VFA) of propionate throughout the 2013 evaluation study.

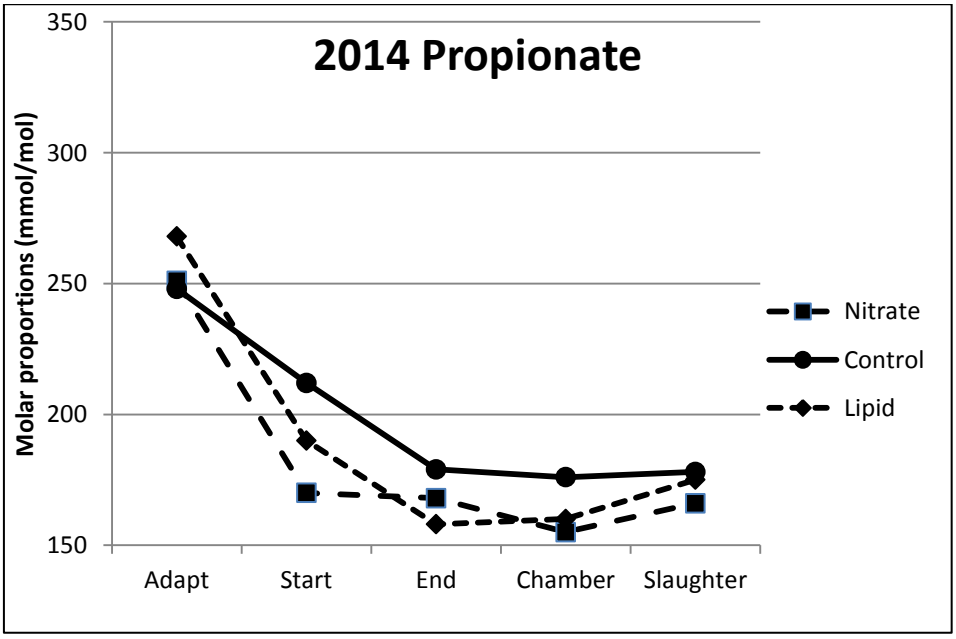


**Figure 9.8:** Change in molar proportions (mmol / mol total VFA) of propionate throughout the 2014 validation study.

## 9.6 Meat quality

### 9.6.1 Vitamin E content

Tables 9.11a shows the Vitamin E content of loin muscle steaks from the two breeds. AAx had more vitamin E than the LIMx. This could be because the AAx on average had more intramuscular fat (IMF) than the LIMx. Vitamin E is present in fat deposits at roughly 5 times that of lean tissue, so fatter animals would tend to have more Vitamin E. Using total fat as covariate reduces the significance from 0.016 to 0.044, still significantly different.

There is no difference in the Vitamin E content of loin steaks derived from animals fed the differing diets (Table 9.11b).

**Table 9.11a.** Effect of breed on Vitamin E content (ug/g) of loin muscle steaks.

| Breed | Count | Mean |
|---|---|---|
| AAx | 36 | 3.48 |
| LIMx | 36 | 3.18 |
| Significance | | * |
| SED | | 0.12 |
| | | |
| With total fat as covariate | | |
| AAx | | 3.50 |
| LIMx | | 3.16 |
| Significance | | * |

AAx, crossbred Aberdeen Angus; LIMx, crossbred Limousin; *, P<0.05.

**Table 9.11b.** Effect of diet treatment on Vitamin E content (ug/g) of loin muscle steaks.

| Treatment | Count | Mean |
|---|---|---|
| Control | 18 | 3.25 |
| Nitrate | 18 | 3.26 |
| Lipid | 18 | 3.43 |
| Combined | 18 | 3.39 |
| Significance | | NS |
| SED | | 0.177 |

NS, not significant.

### 9.6.2 Colour shelf life

Figure 9.9 shows the effect of diet treatment on the colour chroma of loin steaks. Whilst Control, Nitrate and Combined containing diets produced aged loin steaks with a colour shelf-life in modified atmosphere packs of 15-16 days, steaks from animals fed lipid alone in the diet had a shorter colour shelf life of 14-15 days. These values are similar to those obtained in the first trial. When colour shelf-life was assessed as being days taken to reach a chroma value of 18, there was no statistical difference between breeds, diets or breed × diet combinations (P=0.95, 0.074 and 0.063 respectively). This is in line with the Vitamin E concentration of the loin steaks which did not differ due to diet.

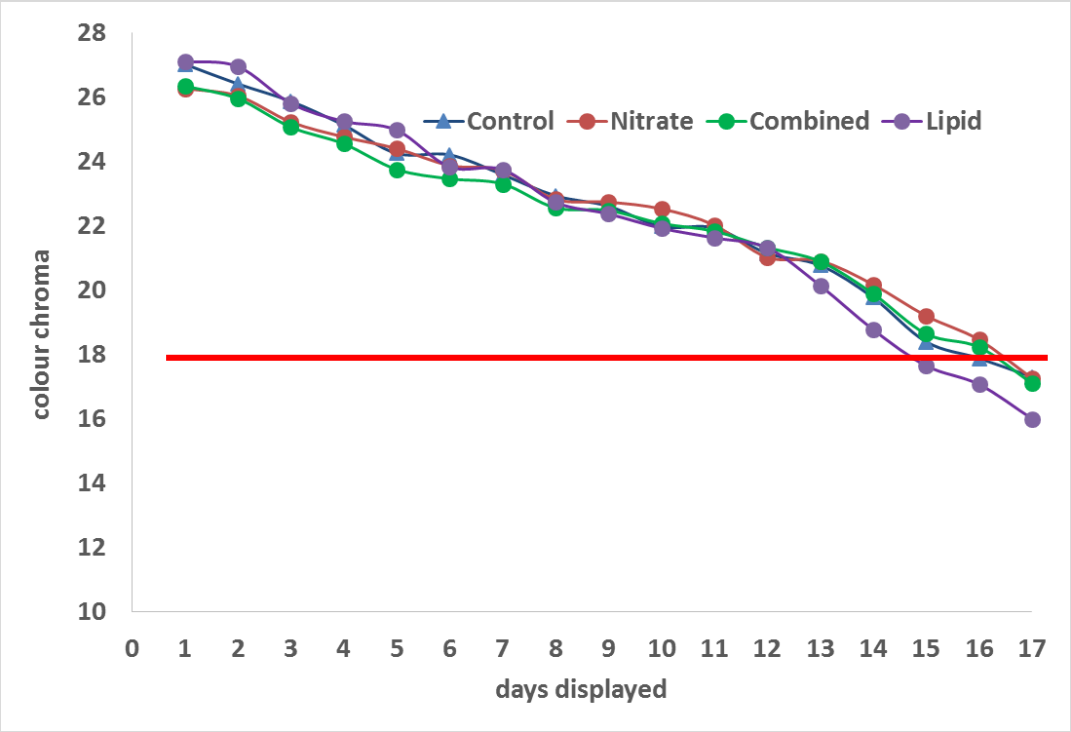


**Figure 9.9:** The effect of diet treatment on the colour chroma of loin steaks displayed in high oxygen modified atmosphere packs.

### 9.6.3 Sensory taste panel assessment

There was no overall effect of diet on the eating quality, tenderness, juiciness or flavour of the beef steaks tested (Table 9.12a).

**Table 9.12a.** Effect of diet treatment on eating quality of grilled beef steak (using the 8 point category scales).

| Treatment<br>Attributes | Control | Nitrate | Lipid | Combined | P | Sig. | SED |
|---|---|---|---|---|---|---|---|
| Tenderness | 5.59 | 5.63 | 5.77 | 5.67 | 0.36 | NS | 0.107 |
| Juiciness | 5.37 | 5.44 | 5.30 | 5.31 | 0.43 | NS | 0.093 |
| Beef Flavour | 4.55 | 4.50 | 4.56 | 4.56 | 0.85 | NS | 0.082 |
| Abnormal Flavour | 2.24 | 2.22 | 2.18 | 2.19 | 0.90 | NS | 0.083 |
| Hedonic<br>Flavour Liking | 5.47 | 5.36 | 5.51 | 5.47 | 0.43 | NS | 0.095 |
| Overall Liking | 5.41 | 5.23 | 5.41 | 5.39 | 0.17 | NS | 0.095 |

NS, not significant.

AAx animals produced steaks which were more tender and juicy than those from LIMx steers, but there was no difference in flavour. The combination of tenderness, juiciness and a tendency for the AAx animals to have more beef flavour resulted in a preference for the Angus steaks both in terms of flavour and overall (Table 9.12b).

**Table 9.12b**. Effect of breed on eating quality of grilled beef steak (using the 8 point category scales).

| Treatment<br>Attributes | AAx | LIMx | P | Significance | SED |
|---|---|---|---|---|---|
| Tenderness | 5.60 | 5.38 | 0.000 | *** | 0.076 |
| Juiciness | 5.53 | 5.18 | 0.000 | *** | 0.066 |
| Beef Flavour | 4.59 | 4.49 | 0.085 | NS | 0.058 |
| Abnormal Flavour | 2.22 | 2.19 | 0.644 | NS | 0.059 |
| Hedonic<br>Flavour Liking | 5.54 | 5.36 | 0.007 | ** | 0.068 |
| Overall Liking | 5.49 | 5.23 | 0.000 | *** | 0.067 |

AAx, crossbred Aberdeen Angus; LIMx, crossbred Limousin;
NS, not significant; **, P<0.01; ***, P<0.001

When each diet × breed value was examined, most of the differences were due to breed (Table 9.12c).

**Table 9.12c.** Effect of breed × treatment group on eating quality of grilled beef steak (using the 8 point category scales).

| Breed | AAx | | | | LIMx | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Combined | Control | Nitrate | Lipid | Combined | P | Sig. | SED |
| Tenderness | 6.02$^{a}$ | 5.78$^{abc}$ | 5.96$^{ab}$ | 6.02$^{ab}$ | 5.13$^{d}$ | 5.48$^{cd}$ | 5.58$^{bcd}$ | 5.32$^{d}$ | 0.000 | *** | 0.152 |
| Juiciness | 5.58$^{ab}$ | 5.71$^{a}$ | 5.39$^{abc}$ | 5.45$^{abc}$ | 5.17$^{c}$ | 5.17$^{c}$ | 5.21$^{bc}$ | 5.18$^{bc}$ | 0.000 | *** | 0.131 |
| Beef Flavour | 4.55 | 4.50 | 4.63 | 4.69 | 4.55 | 4.50 | 4.49 | 4.43 | 0.395 | NS | 0.116 |
| Abnormal Flavour | 2.23 | 2.26 | 2.16 | 2.23 | 2.24 | 2.18 | 2.20 | 2.15 | 0.975 | NS | 0.118 |
| Hedonic | | | | | | | | | | | |
| Flavour Liking | 5.57 | 5.42 | 5.61 | 5.56 | 5.34 | 5.29 | 5.40 | 5.38 | 0.165 | NS | 0.135 |
| Overall Liking | 5.57$^{a}$ | 5.38$^{ab}$ | 5.53$^{a}$ | 5.49$^{ab}$ | 5.25$^{ab}$ | 5.08$^{b}$ | 5.30$^{ab}$ | 5.31$^{ab}$ | 0.005 | ** | 0.134 |

AAx, crossbred Aberdeen Angus; LIMx, crossbred Limousin.
NS, not significant; **, P<0.01; ***, P<0.001.

### 9.6.4 Fatty acids

There were no breed × diet interactions so breed and diet data are presented separately. AAx had more total lipid than LIMx (6.4 *v.* 3.6%, Table 9.13a). This due to more SFA and MUFA, but PUFA concentrations were also significantly higher in AAx than LIMx. When each group was expressed as a proportion of total lipid, SFA and MUFA were still significantly higher in AAx than LIMx. However, PUFA showed a higher proportion in LIMx than AAx. This is because fatter animals with more marbling fat lay down more SFA and MUFA than PUFA.

