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Impact of rearing regime on the sensory quality of sirloin from young bulls

Linda Farmer, Alison White, David Farrell, Colin McRoberts, David Sanderson, Janeen Speers, Declan Devlin, Emma Foster, Alan Gordon, Naomi Rutherford and Francis Lively

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1. Executive Summary

1.1. Background

The DAERA E&I project, “Beef from Grass” investigated the impact on production parameters of an extensive, grass-based diet fed from 3 months (spring born calves) or 6 months (autumn born calves) to ca. 9 months of age, prior to finishing on a concentrate ration.

The use of a grass-fed diet during early life may impact the fatty acid composition, the antioxidants present in the meat and also the flavour of the cooked product. However, it was not known whether these changes would be retained during the finishing stage.

A DAERA-funded PhD student has investigated the effect of grass-fed diets on flavour volatiles and fatty acid composition. Agrisearch agreed to cover the cost of sensory analyses to link sensory quality to the meat composition and the diet.

The aim of this project was, therefore, to determine if there is a residual benefit of early life grass-feeding on the sensory quality of beef sirloins, and to evaluate how this relates to fatty acid composition, flavour and antioxidant capacity.

1.2. Analyses

Home sensory assessments and sensory profiling were funded by this project. These analyses were delayed and adapted due to the effect of the COVID pandemic.

Analyses for pHu, marbling and other measures of meat quality were conducted on the ribeye as part of the original study and these data were incorporated into this study, along with pHu measurements on the sirloin. Antioxidant analyses will not be conducted as part of the PhD but will be added later if possible.

Chemometric methods were used to seek relationships between these analyses and an understanding of differences observed.

1.3. Summary of Findings

1.3.1. Effect of dietary regime

The results of all these analyses showed that differences in dietary regime during the “grower” period had no effect on the sensory quality, and little effect on fatty acid content or flavour volatiles, suggesting that the subsequent finishing period was sufficient to overwrite any impact of grass versus concentrate diets at the grower stage. Only a modest increase in some long chain n-3 fatty acids, significant only in the case of C20:5, carried through to slaughter date. It is unsurprising, therefore, that there were no consistent effects of dietary regime on flavour volatiles and no significant effects on sensory quality.

Commercial significance: If an improvement in fatty acid composition and the associated health benefits and/or sensory quality is required, the treatments will need to be continued into the finishing phase.

1.3.2. Effect of season of birth

There was a difference between autumn and spring born animals in flavour volatiles (but not fatty acids or sensory quality). The flavour volatiles were significantly affected by season of birth, with more volatiles from most compound classes from autumn-born grilled sirloin. This was not explained by differences in fat content. This increase in volatiles was reflected in increased scores for intensity of flavour from both the home panel and the trained panel but neither of these results was statistically significant. This increase in volatiles was not explained by fatty acid analyses nor fat content. This may be explained by antioxidant capacity but this awaits further analyses.

Commercial significance: If there is a consistent increase in flavour volatiles from AB sirloin, there would be benefit in understanding how to manage this to optimise flavour.

1.3.3. Incidence of ultimate pH

As an aside from the main aims of this project, it was observed that ultimate pH (pHu) varied considerably with a higher than expected incidence of high pHu beef across the experiment. Across the two years of the wider trial, both season of birth and kill date appear to influence the incidence of high pHu in these experiments, with higher incidence in spring-born cattle and on certain dates.

The high incidence of high pHu meat and its association with SB cattle and certain dates justifies further consideration. The impact of date is likely associated with animal handling, whether due to transport or conditions at the abattoir, emphasising the need to keep animals calm and unstressed as far as possible. The mechanism by which season should have an effect on pHu and flavour volatiles justifies further consideration.

Commercial significance: A high incidence of high pHu meat is a loss to the industry and it would be worth analysing all available data from Hillsborough cattle to identify any common causes.

1.3.4. Effect of ultimate pH

The results over a wide pHu range confirm clear relationships between pHu and WBSF, cooking loss and colour. These data also indicate that, while the effects on cooking loss and WBSF start at pHu = 6.0, the traditional threshold for dark cutting beef, the impact on colour appears to start nearer pHu = 5.7, the threshold used by Meat Standards Australia.

There are also likely effects on volatile compounds over a narrower pHu range which appeared greater for AB than SB beef. Thus, there appears to be an interaction between these two factors. Further investigation of the mechanisms by which these effects occur may help to optimise flavour quality.

Commercial significance: To achieve consistent meat quality, an ultimate pHu of between 5.5. and 5.7 should be achieved. Further investigation is needed to understand how season of birth and pHu interact to affect flavour.

2. Background

The DAERA E&I project, “Beef from Grass”, led by Francis Lively, investigated the impact on production parameters of an extensive, grass-based diet fed from 3 months (spring born calves) or 6 months (autumn born calves) to ca. 9 months of age, prior to finishing on a concentrate ration. Associated work has been conducted by a PhD student, Naomi Rutherford, part funded by Agrisearch. Details of this study, showing the effects of beef production system on health, carcass characteristics and meat quality have been published (Rutherford et al., 2020).

Rutherford et al (2020) propose that, although bulls are more efficient, they are less well utilised than they could be due to the cost of intensive concentrate feeding. Grazed grass is the cheapest form of ruminant feeding and thus, the inclusion of a grazing period during the first summer could help to reduce production costs. The objective of this study was to compare the health and performance of Holstein bulls on four differing production systems to identify if a grazing period could be included in Holstein bull beef production, and