**Table 9.13a.** Effect of breed on the weights of important groups of fatty acids in *M. longissimus thoracis.*

| Fatty acid | Breed | Mean | Significance |
|---|---|---|---|
| mg/100g tissue | | | |
| Total FA | AAx | 6421 | *** |
| | LIMx | 3632 | |
| SUM SFA | AAx | 2810 | *** |
| | LIMx | 1547 | |
| SUM MUFA | AAx | 2911 | *** |
| | LIMx | 1570 | |
| SUM PUFA | AAx | 212 | ** |
| | LIMx | 190 | |
| SUM n-6 PUFA | AAx | 178 | ** |
| | LIMx | 159 | |
| SUM n-3 PUFA | AAx | 34 | NS |
| | LIMx | 32 | |
| Proportions (g /100g fatty acid) | | | |
| SUM SFA % | AAx | 43.8 | ** |
| | LIMx | 42.3 | |
| SUM MUFA % | AAx | 45.0 | *** |
| | LIMx | 43.0 | |
| SUM PUFA % | AAx | 3.50 | *** |
| | LIMx | 5.58 | |
| SUM n-6 PUFA % | AAx | 2.94 | *** |
| | LIMx | 4.65 | |
| SUM n-3 PUFA % | AAx | 0.56 | *** |
| | LIMx | 0.93 | |
| Ratios | | | |
| P:S ratio | AAx | 0.053 | *** |
| | LIMx | 0.085 | |
| C18;2n-6 to 18:3n-3 | AAx | 8.36 | NS |
| | LIMx | 8.66 | |
| n-6:n-3 ratio | AAx | 5.30 | NS |
| | LIMx | 5.05 | |

NS, not significant; **, P<0.01; ***, P<0.001.

There was no significant difference in the total content of fat, SFA, MUFS or PUFA due to dietary inclusions. The lipid-fed animals had more total and n-6 PUFA and less n-3 PUFA than the other two diets (Table 9.13b). The proportion of n-6 PUFA was still higher for the diet containing only Lipid than the other three, but the n-3 PUFA and total PUFA proportion were not statistically different between diets (Table 9.13c). The n-6 increase was due to C18-2n-6 as seen from the P:S ratio in Table 9.13c and the concentration of C18-2n-6 in Table 9.13d). C18.0 was reduced in all treatment diets compared to the Control. As this is the end product of biohydrogenation of unsaturated fatty acids, it would suggest that some biohydrogenation has been inhibited. However, there was no change in CLA, another product of biohydrogenation, whilst it's intermediate, t18:1n-7 was increased significantly by the Combined diet.

**Table 9.13b.** Effect of diet treatment on the weights of important groups of fatty acids in *M. longissimus thoracis.*

| Fatty acid | Treatment | Mean | SEM | Significance |
|---|---|---|---|---|
| mg/100g tissue | | | | |
| Total FA | Control | 5743 | 375 | NS |
| | Nitrate | 4760 | | |
| | Lipid | 4810 | | |
| | Combined | 4795 | | |
| SUM SFA | Control | 2455 | 166.5 | NS |
| | Nitrate | 2114 | | |
| | Lipid | 2042 | | |
| | Combined | 2102 | | |
| SUM MUFA | Control | 2622 | 180.9 | NS |
| | Nitrate | 2072 | | |
| | Lipid | 2169 | | |
| | Combined | 2100 | | |
| SUM PUFA | Control | 203 | 7.21 | * |
| | Nitrate | 184 | | |
| | Lipid | 215 | | |
| | Combined | 205 | | |
| SUM n-6 PUFA | Control | 165 | 6.41 | ** |
| | Nitrate | 151 | | |
| | Lipid | 183 | | |
| | Combined | 173 | | |
| SUM n-3 PUFA | Control | 36.97 | 1.186 | ** |
| | Nitrate | 32.13 | | |
| | Lipid | 31.41 | | |
| | Combined | 31.14 | | |

NS, not significant; *, P<0.05; **, P<0.01.

**Table 9.13c.** Effect of diet treatment on the proportions and ratios of important groups of fatty acids in *M. longissimus thoracis.*

| Fatty acid | Treatment | Mean | SEM | Significance |
|---|---|---|---|---|
| (mg /100g fatty acid) | | | | |
| SUM SFA % | Control | 42.4 | 0.48 | * |
| | Nitrate | 44.1 | | |
| | Lipid | 42.3 | | |
| | Combined | 43.5 | | |
| SUM MUFA % | Control | 45.3 | 0.47 | ** |
| | Nitrate | 42.8 | | |
| | Lipid | 44.5 | | |
| | Combined | 43.5 | | |
| SUM PUFA % | Control | 3.98 | 0.271 | NS |
| | Nitrate | 4.53 | | |
| | Lipid | 4.96 | | |
| | Combined | 4.70 | | |
| SUM n-6 PUFA % | Control | 3.25 | 0.232 | * |
| | Nitrate | 3.73 | | |
| | Lipid | 4.22 | | |
| | Combined | 3.98 | | |
| SUM n-3 PUFA % | Control | 0.73 | 0.045 | NS |
| | Nitrate | 0.80 | | |
| | Lipid | 0.74 | | |
| | Combined | 0.72 | | |
| Ratios | | | | |
| P:S ratio | Control | 0.06 | 0.004 | * |
| | Nitrate | 0.07 | | |
| | Lipid | 0.08 | | |
| | Combined | 0.07 | | |
| C18;2n-6 to 18:3n-3 | Control | 7.36 | 0.317 | *** |
| | Nitrate | 7.82 | | |
| | Lipid | 7.75 | | |
| | Combined | 9.11 | | |
| n-6:n-3 ratio | Control | 4.52 | 0.173 | *** |
| | Nitrate | 4.75 | | |
| | Lipid | 5.86 | | |
| | Combined | 5.58 | | |

NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

**Table 9.13d.** Effect of diet treatment on the amount of individual fatty acids (mg/100g lean) in *M. longissimus thoracis.*

| Fatty acid | Treatment | Mean | SEM | Significance |
|---|---|---|---|---|
| 14:00 | Control | 167 | 18.4 | NS |
| | Nitrate | 139 | | |
| | Lipid | 130 | | |
| | Combined | 131 | | |
| 15:00 | Control | 19.2 | 2.00 | NS |
| | Nitrate | 15.9 | | |
| | Lipid | 14.0 | | |
| | Combined | 14.9 | | |
| 16:00 | Control | 1520 | 145 | NS |
| | Nitrate | 1324 | | |
| | Lipid | 1252 | | |
| | Combined | 1271 | | |
| 16DMA ald | Control | 35.8 | 0.64 | ** |
| | Nitrate | 33.3 | | |
| | Lipid | 32.9 | | |
| | Combined | 31.5 | | |
| 16:01 | Control | 229 | 23.1 | NS |
| | Nitrate | 191 | | |
| | Lipid | 183 | | |
| | Combined | 176 | | |
| 17:00 | Control | 57.6 | 5.76 | * |
| | Nitrate | 44.8 | | |
| | Lipid | 42.0 | | |
| | Combined | 41.9 | | |
| 18:00 | Control | 763 | 75.2 | NS |
| | Nitrate | 648 | | |
| | Lipid | 656 | | |
| | Combined | 697 | | |
| 18DMA ald | Control | 21.7 | 0.70 | *** |
| | Nitrate | 19.7 | | |
| | Lipid | 22.5 | | |
| | Combined | 22.0 | | |
| tr18:1n-7 | Control | 62.7 | 8.23 | ** |
| | Nitrate | 52.8 | | |
| | Lipid | 61.3 | | |
| | Combined | 84.4 | | |
| 18:1n-9 | Control | 2238 | 220 | NS |
| | Nitrate | 1761 | | |
| | Lipid | 1856 | | |
| | Combined | 1776 | | |
| 18:1n-7 | Control | 79.9 | 6.37 | ** |
| | Nitrate | 59.7 | | |
| | Lipid | 58.5 | | |
| | Combined | 55.2 | | |

NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

**Table 9.13d.** cont.

| Fatty acid | Treatment | Mean | SEM | Significance |
|---|---|---|---|---|
| 18:2n-6 | Control | 116 | 7.9 | ** |
| | Nitrate | 104 | | |
| | Lipid | 131 | | |
| | Combined | 124 | | |
| 18:3n-3 | Control | 16.5 | 1.34 | NS |
| | Nitrate | 13.7 | | |
| | Lipid | 13.7 | | |
| | Combined | 13.8 | | |
| 9c11tCLA | Control | 17.1 | 2.04 | NS |
| | Nitrate | 13.5 | | |
| | Lipid | 14.2 | | |
| | Combined | 18.8 | | |
| 20:4n-6 | Control | 31.6 | 1.06 | NS |
| | Nitrate | 30.2 | | |
| | Lipid | 32.4 | | |
| | Combined | 30.8 | | |
| 20:5n-3 | Control | 4.12 | 0.35 | NS |
| | Nitrate | 3.53 | | |
| | Lipid | 3.53 | | |
| | Combined | 3.23 | | |
| 22:5n-3 | Control | 13.26 | 0.42 | *** |
| | Nitrate | 12.16 | | |
| | Lipid | 11.45 | | |
| | Combined | 11.52 | | |
| 22:6n-3 | Control | 1.514 | 0.127 | NS |
| | Nitrate | 1.251 | | |
| | Lipid | 1.329 | | |
| | Combined | 1.259 | | |

NS, not significant; **, P<0.01; ***, P<0.001.

## 10. VALIDATION STUDY: MAIN CONCLUSIONS

Similar to the evaluation study, following an appropriate adaptation period (four weeks), feeding of nitrate at the level considered here (18 g nitrate/kg diet DM) together with the basal diet type studied did not provide measureable adverse effects in terms of blood MetHb response. The maximum levels reached were 15% and 20.5% of total Hb in the Evaluation and Validation studies, respectively.

In the evaluation study, increasing the concentration of dietary lipid through the use of cold-pressed RSC, did not adversely affect either the performance or feed efficiency of finishing beef steers when used within either a Mixed forage/concentrate diet or a high Concentrate diet. Similarly, in the Validation study, increasing the concentration of dietary lipid within a Mixed forage/concentrate diet (this time through the use of MDG) had no adverse effects on performance or efficiency of finishing beef steers. In comparison to the Evaluation study, however, there were some differences in the performance of steers offered a Mixed diet containing nitrate. Whilst the addition of nitrate to the diet were not shown to adversely affect the performance or efficiency of steers in the evaluation study, steers were shown to have poorer ADG and were less efficient compared to the Control steers in the validation study. It is important to highlight however, that the ADG and FCR values of steers offered Nitrate were within similar ranges to the Evaluation study, but the Control and Lipid fed steers achieved greater levels of performance and efficiency.

The reductions in $CH_4$ observed when either Nitrate or Lipid treatments were imposed on a Mixed forage:concentrate diet were broadly similar for the Evaluation and Validation studies. Nitrate significantly reduced $CH_4$ production in both trials whilst increasing lipid content of the content reduced $CH_4$ numerically but not significantly. The Validation trial further demonstrated that the effects of nitrate and lipid were additive. However, it should be noted that the extent of reductions in $CH_4$ emissions were less marked in the Validation study than the Evaluation study for both nitrate (17 *v.* 9 %) and lipid (7 *v.* 4 %) addition.

**Table 10.1.** Comparison of reductions in $CH_4$ emissions (g/kg DMI) in evaluation and validation trials.

| Trial | Nitrate | $CH_4$ (g / kg DMI) | Lipid | $CH_4$ (g / kg DMI) |
|---|---|---|---|---|
| Evaluation | - | 25.0 | - | 25.0 |
| | + | 20.6 | + | 23.1 |
| | Reduction % | 17 | Reduction % | 7 |
| Validation | - | 23.7 | - | 23.0 |
| | + | 21.5 | + | 22.1 |
| | Reduction % | 9 | Reduction % | 4 |

Overall and in common with the Evaluation study, adding nitrate to the diet increased acetate and decreased propionate molar proportions. Significant increases in butyrate molar proportions were also observed when nitrate was added to the diet in the Validation trial. Whereas in the evaluation trial, increasing dietary lipid had no effect on VFA molar proportions, in the Validation trial, lipid increased acetate and decreased propionate proportions; these differences between trials may result from increased replication for individual treatments in the Validation trial. Whereas in the Evaluation trial, there were no consistent changes in VFA as the trial progressed, in the Validation trial the proportions of acetate increased and propionate decreased as the trial progressed, perhaps suggesting

that there long term changes in the rumen environment in the Validation trial not observed in the Evaluation trial.

The same meat quality tests were applied and compared between treatments. In this validation study there were no significant Vitamin E concentration differences across treatments, contrasting with the previous trial where elevated concentrations were measured in the Lipid diet. Colour shelf life was less in the Lipid treatment compared with all other diet treatments, similar to the findings of the previous trial. Again, similar to the Evaluation study, there were significant breed differences in sensory quality, in this case, AAx having higher overall liking than LIMx. However, there was no treatment effect on any of the sensory attributes of loin meat, confirming there are no antagonistic effects of the dietary $CH_4$ mitigation strategies, including the Combined diet treatment. This was in agreement with the previous trial treatment comparisons. In agreement with the Evaluation study, there were no significant differences in the total content of fat, SFA, MUFA or PUFA due to dietary inclusions, apart from the Lipid treatment, which included more total and n-6 PUFA and less n-3 PUFA than the other treatments.

## 11. REPORT ON METHANE HOODS: EVALUATION AND VALIDATION TRIALS

### 11.1 Material and Methods

$CH_4$ measurements were taken using the hoods during the 56 day performance test period across both experimental years. In order to measure $CH_4$ concentrations a polycarbonate hood was built over each electronic feeder (Figure 11.1 and 11.2). Each hood was positioned approximately 20 mm above the top of each feed bin, so as not to interfere with operation of the feeder. The hoods enclosed the volume above the feed bin on three sides. The fourth side was left open to allow the animals' heads to enter the bins, through an automatic door. The air below each hood was constantly exhausted by a fan located in an opening on top of the hood at a rate of approximately 1 $m^3$/min (Figure 11.1). Exhaust air velocity was measured every second using in-line velocity meters placed in each exhaust air duct. These velocity meters were calibrated weekly using a UKAS calibrated vane anemometer (Testo 417 Anemometer, Testo Ltd, Hampshire, UK), in order to determine air flow ($m^3$/hr) from the velocity measurements. The exhaust air was vented away from the pens and the opening to the hoods. Wind speed in the group pens was recorded as the average speed over a 1 minute interval using a weather station.

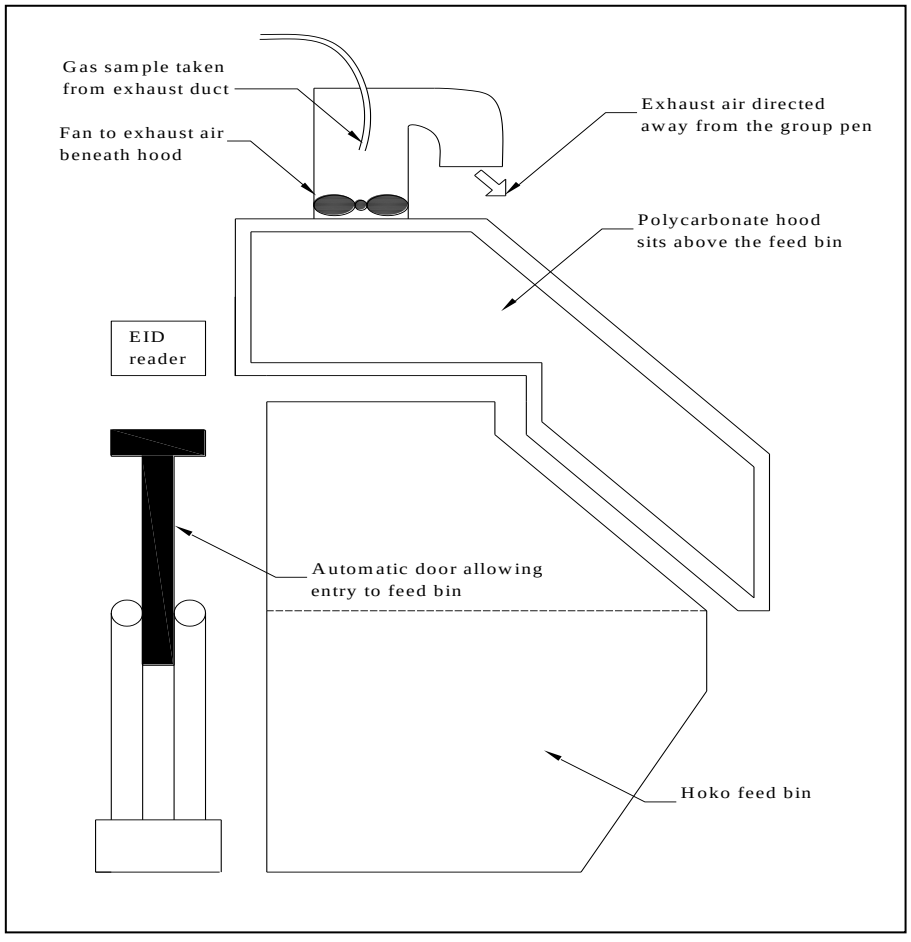


**Figure 11.1**: Schematic of the Hood system used to measure $CH_4$ during feeding.


**Figure 11.2**: Polycarbonate hood positioned above a feed bin.

$CH_4$ concentrations were measured using four infrared multigas analysers, with each analyser measuring samples from eight feeders. Air was drawn continuously from the exhaust air duct above each hood (from between the fan and outlet) to the inlet of one of the gas analysers, using four twin diaphragm pumps. Using a series of solenoid valves, air from each of the eight hoods connected to each analyser was directed sequentially through the analyser. An equilibrium period of 45 sec was allowed before the $CH_4$ concentration was recorded. Since there were eight channels per analyser the concentration of $CH_4$ from each hood was recorded every 6 min. Methane production rates were calculated by multiplying gas concentration by volumetric dry air flow and correcting to standard temperature and pressure (25 $^{o}$C and 101300 Pa). The analysers were calibrated three times per week and the drift in accuracy between calibration bouts was found to be negligible. The hoods and analysers ran continuously during the 56-d measurement phase, apart from a period of approximately 1 h each morning when the hoods were lifted to allow refilling of the feed bins.

The Hoko feeder entry and exit times for each animal were matched with the analyser $CH_4$ concentration for each hood. Feeding bouts of less than 1 min in duration were removed as there was insufficient time to allow the analyser to equilibrate. Feeding bouts greater than 1 minute in duration were used if a gas measurement was taken from the relevant feed bin between 1 minute after the animal's head first entered the feed bin and 30 seconds after the animals head was removed from the feed bin. This 1 min initial cut-off period was required as it took approximately 15 sec to pump the sample from the hood exhaust to the analyser and the analyser then required 45 sec to stabilise. The extra 30 sec following the feeding bout was included again to allow for pumping time to the analysers and also because the $CH_4$ concentrations did not begin to reduce until at least 30 sec after the animal had removed its head from the bin. The average of all $CH_4$ measurements for each steer over the 8 week sampling period was used to calculate an average MH $CH_4$ production rate for each animal (MH-MPR, g/d). Methane yields corrected for DMI were also computed for each animal

(MH-Yld, g/kg DMI). Weekly, fortnightly and four-weekly means for both MH-MPR and MH-Yld were also computed to compare the repeatability of the MH technique across sampling periods, and to determine the minimum sampling time required. Weekly, fortnightly and four-weekly means for both emission values were also computed to compare the repeatability of the hood technique over time, and to determine the minimum sampling time required.

In Experiment 1 (Evaluation study), 10 of the 56 days of hood measurements were discarded due to problems with gas analysers, while in Experiment 2 (Validation study), 10 days were also lost as the Hoko feed bin and gas analyser times were not synchronised. Gas samples taken when the average wind speed was greater than 1.5 m/sec were discarded as high winds reduced the concentration of $CH_4$ measured by the hood.

Following the 56-d measurement phase in both experiments, the steers were incrementally moved, in groups of six per week, from the group pens to the GreenCow respiration chamber facility on the same site. In Experiment 1 (year 2013), 76 animals were subsequently measured in the chamber measurement phase, while in Experiment 2 (year 2014), 72 animals were measured. The animals remained in grouping housing until it was their turn to enter the respiration chamber facility, during which time DMI monitoring continued.

All statistical analyses were performed using SAS. Hood and chamber measurements were analysed using the Mixed procedure in SAS with the fixed effects of breed, diet, and treatment for Experiment 1, and breed, nitrate and lipid for Experiment 2. For the respiration chamber measurements the random effects of week and chamber were included. The interaction effects of breed × diet, diet × treatment and breed × treatment, or breed × nitrate, breed × lipid and nitrate × lipid were also included in the model when these effects proved significant ($P<0.05$). Repeatability of average weekly, fortnightly and four-weekly hood measurements for individual steers and for treatments across all eight sampling weeks were investigated. The data from Experiments 1 and 2 were combined to predict and validate individual animal $CH_4$ outputs (g/day) using measurements taken during the 56-d phase only. To achieve this, approximately 60% of individual animal measurements from each breed × diet × treatment combination across both experiments were randomly allocated to the prediction group (n=88), while the rest were allocated to the validation group (n=58). Models for predicted chamber $CH_4$ outputs (g/day) were computed by linear regression. The prediction models were validated by applying them to the hood measurements for the animals in the validation group, and comparing the level of agreement with chamber measurements. The accuracy of the individual animal predicted $CH_4$ emissions compared to those measured by the chambers were estimated by computing the concordance correlation coefficient, with a value of 1 denoting perfect agreement and 0 denoting no agreement.

## 11.2 Results

### 11.2.1 Repeatability of the Hood system

In Experiment 1, a greater number of daily hood measurements were recorded from animals receiving the Mixed diet (18.4±0.47) compared with animals fed the Concentrate diet (14.8±0.66; $P<0.001$). Treatment had no effect on the number of hood samples captured from each animal ($P>0.05$). The rate of sample capture depended on the amount of time spent eating per day and the length of feeding bouts. For Experiment 2, on average 14.3±0.35 hood measurements per day were captured from each animal on the Mixed diet. Again, treatment had no effect on the number of hood samples captured from each animal ($P>0.05$).

For both Experiment 1 and Experiment 2 there was significant differences in individual animal average weekly MH-MPR values (P<0.001), average fortnightly MH-MPR values (P<0.001), and average four-weekly MH-MPR values (P<0.01). For Experiment 1, the ranking of individual animals based on weekly, fortnightly and four-weekly MH-MPR values was more consistent for animal fed the Mixed diet than those fed the Concentrate diet. For animals fed the Mixed diet each individual weekly, fortnightly and four-weekly MH-MPR ranking deviated from the 8-week MH-MPR ranking by an average of 5.2, 3.2 and 2.1 ranking places, respectively. However, for those animals fed on the Concentrate diet, this average deviation was 8.0, 6.7 and 4.2 ranking places, for weekly, fortnightly and four-weekly MH-MPR values respectively. Similarly for Experiment 2, the ranking of animals based on MH-MPR values improved when longer time-periods were used; each individual MH-MPR ranking deviated from the 8-week MH-MPR ranking by an average of 7.2, 5.5 and 3.5 ranking places, for weekly, fortnightly and four-weekly rankings, respectively.

### 11.2.2 Comparison of dietary effects across both methods

*Dry matter intake*

For Experiment 1, during the hood measurement phase steers offered the Mixed diet had a greater daily DMI than those offered the Concentrate diet (P<0.001, Table 11.1). This also tended to be the case during the chamber measurement phase (P=0.05). The addition of nitrate or rapeseed cake to either basal diet did not affect DMI during either measurement phase (P>0.05). Dry matter intake measured during the hood measurement phase (11.6 kg/day) was similar to average DMI measured in the group pens in the 4 weeks prior to individual animals' chamber measurement phase (11.3 kg/day; P=0.60). However, individual animal DMI during the chamber measurement phase was on average 14% lower than the hood measurement phase, and 12.5% lower than the DMI measured in the 4 weeks pre-chamber measurement (P<0.001).

**Table 11.1.** Dry matter intakes (DMI, kg/day), hood (MH) and respiration chamber (RC) measurement phases for Experiment 1.

| Basal Diet | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SEM | D | T | D × T[1] |
| MH-DMI | 12.0 | 12.1 | 11.9 | 11.0 | 10.8 | 11.0 | 0.34 | *** | NS | NS |
| DMI pre-RC[2] | 11.6$^{ab}$ | 12.5$^{b}$ | 11.5$^{ab}$ | 11.4$^{ab}$ | 10.1$^{a}$ | 11.1$^{ab}$ | 0.41 | ** | NS | * |
| RC-DMI | 9.8 | 10.4 | 10.5 | 10.0 | 9.1 | 9.7 | 0.53 | * | NS | NS |

D, Diet, T; Treatment, D × T; Diet × Treatment interaction.
[1] Where there are significant diet x treatment effects, differences are displayed using superscripts: within each row, means without a common superscript differ (P < 0.05).
[2] Average DMI as measured in the group pen environment for the 4 weeks immediately prior to the animal entering the chamber facility.
NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

For Experiment 2, during the hood and chamber measurement phases neither the addition of nitrate nor increasing the dietary lipid content adversely affected DMI (Table 11.2). Dry matter intake measured during the hood measurement phase (11.6 kg/day) was similar to average DMI measured in the group pens in the 4 weeks prior to individual animals' chamber measurement phase (11.3 kg/day; P=0.33). However, individual animal DMI during the chamber measurement phase was on average 12.4% lower than during both the hood measurement phase, and 9.7% lower than the DMI measured in the 4 weeks pre-chamber measurement (P<0.001).

**Table 11.2.** Dry matter intakes (DMI, kg/day), from the hood (MH) and respiration chamber (RC) measurement phases for Experiment 2.

| Basal Diet | Mixed | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Combined | SEM | Nitrate | Lipid | Nitrate × Lipid[1] |
| MH-DMI | 11.8 | 11.4 | 11.8 | 11.6 | 0.32 | NS | NS | NS |
| DMI pre-RC[2] | 11.3 | 10.9 | 11.4 | 11.5 | 0.28 | NS | NS | NS |
| RC-DMI | 10.4 | 9.8 | 10.2 | 10.2 | 0.43 | NS | NS | NS |

[1] Where there are nitrate x fat interaction effects, differences are displayed using superscripts: within each row, means without a common superscript differ (P < 0.05).
[2] Average DMI as measured in the group pen environment for the 4 weeks immediately prior to the animal entering the chamber facility.
NS, not significant.

*Methane measurement*

For experiment 1, MH-MPR and MH-Yld values from the animals which received the Mixed diet were significantly higher than from the animals fed the Concentrate diet (P<0.001; Table 11.3). Similarly, chamber $CH_4$ emissions were higher from the steers who received the Mixed diet compared with those steers who received the Concentrate diet, when measured per day and per kg DMI (P<0.001).

The addition of nitrate to the Mixed diet resulted in reduced MH-MPR and MH-Yld values compared with the Control (P<0.001). However, when nitrate was added to the Concentrate diet there was no reduction in MH-MPR (P=1) or MH-Yld (P=1). Similarly, when measured in the chambers, the animals who received the Nitrate treatment had lower $CH_4$ output (g/day) and yield (g/kg DMI) than the animals who received the Control treatment (P<0.01), however the addition of nitrate to the Concentrate diet caused no reduction in daily $CH_4$ output (P=0.28) or $CH_4$ yield (P=0.62). The addition of rapeseed cake to both diets did not reduce MH-MPR (P=0.54) or MH-Yld (P=0.69) values, nor was there a reduction in daily chamber $CH_4$ output (P=0.90) or yield (P=0.67), when compared with the Control treatments.

**Table 11.3.** Average $CH_4$ concentration (MH-MPR, g/day) and $CH_4$ concentration corrected for DMI (MH-Yld, g/kg DMI) from the hood measurement phase, and $CH_4$ output (g/day) and yield (g/kg DMI) measured in chambers for Experiment 1.

| Basal Diet | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SEM | D | T | D×T[1] |
| Hood Measurement Phase | | | | | | | | | | |
| MH-MPR | 88.8[c] | 61.4[b] | 84.8[c] | 39.3[a] | 36.8[a] | 38.1[a] | 2.42 | *** | *** | *** |
| MH-Yld | 7.4[c] | 5.1[b] | 7.2[c] | 3.6[a] | 3.4[a] | 3.5[a] | 0.19 | *** | *** | *** |
| Chamber Measurement Phase | | | | | | | | | | |
| $CH_4$, g/day | 241 | 212 | 242 | 149 | 136 | 150 | 9.97 | *** | * | NS |
| $CH_4$,g/kg DMI | 25.1[c] | 20.6[b] | 23.2[c] | 14.6[a] | 15.3[a] | 15.8[a] | 0.96 | *** | NS | * |

[1] Where there are significant diet x treatment effects, differences are displayed using superscripts: within each row, means without a common superscript differ (P<0.05).
NS, not significant; *, P<0.05; ***, P<0.001.

The dietary and treatment effects on MH-MPR values were persistent over time; when the 8 week Hd measurement phase is split into two 4-week periods and analysed separately, similar reductions associated with feeding the Concentrate diet compared to the Mixed diet (P<0.001), and with the addition of nitrate to the Mixed diet are found in both periods (P<0.001). Similarly, neither period showed a decrease in MH-MPR values when nitrate was added to the Concentrate diet (P=0.98 and P=0.97 for periods 1 and 2, respectively), or when lipid was added to either diet (P=0.86 and P=0.27 for periods 1 and 2, respectively).

For Experiment 2, the addition of nitrate resulted in reduced MH-MPR and MH-Yld values compared with treatments which contained no nitrate (P<0.001; Table 11.4). Similarly, during the chamber phase steers who received nitrate amended treatments had lower daily $CH_4$ output and $CH_4$ yield (P<0.001). MH-MPR (P<0.05) and MH-Yld (P<0.01) values were both lower for the Lipid treatment when compared with the Control. However, increasing the lipid content did not result in any reductions in daily chamber $CH_4$ output (P=0.37) or $CH_4$ yield (P=0.12).

**Table 11.4.** Average $CH_4$ concentration (MH-MPR, ppm) and $CH_4$ concentration corrected for DMI (MH-Yld, g/kg DMI) from the hood measurement phase, and $CH_4$ output (g/day) and yield (g/kg DMI) measured in chambers for Experiment 2.

| Basal Diet | Mixed | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Combined | SEM | Nitrate | Lipid | Nitrate × Lipid[1] |
| Hood Measurement Phase | | | | | | | | |
| MH-MPR | 109.2[c] | 67.6[a] | 98.0[b] | 73.9[a] | 2.57 | *** | NS | ** |
| MH-Yld | 9.3[c] | 6.0[a] | 8.3[b] | 6.4[a] | 0.20 | *** | NS | ** |
| Chamber Measurement Phase | | | | | | | | |
| $CH_4$, g/day | 245.5 | 218.6 | 238.2 | 209.9 | 9.35 | *** | NS | NS |
| $CH_4$, g/kg DMI | 24.0 | 22.1 | 23.4 | 20.9 | 0.69 | *** | NS | NS |

[1] Where there are nitrate x fat interaction effects, differences are displayed using superscripts: within each row, means without a common superscript differ ($P < 0.05$).
Similarly to Experiment 1, the effects of the addition of nitrate on MH-MPR values were persistent over time when they are analysed separately as 2 four-week periods ($P<0.001$). However, increasing the fat content of the diet had no effect on MH-MPR emissions in the first period ($P=0.89$), while there was a tendency for the treatments with higher fat to reduce MH-MPR values in the second period ($P=0.08$).
NS, not significant; **, $P<0.01$; ***, $P<0.001$.

### 11.2.3 Prediction of individual animal emissions from methane Hood system

There was a gap of between 2 and 13 weeks between the final week of hood measurement phase and the individual animal measurements in the chamber. Despite this gap, there was good agreement when the individual animal MH-MPR values were directly correlated with their subsequent daily chamber $CH_4$ output (Experiment 1: $R^2=0.64$, Experiment 2: $R^2=0.24$; $P<0.001$). Similarly, when both measurements were corrected for DMI there was good agreement between individual animal MH-Yld values and the subsequent chamber $CH_4$ yield (g/kg DMI; Experiment 1: $R^2=0.64$, Experiment 2: $R^2=0.31$; $P<0.001$). Experiment 2 had a smaller range of $CH_4$ emissions than Experiment 1 as a result of only having 1 basal diet type. This resulted in poorer correlations when the individual animal hood values were directly compared with their subsequent chamber measurements.

The models used to predict individual animal $CH_4$ emissions (g/day) from hood measurements are given in Table 11.5. The model (M6) with the highest correlation used MH-MPR, MH-DMI, DMI-Ratio and Diet factors. The DMI-Ratio factor is used to correct for the change in individual animal chamber-DMI compared to MH-DMI. The best model which did not use the diet factor was model M4. Using models M4 and M6 on all the data across both experiments, there is good correlation between Predicted-$CH_4$ and RC measured $CH_4$ (M4: expt. 1 $R^2=0.71$, expt. 2 $R^2=0.63$; M6: expt. 1 $R^2=0.74$, expt. 2 $R^2=0.68$). Using M6 only the concordance correlation coefficient between measured and predicted $CH_4$ output was 0.83 for experiment 1, 0.79 for experiment 2, and 0.85 when both experiments were combined.

Using M6 only the concordance correlation coefficient between measured and predicted $CH_4$ output was 0.83 for experiment 1, 0.79 for experiment 2, and 0.85 when both experiments were combined. When all animals measured in the RC phase were ranked by both RC-$CH_4$ (g/d) and M6 predicted chamber $CH_4$ (g/d), the average deviation of animals predicted $CH_4$ ranking from the measured $CH_4$ ranking was 8.5 in experiment 1 and 10.8 ranking places and experiment 2.

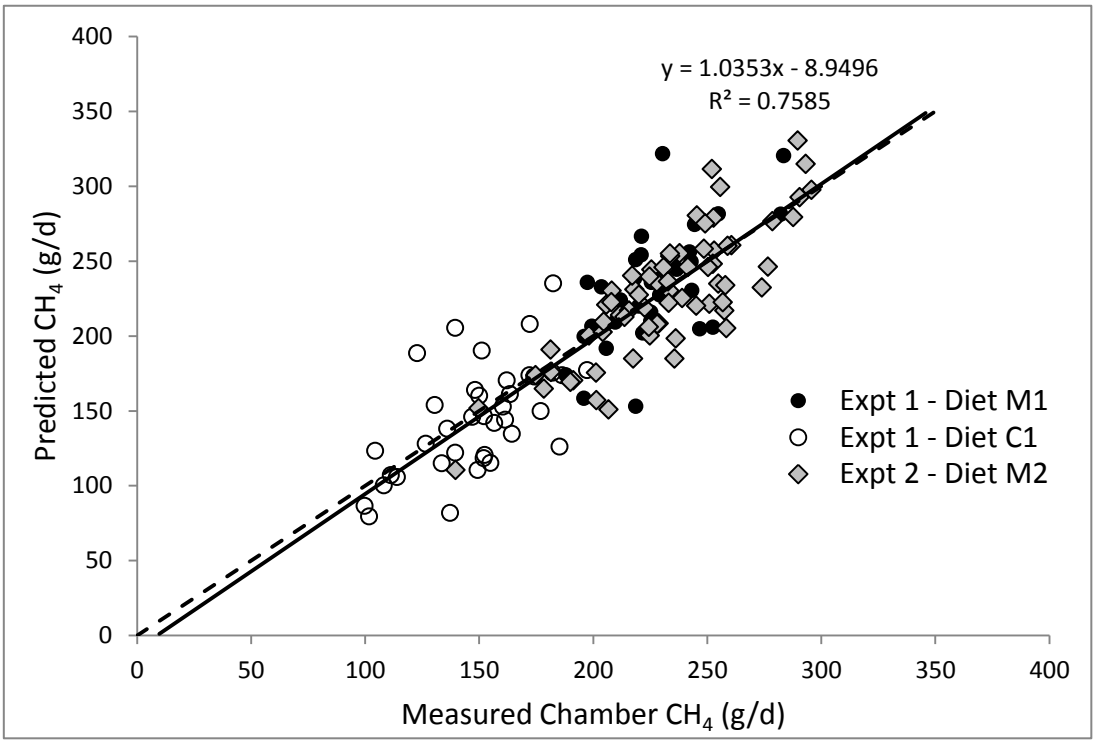


**Figure 11.3:** Relationship between individual animal predicted $CH_4$ output (Model M6) and $CH_4$ output as measured by the chambers in two experiments (n = 147). Dashed line indicated x = y. Lin's concordance coefficient = 0.86. For individual experiments $R^2$=0.74 for both Experiment 1 and 2. M1 refers to the Mixed diet in Experiment 1; C1, the Concentrate diet in Experiment 1; M2 the Mixed diet in Experiment 2.

**Table 11.5.** Accuracy of models to predict and validate methane (g/d) from respiration chambers (RC) based on measurements taken during the methane hood (MH) measurement period.

| Model | Methane Prediction Models[1] | $R^2$ (prediction group)[2] | $R^2$ (validation group)[2] | P (validation group)[3] | Concordance (validation group) |
|---|---|---|---|---|---|
| M1 | 107.1 + 10.4 MH-DMI – 71.6 Diet | 0.56 | 0.64 | < 0.0001 | 0.79 |
| M2 | 75.73 + 0.77 MH-MPR + 7.30 MH-DMI – 40.6 Diet | 0.63 | 0.67 | < 0.0001 | 0.78 |
| M3 | -123.8 + 18.5 MH-DMI + 134.6 DMI-Ratio | 0.30 | 0.39 | < 0.0001 | 0.55 |
| M4 | -121.4 + 1.34 MH-MPR + 8.23 MH-DMI + 153.4 DMI-Ratio | 0.72 | 0.70 | < 0.0001 | 0.82 |
| M5 | -20.2 + 11.6 MH-DMI + 129.6 DMI-Ratio – 70.8 Diet | 0.67 | 0.68 | < 0.0001 | 0.79 |
| M6 | -72.1 + 0.91 MH-MPR + 8.20 MH-DMI + 144.9 DMI-Ratio – 34.3 Diet | 0.77 | 0.75 | < 0.0001 | 0.84 |
| M7 | 27.0 + 1.28 MH-MPR + 7.27 MH-DMI | 0.54 | 0.61 | < 0.0001 | 0.74 |
| M8 | 151.0 + 0.90 MH-MPR – 40.5 Diet | 0.60 | 0.57 | < 0.0001 | 0.70 |

[1] MH-MPR is the average $CH_4$ output over the 8 week MH sampling period (g/d), MH-DMI is the average daily DMI during this 8 week sampling period (kg), DMI-Ratio is the ratio of DMI during the RC measurement phase with DMI during the MH measurement phase (RC-DMI/ MH-DMI), and diet type is a correction for diets (mixed forage and concentrate diet = 0 and high concentrate diet = 1).

[2] Prediction group, n=88; validation group, n=58.

[3] The P-value here tells us whether the relationship between the measured chamber methane output and the chamber output as predicted from the model are significant for the validation group; with the 'null hypothesis' being 'hood values and chamber values are unrelated'.

The dietary effects calculated using each individual animals' Predicted-$CH_4$ emissions are similar those calculated from the measured chamber $CH_4$ emissions (see Table 11.6 for Experiment 1 and Table 11.7 for Experiment 2).

**Table 11.6.** $CH_4$ output (g/day) predicted using Model M4 and measured using chambers for Experiment 1.

| Basal Diet | Mixed | | | Concentrate | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Control | Nitrate | Lipid | SEM | D | T | D×T |
| Predicted-$CH_4$ | 232 | 213 | 238 | 159 | 145 | 150 | 5.9 | *** | * | NS |
| Chamber-$CH_4$ | 241 | 212 | 242 | 149 | 136 | 150 | 9.97 | *** | * | NS |

D, Basal Diet; T, Treatment; D×T, Basal Diet × Treatment interaction.
NS, not significant; *, P<0.05; ***, P<0.001.

**Table 11.7.** $CH_4$ output (g/day) predicted using Model M4 and measured using chambers for Experiment 2.

| Basal Diet | Mixed | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Control | Nitrate | Lipid | Combined | SEM | Nitrate | Lipid | Nitrate×Lipid |
| Predicted-$CH_4$ | 263 | 221 | 232 | 217 | 5.8 | *** | * | NS |
| Chamber-$CH_4$ | 246 | 219 | 238 | 210 | 9.4 | *** | NS | NS |

NS, not significant; *, P<0.05; ***, P<0.001.

## 11.3 Conclusions

The hood system can be used to estimate $CH_4$ output from individual beef cattle in a group housed environment. The predicted $CH_4$ output has been shown to be consistent with the $CH_4$ output measured in respiration chambers. The consistency of this ranking of individual animal emissions and the high throughput possible using the hood system supports the use of this system as a tool for the genetic selection of cattle based on $CH_4$ emissions. Furthermore, the timescale required to gather the hood data is similar to the timescale required to determine performance (56-d), and both sets of measurements can be taken simultaneously. The average ranking based on hood measured $CH_4$ concentrations for individual animals were similar for 4 and 8 week sampling times. Therefore, in future a measurement period of 4 weeks may be sufficient to rank animals based on $CH_4$ emissions. However, an 8-week sampling period is advised to prediction individual animal $CH_4$ yield from hood measurements.

## 12. PROFITABILITY, ECONOMIC ANALYSIS AND RECOMMENDATIONS

As part of the overall evaluation of potential dietary $CH_4$ mitigation strategies, a full Gross and Net Margin calculation has been undertaken for all treatments studied during both experiments carried out during 2013 and 2014. The main factors in each experiment for which the financial evaluation has been obtained are:-

| | |
|---|---|
| Breed of steer | (CHx and LU in 2013: AAx and LIMx in 2014) |
| Basal diet used (2013 only) | Forage to Concentrate ratios (g/kg DM): (i) 50:50 (Mixed) and (ii) 8:92 (Concentrate) |
| Mitigation strategy used | Nitrate (Calcinit) or Lipid (RSC in 2013 or MDG in 2014) |

The statistical significance of these main treatment factors along with their major interactions has been assessed.

### 12.1 Approaches taken to calculate profitability and economic analysis

In both years, the actual variable costs incurred from the start of daily DMI recording until slaughter for all animals reaching the end of the trial have been used to calculate the financial performance up to the Gross Margin stage.

In contrast, the fixed costs at a research unit such as the Beef and Sheep Research Centre (BSRC) are atypical and unrepresentative of most farming situations. Consequently, the average fixed costs from both the AHDB Beef and Lamb "Stocktake Report" and the QMS "Cattle and Sheep Enterprise Profitability in Scotland" report have been assumed and applied to the actual Gross Margin figures obtained to calculate a Net Margin figure.

Fixed costs from the 2013 Stocktake Report (AHDB, 2013) and the 2014 edition of the QMS publication (QMS, 2014) have been used since at the time of calculation, they are the most recent figures available that relate to the year ending March 2013 in both cases.

Once relevant output, input and margin calculations were completed the results were statistically analysed by analysis of variance using Genstat 16 to allow the main experimental factors to be compared.

#### 12.1.1 Gross Margin calculations

Actual sale prices on a CCW basis (p/kg CCW) and total sale values (£/head) were obtained from the commercial abattoir where all cattle were slaughtered. In both years an initial "Feeders Margin" was calculated on both a £/head basis and a £/head/day basis. This feeder's margin was simply the difference between the sale value of the animals obtained from the abattoir and the "store" value of the animal at the start of the DMI recording period.

The store value was calculated using the BW at the start and the average price per kg BW paid for the animals when they had been purchased a few weeks earlier. Where animals were home bred then the average price for the value of the animals purchased was assumed to apply also to home bred animals of the same breed type.

The next stage in the financial calculations was to calculate total feed costs from the actual DMI figures from the start to slaughter obtained using the SRUC Beef and Sheep Research Centre feed intake facilities and the actual prices paid by the farm for each feed used in the trial.

Average DMI/day and total DMI consumed throughout the period were calculated and the total feed cost per tonne of complete diet DM applied to these total feed usage figures to obtain a total feed cost on a £/head and a £/head/day basis.

Margin over Feed and Forage (MOFF) figures on a £/head and a £/head/day basis were then calculated.

Estimates of wood-fine bedding material usage (10.9 kg/head/day) was combined with its purchase price and days on trial for each animal to calculate a bedding cost along with a Margin over Feed, Forage and Bedding (MOFFB) figure, again on a £/head and a £/head/day basis.

Other variable costs were then assessed and included vet and medical costs (£3.40 /head for 1 respiratory vaccination), a haulage charge at £28 /head in 2013 or £19 /head in 2014, an abattoir killing charge at £11.60 /head, a levy payment at £4.20 /head and a livestock sundries charge assumed to be £1 /head to cover items such as replacement tags. Since the farm had its own borehole, no water charges were included. Each of these figures did actually apply or were assumed to apply to each steer on trial.

Total variable costs on a £/head and a £/head/day basis were then calculated and subtracted from the Feeders Margin to give a Gross Margin figure on a £/head or a £/head/day basis for each animal.

### 12.1.2 Net Margin calculations

Following the Gross Margin calculation, an average fixed cost daily rate was assumed from figures published by both AHDB Beef and Lamb and QMS as noted above. These daily fixed cost rates cover labour, buildings, machinery, land and capital costs associated with various classes of beef finishing business surveyed by these respective organisations each year. A total of five categories of beef finishing business ranging from short duration, mainly concentrate fed systems to longer term, mainly forage fed enterprises were included in the datasets yielding these figures. The average daily fixed cost rate was 0.73 p/head/day across the reported systems and this figure was applied along with the number of days from start of DMI recording to slaughter to calculate a total fixed cost figure for each animal on trial on a £/head and a £/head/day basis.

A Net Margin or "profit" figure was then calculated for each animal as the difference between the Gross Margin and fixed cost figures, again expressed on a £/head and a £/head/day basis.

## 12.2 Financial analysis results

To ensure both completeness and ease of reading the results from both 2013 and 2014 have been presented firstly as a summary table of all diets studied along with an assessment of the main factors of significance and then each of the main factor means (breed, basal diet and mitigation strategy) and their statistical analysis have been presented separately in a subsequent table for the major margin figures only. Since only one basal diet was used in 2014, the results have been presented as summary

tables for each of the four diets studied with the main experimental factor means (breed and mitigation strategy) being listed in a final table.

### 12.2.1 Evaluation study (2013): evaluation of Nitrate (Calcinit) and Lipid (Rapeseed cake) as dietary $CH_4$ mitigation strategies

The sale prices, values and a Feeders Margin calculation are given in Table 12.1 whilst the feed inputs, feed costs and Margin over Feed and Forage (MOFF) calculations are given in Table 12.2. The bedding inputs and costs along with a MOFF plus bedding (MOFFB) calculation are given in Table 12.3 along with the total variable cost figures for each of the breed × diets studied. Similarly, Gross Margin, fixed cost estimates and Net Margin calculations for each basal diet × breed × treatment interaction are given in Table 12.4. The main breed (CHx and LU) along with the main basal diet (Mixed and Concentrate) and mitigation treatment (Control, Nitrate and Lipid) effects for the major margin calculations are given in Table 12.5.

Days on trial were not significantly different for all groups of steers at 165 days (5.4 months) with a small price premium of approximately 4.8 p/kg CCW obtained for the CHx compared with the LU steers (416.5 *v.* 411.7 p/kg CCW respectively). This mainly reflects the price premium paid by the abattoir in relation to the superior carcass conformation of the CHx compared with the LU steers.

Sale values ranged from £1422 – £1792 /head leaving a Feeders Margin ranging from £415 – £532 /head after the initial store value had been taken into account. When expressed on a daily basis the Feeders Margin ranged from £2.58 – £3.16 /head/day.

Due to the high feed barley price of £200/t during 2013, total feed costs were also high during this trial, particularly for the concentrate basal diet type as might be expected. Overall feed costs per tonne fresh weight (£/t FW) were 92.03, 88.15 and 84.96 for the Forage based Control, Nitrate and Lipid diets respectively whilst they were 210.81, 204.72 and 202.10 for the Concentrate Control, Nitrate and Lipid diets. Similarly feed costs on a dry matter (£/t DM) basis were 189.36, 183.26 and 175.53 for the Forage based Control, Nitrate and Lipid diets respectively whilst they were 245.99, 239.44 and 234.73 for the Concentrate Control, Nitrate and Lipid diets.

Feed costs ranged from £2.04 – £2.77 /head/day on average across the trial groups (Table 12.2). This left a MOFF ranging from £0 – £150 /head or £0 – £0.87 /head/day. Once the bedding and other variable cost had been added the total variable costs ranged from £422 – £558 /head or £2.64 – £3.38 /head/day across the trial groups under study (Table 12.3).

Gross Margin figures (Table 12.4) ranged from its highest level of £49.70 /head to a low of £-97.40 /head depending on the particular group in question. Fixed costs were fairly similar at £113-126/head and since the daily fixed costs were assumed from published values this reflected the slightly different number of days on trial for animals across the groups. As expected, Net Margin figures were negative for all groups studied in this trial and ranged from -£74 to -£216 /head or -£0.47 to -£1.34 /head/day.

**Table 12.1.** Sale prices and cattle values along with a "Feeders Margin" calculation for Nitrate and Lipid (rapeseed cake) dietary $CH_4$ mitigation strategies in Charolais crossbreds (CHx) and Luing (LU) finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

| Basal diet | Mixed | | | | | | Concentrate | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | | Nitrate | | Lipid | | Control | | Nitrate | | Lipid | | SED | | | Significance | | |
| Breed | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | B | T | D | B | T | D |
| Days on trial | 170 | 167 | 160 | 170 | 160 | 172 | 162 | 167 | 170 | 162 | 170 | 155 | 5.2 | 6.3 | 5.2 | | | |
| Sale Price (p/kg CCW) | 416.7 | 412.0 | 416.6 | 413.3 | 416.6 | 410.7 | 416.1 | 413 | 416 | 409.1 | 416.7 | 412.3 | 1.05 | 1.28 | 1.05 | *** | | |
| Sale value (£/hd) | 1792 | 1524 | 1699 | 1581 | 1690 | 1582 | 1736 | 1509 | 1740 | 1422 | 1711 | 1487 | 23.5 | 28.7 | 23.5 | *** | | |
| Store value (£/hd) | 1283 | 1104 | 1284 | 1117 | 1226 | 1101 | 1246 | 1021 | 1207 | 1007 | 1189 | 1038 | 27.4 | 33.5 | 27.4 | *** | * | |
| Feeders Margin (£/hd) | 509 | 420 | 415 | 464 | 464 | 482 | 490 | 488 | 532 | 416 | 522 | 449 | 21.5 | 26.3 | 21.5 | | | |
| Feeders Margin (£/hd/d) | 2.99 | 2.65 | 2.58 | 2.77 | 2.91 | 2.79 | 3.02 | 2.95 | 3.16 | 2.60 | 3.08 | 2.89 | 0.111 | 0.136 | 0.111 | | | |

B = Breed effects, T = dietary treatment effects and D = basal diet effects.
*, P<0.05; ***, P<0.001.

**Table 12.2.** Feed inputs and costs along with a "Margin over feed and forage" calculation for Nitrate and Lipid (rapeseed cake) dietary $CH_4$ mitigation strategies in Charolais crossbreds (CHx) and Luing (LU) finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

| Basal diet | Mixed | | | | | | Concentrate | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | | Nitrate | | Lipid | | Control | | Nitrate | | Lipid | | SED | | | Significance | | |
| Breed | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | B | T | D | B | T | D |
| Total DMI (t) | 1.90 | 1.94 | 1.85 | 2.03 | 1.85 | 1.99 | 1.80 | 1.87 | 1.82 | 1.74 | 1.86 | 1.71 | 0.037 | 0.045 | 0.037 | | ** | |
| Feed costs (£/hd) | 359 | 368 | 339 | 372 | 325 | 349 | 443 | 459 | 436 | 416 | 436 | 401 | 7.8 | 9.5 | 7.8 | | *** | |
| Feed costs (£/hd/day) | 2.13 | 2.32 | 2.15 | 2.20 | 2.04 | 2.05 | 2.77 | 2.77 | 2.59 | 2.61 | 2.59 | 2.61 | 0.056 | 0.068 | 0.056 | | * | *** |
| MOFF (£/hd) | 150 | 53 | 76 | 92 | 139 | 132 | 47 | 29 | 96 | 0 | 86 | 48 | 18.4 | 22.4 | 18.4 | * | | * |
| MOFF (£/hd/d) | 0.85 | 0.33 | 0.43 | 0.56 | 0.87 | 0.75 | 0.25 | 0.18 | 0.57 | 0.00 | 0.50 | 0.28 | 0.110 | 0.135 | 0.110 | * | | ** |

B = Breed effects, T = dietary treatment effects and D = basal diet effects.
Complete diet feed costs (£/t DM) were: Forage : Control - 189.36; Nitrate – 183.26; Lipid – 175.53:
Concentrate : Control - 245.99; Nitrate – 239.44; Lipid – 234.73:
MOFF = Margin over feed and forage costs.
*, P<0.05; **, P<0.01; ***, P<0.001.

**Table 12.3.** Bedding and other variable costs along with a "Margin over feed, forage and bedding" calculation for Nitrate and Lipid (rapeseed cake) dietary $CH_4$ mitigation strategies in Charolais crossbreds (CHx) and Luing (LU) finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

| Basal diet | Mixed | | | | | | Concentrate | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | | Nitrate | | Lipid | | Control | | Nitrate | | Lipid | | SED | | | Significance | | |
| Breed | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | B | T | D | B | T | D |
| Bedding cost (£/hd) | 52 | 49 | 49 | 52 | 49 | 52 | 49 | 51 | 52 | 49 | 52 | 48 | 1.6 | 1.9 | 1.6 | | | |
| MOFFB (£/hd) | 97.9 | 3.9 | 27.4 | 40.1 | 90.6 | 79.9 | -2.7 | -22.0 | 44.0 | -49.2 | 34.3 | 1.0 | 17.91 | 21.87 | 17.91 | * | | ** |
| MOFFB (£/hd/d) | 0.54 | 0.02 | 0.13 | 0.26 | 0.56 | 0.44 | -0.06 | -0.13 | 0.26 | -0.31 | 0.19 | -0.03 | 0.10 | 0.135 | 0.110 | * | | ** |
| Total variable costs (£/hd) | 459 | 465 | 436 | 472 | 422 | 450 | 541 | 558 | 537 | 513 | 536 | 496 | 9.0 | 10.9 | 9.0 | | * | *** |
| Total variable costs (£/hd/d) | 2.73 | 2.94 | 2.76 | 2.80 | 2.66 | 2.64 | 3.38 | 3.37 | 3.18 | 3.22 | 3.18 | 3.24 | 0.064 | 0.078 | 0.064 | | | *** |

B = Breed effects, T = dietary treatment effects and D = basal diet effects.
Bedding costs (£/t DM) were: wood fines bedding material at £28/t and an assumed daily usage of 10.9 kg/day for all animals.
MOFFB = Margin over feed, forage and bedding costs. Other variable costs included Vet and med @ 3.40, Haulage @ 28.0, abattoir charge @ 11.6, QMS levy @ 4.20, livestock sundries @ 1.00 giving a total other variable cost of £48.20/head.
*, P<0.05; **, P<0.01; ***, P<0.001.

**Table 12.4.** Gross and Net Margin calculation for Nitrate and Lipid (rapeseed cake) dietary $CH_4$ mitigation strategies in Charolais crossbreds (CHx) and Luing (LU) finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

| Basal diet | Mixed | | | | | | Concentrate | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | | Nitrate | | Lipid | | Control | | Nitrate | | Lipid | | SED | | | Significance | | |
| Breed | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | CHx | LU | B | T | D | B | T | D |
| Gross Margin (£/hd) | 49.7 | -44.3 | -20.8 | -8.1 | 42.4 | 31.7 | -50.9 | -70.2 | -4.2 | -97.4 | -13.9 | -47.2 | 17.9 | 21.9 | 17.9 | * | | * |
| Gross Margin (£/hd/d) | 0.26 | -0.29 | -0.18 | -0.03 | 0.25 | 0.16 | -0.36 | -0.42 | -0.02 | -0.62 | -0.10 | -0.35 | 0.113 | 0.138 | 0.113 | * | | ** |
| Fixed costs (£/hd) | 124 | 117 | 117 | 124 | 117 | 126 | 118 | 122 | 124 | 118 | 124 | 113 | 3.8 | 4.6 | 3.8 | | | |
| Net Margin (£/hd) | -74 | -161 | -138 | -132 | -74 | -94 | -169 | -192 | -128 | -216 | -138 | -132 | 17.4 | 21.2 | 17.4 | * | | ** |
| Net Margin (£/hd/d) | -0.47 | -1.01 | -0.91 | -0.76 | -0.47 | -0.57 | -1.09 | -1.15 | -0.75 | -1.34 | -0.83 | -1.08 | 0.113 | 0.138 | 0.113 | * | | ** |

B = Breed effects, T = dietary treatment effects and D = basal diet effects.
Fixed costs = £0.73/day x days on trial (average data for AHDB and QMS surveys for the 2013 financial year).
*, P<0.05; **, P<0.01.

**Table 12.5.** Gross and Net Margin calculations for the main breed and dietary $CH_4$ mitigation strategies in finishing steers during the evaluation study (2013) managed on two contrasting basal diets.

| | Breed | | | | Treatment | | | | | Diet | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CHx | LU | SED | Sig. | Control | Nitrate | Lipid | SED | Sig. | Mixed | Concentrate | SED | Sig. |
| Gross Margin (£/hd) | 0.79 | -38.62 | 17.910 | * | -27.40 | -31.69 | 4.34 | 21.870 | | 8.51 | -46.73 | 17.910 | * |
| Gross Margin (£/hd/d) | -0.02 | -0.25 | 0.113 | * | -0.20 | -0.21 | 0.00 | -0.138 | | 0.03 | -0.31 | 0.113 | ** |
| Fixed costs (£/hd) | 120.70 | 120.00 | 3.780 | | 120.20 | 120.80 | 119.90 | 4.613 | | 120.70 | 120.00 | 3.780 | |
| Net Margin (£/hd) | -120 | -159 | 17.4 | * | -148 | -153 | -116 | 21.2 | | -112 | -167 | 17.4 | ** |
| Net Margin (£/hd/d) | -0.75 | -0.98 | 0.113 | * | -0.93 | -0.94 | -0.73 | 0.138 | | -0.70 | -1.04 | 0.113 | ** |

B = Breed effects, T = dietary treatment effects and D = basal diet effects.
Fixed costs = £0.73/day x days on trial (average data for AHDB and QMS surveys for the 2013 financial year).
*, P<0.05; **, P<0.01.

Results from the main experimental factor analysis in Table 12.5 show that breed had a significant effect ($P<0.05$) on both the Gross and Net Margin figures. CHx steers made a Gross Margin of approximately zero whilst LU steer GM was -£39 per head on average. Once fixed costs of approximately £120 per head were taken into account the respective Net Margin figures were -£120 and -£159 per head for the CHx and LU steers on average across all diets studied.

This was mainly due to the significantly higher ($P<0.001$) sale value of the CHx compared with the LU steers (£1728 *v.* £1518) and the slightly lower feed costs of the CHx cattle as a result of lower total feed intakes, primarily of the Mixed basal diet (1.86 *v.* 1.99 t DMI for CHx *v.* LU, respectively).

With respect to the comparison between the basal diets, the Mixed diet showed an average Gross Margin of £8.51/head whilst the Concentrate diet average figure was -£46.73/head. Fixed costs again averaged £120/head for both basal diets leaving a significantly different ($P<0.01$) Net Margin for the Mixed and Concentrate diets of -£112 and -£167 per head respectively. Concentrate price per tonne for barley and other concentrate feeds was mainly responsible for this difference between basal diets.

As shown in Table 12.5, neither the Nitrate nor the Lipid $CH_4$ mitigation strategies had any statistically significant effect on any of the Gross or Net Margin parameters in this study.

Gross Margin figures were approximately -£27, -£32 and +£4 per head for the Control, Nitrate and Lipid treatments, respectively. With fixed costs again averaging approximately £120/head the corresponding Net Margin figures were -£148, -£153 and -£116 per head across the same three mitigation strategy dietary treatments.

Overall the main factors leading to statistically significant differences in this study were the main breed and/or basal diet effects. The mitigation strategy treatments had relatively little statistical significance to the bottom line profitability figures.

The only slight effect of mitigation strategy treatments was in feed costs where the relative costs of the various treatments sometimes had a statistically significant ($P<0.05$), albeit small influence on feed cost levels.

### 12.2.2 Validation study (2104): evaluation of Nitrate and Lipid either alone or in combination in finishing diets for steers

The sale prices, values and a Feeders Margin calculation are given in Table 12.6 whilst the feed inputs, feed costs and Margin over Feed and Forage (MOFF) calculations are given in Table 12.7. The bedding inputs and costs along with a MOFF plus bedding (MOFFB) calculation are given in Table 12.8 along with the total variable cost figures for each of the breed × diet studied. Similarly, Gross Margin, fixed cost estimates and Net Margin calculations for each breed × diet interaction are given in Table 12.9.

Finally, the main breed (AAx and LIMx), along with the main treatment (Control, Nitrate, Lipid and Combined), effects for the major margin calculations are given in Table 12.10.

Days on trial were similar for all groups of animals at 176 days (5.8 months) with a price premium of approximately 17.5 p/kg CCW obtained for the AAx compared with the LIMx steers (393.1 *v.* 373.6 p/kg CCW, respectively). This reflects the price premium paid by the abattoir rather than any general price premium paid for AA sired cattle across the industry.

Sale values ranged from £1402 – £1517 /head leaving a Feeders Margin ranging from £298 – £407 /head after the initial store value had been taken into account. When expressed on a daily basis the Feeders Margin ranged from £1.67 – £2.49 /head/day.

Due to the lower feed barley price of £134 /t during 2014 rather than the £200 /t during 2013 feed costs were lower during this trial and ranged from £1.43 – £1.76 /head/day on average across the trial groups. This left a MOFF ranging from £29 – £133 /head or £0.12 – £0.73 /head/day. Once the bedding and other variable cost had been added the total variable costs ranged from £340 – £398 £/head or £1.96 – £2.29 /head/day across the trial groups under study. Overall feed costs per tonne fresh weight (£/t FW) were 77.47, 73.70, 79.77 and 77.58 for the Control, Nitrate, Lipid and Combined diets respectively. Similarly feed costs on a dry matter (£/t DM) basis were 145.34, 138.80, 149.67 and 145.56 for the Control, Nitrate, Lipid and Combined diets respectively.
Gross Margin figures ranged from its highest level of £41.60 /head to a low of -£63.10 /head depending on the particular group in question.

Fixed costs were fairly similar at £126-£131 /head and since the daily fixed costs were assumed from published values this reflected the slightly different number of days on trial for animals across the groups.

As expected, Net Margin figures were negative for all groups studied and ranged from -£85 to -£191 /head or -£0.53 to -£1.14 /head/day.

**Table 12.6.** Sale prices and cattle values along with a "Feeders Margin" calculation for Nitrate, Lipid (Maize Distillers Dark Grains) and the Combined (Nitrate and Lipid) dietary $CH_4$ mitigation strategies in crossbred Aberdeen Angus (AAx) and crossbred Limousin (LIMx) finishing steers during the validation study (2014).

| Treatment | Control | | Nitrate | | Lipid | | Combined | | SED | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Breed | AAx | LIMx | AAx | LIMx | AAx | LIMx | AAx | LIMx | B | T | B×T | B | T | B×T |
| Days on trial | 177 | 177 | 177 | 175 | 173 | 179 | 175 | 175 | 8.0 | 5.6 | 11.3 | | | |
| Sale Price (p/kg CCW) | 394.3 | 374.2 | 389.0 | 373.6 | 393.3 | 374.5 | 387.8 | 372.3 | 2.23 | 1.58 | 3.15 | *** | | |
| Sale value (£/hd) | 1517 | 1486 | 1480 | 1417 | 1491 | 1445 | 1488 | 1402 | 24.8 | 17.6 | 35.1 | ** | * | |
| Store value (£/hd) | 1110 | 1116 | 1097 | 1090 | 1055 | 1107 | 1119 | 1104 | 41.2 | 29.2 | 58.3 | | | |
| Feeders Margin (£/hd) | 407 | 370 | 383 | 327 | 436 | 338 | 369 | 298 | 36.2 | 25.7 | 51.3 | * | | |
| Feeders Margin (£/hd/d) | 2.25 | 2.09 | 2.14 | 1.85 | 2.49 | 1.86 | 2.10 | 1.67 | 0.154 | 0.109 | 0.218 | *** | | |

B = Breed effects, T = dietary treatment effects and B x T = their interaction.
*, P<0.05; **, P<0.01; ***, P<0.001.

**Table 12.7.** Feed inputs and costs along with a "Margin over feed and forage" calculation for Nitrate, Lipid (Maize Distillers Dark Grains) and the Combined (Nitrate and Lipid) dietary $CH_4$ mitigation strategies in crossbred Aberdeen Angus (AAx) and crossbred Limousin (LIMx) finishing steers during the validation study (2014).

| Treatment | Control | | Nitrate | | Lipid | | Combined | | SED | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Breed | AAx | LIMx | AAx | LIMx | AAx | LIMx | AAx | LIMx | B | T | B×T | B | T | B×T |
| Total DMI (t) | 2.05 | 1.93 | 2.08 | 1.79 | 2.02 | 1.91 | 2.10 | 1.84 | 0.074 | 0.052 | 0.104 | *** | | |
| Feed costs (£/hd) | 298 | 280 | 289 | 248 | 303 | 286 | 305 | 268 | 10.7 | 7.5 | 15.1 | *** | * | |
| Feed costs (£/hd/day) | 1.70 | 1.60 | 1.66 | 1.43 | 1.76 | 1.55 | 1.76 | 1.61 | 0.058 | 0.041 | 0.082 | *** | * | |
| MOFF (£/hd) | 109 | 90 | 94 | 79 | 133 | 52 | 64 | 29 | 32.5 | 23.0 | 46.0 | | | |
| MOFF (£/hd/d) | 0.55 | 0.49 | 0.47 | 0.42 | 0.73 | 0.25 | 0.34 | 0.12 | 0.178 | 0.126 | 0.252 | | | |

B = Breed effects, T = dietary treatment effects and B x T = their interaction.
Complete diet feed costs (£/t DM) were: Control - 145.34; Nitrate – 138.80; Lipid – 149.67; Combined – 145.56.
MOFF = Margin over feed and forage costs.
*, P<0.05; ***, P<0.001.

**Table 12.8.** Bedding and other variable costs along with a "Margin over feed, forage and bedding" calculation for Nitrate, Lipid (Maize Distillers Dark Grains) and the Combined (Nitrate and Lipid) dietary $CH_4$ mitigation strategies in crossbred Aberdeen Angus (AAx) and crossbred Limousin (LIMx) finishing steers during the validation study (2014).

| Treatment | Control | | Nitrate | | Lipid | | Combined | | SED | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Breed | AAx | LIMx | AAx | LIMx | AAx | LIMx | AAx | LIMx | B | T | B×T | B | T | B×T |
| Bedding cost (£/hd) | 53.9 | 53.9 | 53.9 | 53.2 | 52.6 | 54.5 | 53.2 | 53.2 | 2.43 | 1.72 | 3.44 | | | |
| MOFFB (£/hd) | 55.4 | 35.9 | 40.2 | 25.6 | 80.7 | -2.1 | 10.4 | -23.9 | 31.07 | 21.97 | 43.94 | | | |
| MOFFB (£/hd/d) | 0.24 | 0.19 | 0.17 | 0.12 | 0.43 | -0.05 | 0.03 | -0.18 | 0.178 | 0.126 | 0.252 | | | |
| Total variable costs (£/hd) | 391 | 373 | 382 | 340 | 395 | 380 | 398 | 361 | 12.3 | 8.7 | 17.5 | ** | * | |
| Total variable costs (£/hd/d) | 2.24 | 2.13 | 2.19 | 1.96 | 2.29 | 2.14 | 2.29 | 2.09 | 0.065 | 0.046 | 0.092 | *** | | |

B = Breed effects, T = dietary treatment effects and B x T = their inter-action.
Bedding costs (£/t DM) were: wood fines bedding material at £28/t and an assumed daily usage of 10.9 kg/day for all animals.
MOFFB = Margin over feed, forage and bedding costs. Other variable costs included Vet and med @ 3.40, Haulage @ 19.0, abattoir charge @ 11.6, QMS levy @ 4.20, livestock sundries @ 1.00 giving a total other variable cost of £39.20/head.
*, P<0.05; **, P<0.01; ***, P<0.001.

**Table 12.9.** Gross and Net Margin calculations for Nitrate, Lipid (Maize Distillers Dark Grains) and the Combined (Nitrate and Lipid) dietary $CH_4$ mitigation strategies in crossbred Aberdeen Angus (AAx) and crossbred Limousin (LIMx) finishing steers during the validation study (2014).

| Treatment | Control | | Nitrate | | Lipid | | Combined | | SED | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Breed | AAx | LIMx | AAx | LIMx | AAx | LIMx | AAx | LIMx | B | T | B×T | B | T | B×T |
| Gross Margin (£/hd) | 16.2 | -3.3 | 1.0 | -13.6 | 41.6 | -41.3 | -28.9 | -63.1 | 31.07 | 21.97 | 43.94 | | | |
| Gross Margin (£/hd/d) | 0.02 | -0.04 | -0.06 | -0.11 | 0.20 | -0.28 | -0.20 | -0.41 | 0.184 | 0.130 | 0.261 | | | |
| Fixed costs (£/hd) | 129 | 129 | 129 | 127 | 126 | 131 | 127 | 128 | 5.8 | 4.1 | 8.2 | | | |
| Net Margin (£/hd) | -113 | -132 | -128 | -141 | -85 | -172 | -156 | -191 | 28.2 | 19.9 | 39.8 | | | |
| Net Margin (£/hd/d) | -0.71 | -0.77 | -0.79 | -0.84 | -0.53 | -1.01 | -0.93 | -1.14 | 0.184 | 0.13 | 0.261 | | | |

B = Breed effects, T = dietary treatment effects and B × T = their interaction.
Fixed costs = £0.73/day x days on trial (average data for AHDB and QMS surveys for the 2013 financial year).

**Table 12.10.** Gross and Net Margin calculations for the main breed and dietary treatment effects in finishing steers during the validation study (2014).

| | Breed effects | | | | Treatment effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AAx | LIMx | SED | Sig. | Control | Nitrate | Lipid | Combined | SED | Sig. |
| Gross Margin (£/hd) | 7.49 | -30.33 | 21.972 | | 6.47 | -6.31 | 0.10 | -45.96 | 31.073 | |
| Gross Margin (£/hd/d) | -0.01 | -0.21 | 0.130 | | -0.01 | -0.08 | -0.04 | -0.31 | 0.184 | |
| Fixed costs (£/hd) | 127.80 | 128.60 | 4.110 | | 129.00 | 128.20 | 128.20 | 127.50 | 5.820 | |
| Net Margin (£/hd) | -120.53 | -158.94 | 19.917 | | -122.52 | -134.53 | -128.12 | -173.42 | 28.168 | |
| Net Margin (£/hd/d) | -0.74 | -0.94 | 0.130 | | -0.74 | -0.81 | -0.77 | -1.04 | 0.184 | |

Fixed costs = £0.73/day x days on trial (average data for AHDB and QMS surveys for the 2013 financial year).

Results from the main experimental factor analysis in Table 12.10 show that, neither breed nor dietary mitigation treatment had any statistically significant effect on the Gross or Net Margin figures in this study.

However, once again, breed did influence both the Gross and Net Margin figures albeit at a non-statistically significant level. AAx steers made a Gross Margin of approximately £7 whilst LIMx steer GM was -£30 per head on average. Once fixed costs of approximately £128 per head were taken into account the respective Net Margin figures were -£120 and -£159 per head for the AAx and LIMx steers on average across all diets studied.

These Net Margin figures were, almost unbelievably exactly the same as the Net Margin figures of -£120 and -£159 per head for the CHx and Luing steers in 2013.

Despite the non-significance of the breeds on the overall Gross Margin and Net Margin figures, it is worth pointing out that they were achieved via slightly different routes. The AAx animals did have significantly higher ($P<0.01$) sale values (£1494 *v.* £1437 /head respectively) but also had higher feed costs (£299 *v.* £271 /head respectively) due to lower overall feed consumption levels by the LIMx animals.

Similar to the results from 2013, neither the Nitrate, Lipid nor the Combined dietary $CH_4$ mitigation strategies had any statistically significant effect on any of the Gross or Net Margin parameters in this study.

Gross Margin figures were approximately +£6, -£6, £0 and -£46 per head for the Control, Nitrate, Lipid and Combined treatments, respectively. With fixed costs again averaging approximately £128/head the corresponding Net Margin figures were -£123, -£135, -£128 and -£173 per head across the same four dietary treatments respectively. This once again confirms that alternative $CH_4$ dietary mitigation strategies have relatively little statistical significance to the bottom line profitability figures.

However, it is probably worth highlighting that the Combined treatment where both nitrate and lipid feeds were fed was the diet where the lowest numerical Gross Margin and Net Margin figures were recorded.

## 12.3 Discussion, conclusions and key financial recommendations

(1) The profitability of finishing steers in both years was highly dependent on four key factors. These were: sale value, "store" value at start and both total feed and fixed costs. Farmers should focus on the factors from this list that are directly within their own control when seeking to optimise profit from their beef finishing operations. In particular the importance of days to finish should be stressed rather than over-emphasising features such as £/tonne of barley or deadweight sale price per kg CCW. The "store" values (pence/kg LW) were £2.65 for CHx, £2.30 for Luing and £2.60 for both AAx and LIMx steers respectively and were representative of typical purchase values at the start of the trials. Using the AHDB levy charge of £4.05 per head rather than the QMS charge of £4.20 per head would have made a minimal difference to the bottom line profitability figures. However, using the AHDB only fixed costs of £0.98 per head per day rather than the average of both QMS and AHDB figures at £0.73 per head per day would have added an average of £41 per head to the fixed costs in the evaluation study. Similarly, using the AHDB only fixed costs would have added an average of £44 per head to the fixed costs in the validation study.

(2) Breed of cattle can have a significant impact on profitability of finishing enterprises on farm. Depending on the circumstances either continental sired cattle or native UK sired cattle can yield better margin figures. In 2013 CH sired cattle returned better margin figures than LU sired cattle, whereas in 2014 AA sired cattle returned better margin figures than LIM sired cattle. The key features determining overall margin superiority of any breed type were size and conformation of carcass along with any price premiums obtained (carcass value) along with both feed and fixed costs. Aligning cattle type to market outlet will also be an important feature of financial success in beef finishing units.

(3) The suitability of basal diet type can vary with year depending on the cost of concentrate feeds. Barley price was £200/t in 2013 but dropped to £134/t in 2014. This had a major impact on the total feed costs across the years, even in the Mixed based diet where concentrates still made up approximately 50% of the diet DM. However, the price of barley per tonne should not be the only important feature to consider here since changing the basal diet can have fundamental impacts on the overall nature of the production system on the farm, especially in relation to the number of days to slaughter under practical farming conditions. Balancing feed costs per tonne along with total feed and fixed costs per carcass is one of the key arts in the success or failure of a beef cattle finishing business. Advice and management on farm should focus on these areas to a significant degree.

(4) Incorporating feedstuffs with high lipid content into diets for finishing cattle can be recommended on the basis of the results from this body of work with a number of qualifying remarks. Both RSC and MDG proved suitable feeds from these studies with no practical problems or adverse financial consequences. Both Gross and Net Margin levels were generally similar to Control diets used here. However, care should be taken to ensure that overall lipid levels do not exceed 6% of complete diet DM so as to avoid any impairments of rumen digestive function which can occur with excessively high lipid diets. In addition, the prevailing price of high lipid feeds should be compared with alternatives such as Rapeseed Meal when feed purchasing decisions are being made to ensure the most financially attractive choices are being made.

(5) Although the Nitrate diets did not have major adverse consequences for animal health or performance compared with the Control diets in both studies, the overall financial figures were slightly lower in both cases. Given that there is no financial upside to feeding nitrate as a $CH_4$ mitigation strategy and no financial reward for reducing $CH_4$ output at present from any market or subsidy driven income stream the question remains: can feeding nitrate to finishing cattle be recommended? Given that there is no financial upside and that the potential downside of nitrate toxicity is dramatic if its inclusion in diets is managed badly; then it can be concluded that feeding nitrate to finishing cattle could only be considered and recommended following some change to income streams that would provide farmers with an incentive to mitigate $CH_4$.

(6) It cannot be recommended that both the Nitrate and Lipid dietary $CH_4$ mitigation strategies be combined together at the same time since this did result in a slightly poorer financial performance overall. The Combined treatment did reduce the overall Net Margin by £42 per head compared with the average of either the Nitrate or the Lipid diets fed singly. Whilst this difference was not statistically significant it is financially important. Any finishing farm with 1000 cattle per year to finish would regard an additional loss of £42/head important (i.e. a total of £42,000 less profit per year).

(7) From an overall financial perspective, the feeding of high lipid straight feedstuffs to finishing cattle can be recommended since they have few adverse consequences for productivity provided

excessive total diet lipid levels are avoided (> 6% lipid). Whilst they will reduce $CH_4$ output to some extent, there are currently no financial incentives for farmers to do so. Consequently, any decision on feeding high lipid feeds (e.g. RSC or MDG studied here) should be made on the relative feed costs associated with using these feeds compared to alternative straight ingredients. On this basis it can be recommended that these feeds are used by practicing farmers.

(8) Overall recommendation based on financial outcomes

Whilst nitrate feeding has some advantages in terms of reducing $CH_4$, in both trials studied here its use was less financially attractive. In addition, to avoid the potential downside in terms of animal toxicity, a careful diet preparation and an appropriate adaptation period has to be considered. Consequently, nitrate feeding cannot be recommended to practical farmers at this stage. Should any of the financial incentives surrounding the reduction of $CH_4$ emissions change however, then this recommendation can be reviewed at any time. Feeding high lipid feedstuffs in finishing cattle diets can be recommended provided its use is economically competitive and excessive lipid levels in the total diet are avoided.

## 13. LIST OF PROJECT OUTPUTS: REFEREED PUBLICATIONS


Duthie C-A, Rooke JA, Hyslop JJ, Ross DW, Waterhouse A and Roehe R 2014. Relationships between methane ($CH_4$) production and residual feed intake (RFI) of two divergent breeds of finishing steers offered either a concentrate-straw based diet or a silage based diet, Advances in Animal Biosciences 5,126

Duthie C-A, Rooke JA, Troy S, Hyslop JJ, Ross DW, Waterhouse A and Roehe R 2015. Impact of adding nitrate or increasing the lipid content of two contrasting diets on blood methaemoglobin and performance of two breeds of finishing steers. Animal, doi:10.1017/S1751731115002657.

Duthie C-A, Rooke J, Troy S, Hyslop JJ, Ross D, Waterhouse A and Roehe R 2015. Animal performance and efficiency of two divergent breeds of finishing steers offered either a concentrate-straw or a silage based diet *ad libitum* with either nitrate or increased dietary oil. Advances in Animal Biosciences 6, 176.

Rooke JA, Duthie C-A, Troy S, Broadbent C, Waterhouse A and Roehe R 2014. Responses in blood met-haemoglobin (Met-Hb) concentrations of steers during adaptation to inclusion of nitrate in the diet and subsequent long-term feeding of nitrate, Advances in Animal Biosciences 5, 40.

Rooke JA, Troy S, Duthie C-A, Wallace RJ, Hyslop J, Ross D, Waterhouse T and Roehe R 2015. Effects of dietary nitrate or oil concentration on methane (CH4) and hydrogen (H2) emissions from beef cattle are basal diet dependant. Advances in Animal Biosciences 6, 139.

Troy S, Duthie C-A, Hyslop JJ, Roehe R, Ross DW, Wallace RJ, Waterhouse A and Rooke JA. 2015. Effectiveness of nitrate addition and increased oil content as methane mitigation strategies for beef cattle fed two contrasting basal diets. Journal of Animal Science 93, 1-9.

Troy SM, Duthie C-A, Hyslop JJ, Roehe R, Ross DW, Waterhouse A and Rooke JA 2015. A novel method of measuring methane (CH4) emissions from beef cattle in group housing. Advances in Animal Biosciences 6, 140

Troy SM, Duthie C-A, Ross DW, Hyslop, JJ, Roehe R, Waterhouse A, and Rooke JA 2016. A comparison of methane emissions from beef cattle measured using methane hoods with those measured using respiration chambers. Animal Feed Science and Technology, 211: 227-240.

Yang C, Rooke JA, Cabeza I and Wallace RJ 2016. Nitrate and inhibition of ruminal methanogenesis: microbial ecology, obstacles and opportunities for lowering methane emissions from ruminant livestock . Frontiers in Microbiology ( Accepted).

## 14. REFERENCES

AHDB Beef and Lamb (formerly Eblex) 2013.. Stocktake Report 2013. *AHDB Beef and Lamb, Stoneleigh Park, Kenilworth, Warwickshire CV8 2TL.*

Basarab JA, Price MA, Aalhus JL, Okine EK, Snelling WM and Lyle KL 2003. Residual feed intake and body composition in young growing cattle. Canadian Journal of Animal Science 83, 189–204.

Bauchart D, Gladine C, Gruffat D, Leloutre L and Durand D 2005. Effects of diets supplemented with oil seeds and vitamin E on specific fatty acids of rectus abdominis muscle in charolais fattening bulls. In "Indicators of milk and beef quality". EAAP Publication No. 112, 431-436

BSI (1993). Assessors for sensory analysis. Guide to selection, training and monitoring of selected assessors. BSI 7667. London: British Standards Institution.

Brask M, Lund P, Weisbjerg MR, Hellwing ALF, Poulsen M, Larsen MK and Hvelplund T 2013. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. Journal of Dairy Science 96, 2356–2365.

Bruning-Fann CS and Kaneene JB 1993. The effects of nitrate, nitrite, and N-nitroso compounds on animal health. Veterinary and Human Toxicology 35, 237–253.

CIE (1978) International Commission of Illumination. Recommendations on uniform colour spaces, colour difference equations, psychrometric colour terms. Bureau de la CIE, Paris.

Cockburn A, Brambilla G, Fernández M-L, Arcella D, Bordajandi LR, Cottrill B, van Peteghem C and Dorne J-L 2013. Nitrite in feed: from animal health to human health. Toxicology and Applied Pharmacology 270, 209–217.

Cottle DJ, Nolan JV and Wiedemann SG 2011. Ruminant enteric methane mitigation: a review. Animal Production Science 51, 491–514.

Craigie CR, Navajas EA, Purchas RW, Maltin CA, Bunger L, Hoskin SO, Ross DW, Morris ST and Roehe R 2012. A review of the development and use of video image analysis (VIA) for beef carcass evaluation as an alternative to the current EUROP system and other subjective systems. Meat Science 92, 307-318.

EBLEX Report 2010. “Testing the Water”: The English Beef and Sheep Production Environmental Roadmap – Phase 2. EBLEX: Warwickshire, UK.

EBLEX Report 20012: “Down to earth. The beef and sheep roadmap – phase 3”.

Fisher, AL 2007. Beef carcass classification in the EU: an historical perspective. In: Lazzaroni, C., S. Gigli, and D. Gabiña. (Eds.), Evaluation of Carcass and Meat Quality in Beef and Sheep, Wageningen Academic Publishers, Wageningen, pp.19–30. (EAAP publication No. 123).

Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A and Tempio G 2013. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Grainger C and Beauchemin KA 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Animal Feed Science and Technology 166-167, 308–320.

Hook SE, Wright AD, and McBride BW 2010. Methanogens: methane producers of 683 the rumen and mitigation strategies. *Archaea* 2010, 945785.

Hulshof RBA, Berndt A, Gerrits WJJ, Dijkstra J, van Zijderveld SM, Newbold JR and Perdok HB 2012. Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. Journal of Animal Science 90, 2317–2323.

Hyslop JJ, Duthie C-A, Ross DW, Rooke JA and Roehe R 2012. An assessment of alternative test length periods when measuring liveweight change in finishing cattle during feed efficiency studies. Advances in Animal Biosciences 3, 46.

Jeyanathan J, Martin C and Morgavi DP 2014. The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. Animal 8, 250–261.

Johnson KA and Johnson DE 1995. Methane emissions from cattle. Journal of Animal Science 73, 2483–2492.

Kempster AJ, Cook GL and Grantley-Smith M 1986. National estimates of the body composition of British cattle, sheep and pigs with special reference to trends in fatness: a review. Meat Science 17, 107–138.

Lafontan M, Berlan M, Stich V, Crampes F, Riviere D, de Glisezinski I, Sengenes C and Galitzky J 2002. Recent data on the regulation of lipolysis by catecholamines and natriuretic peptides. Annales d'Endocrinologie 63, 86-90.

Li L, Davis J, Nolan J and Hegarty R 2012. An initial investigation on rumen fermentation pattern and methane emission of sheep offered diets containing urea or nitrate as the nitrogen source. Animal Production Science 52, 653–658.

Liu, Q., Scheller, K.K. and Schaefer, D.M. (1996). Technical Note: A simplified procedure for vitamin E determination in beef muscle. Journal of Animal Science 74: 2406-2410.

Ministry of Agriculture Fisheries and Food 1992. Analysis of Agricultural Materials., 2nd Edition . Her Majesty's Stationary Office., London.

Martin C, Morgavi DP and Doreau M 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4, 351–365.

Nolan JV, Hegarty RS, Hegarty J, Godwin IR and Woodgate R 2010. Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. Animal Production Science 50, 801–806.

Patra AK 2013. The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. Livestock Science 155, 244–254.

QMS 2014. Cattle and Sheep Enterprise Profitability in Scotland. 2014 edition. *Quality Meat Scotland, The Rural Centre, West Mains, Ingliston, Newbridge EH28 8NZ.*

Rooke JA, Wallace RJ, Duthie C-A, McKain N, de Souza SM, Hyslop JJ, Ross DW, Waterhouse T and Roehe R 2014. Hydrogen and methane emissions from beef cattle and their rumen

microbial community vary with diet, time after feeding and genotype. British Journal of Nutrition 112, 398–407.

Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G and Jiang Y 2004. Saponins from edible legumes: Chemistry, processing and health benefits. Journal of Medicinal Food 7, 67-78.

Teye, G.A., Sheard, P.R., Whittington, F.M., Nute, G.R., Stewart, A. and Wood, J.D. (2006) Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. Meat Science, 73, 157-165.

Van Zijderveld SM, Gerrits WJJ, Apajalahti JA, Newbold JR, Dijkstra J, Leng RA and Perdok HB 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. Journal of Dairy Science 93, 5856–5866.

Van Zijderveld SM, Gerrits WJJ, Dijkstra J, Newbold JR, Hulshof RBA and Perdok HB 2011. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. Journal of Dairy Science 94, 4028–4038.

Wallace RJ 2004. Antimicrobial properties of plant secondary metabolites. In: Proceedings of the Nutrition Society 63, 621-629.

Wallace RJ, Rooke JA, Duthie C-A, Hyslop JJ, Ross DW, McKain N, Motta de Souza S, Snelling TJ, Waterhouse A and Roehe R 2014. Archaeal abundance in post-mortem ruminal digesta may help predict methane emissions from beef cattle. Scientific Reports 4, 5892.

Yang C, Rooke JA, Cabeza I and Wallace RJ 2016. Rumen microbial ecology, methane and nitrate salts: obstacles and opportunities for lowering methane emissions from ruminant livestock. Frontiers in Microbiology (submitted).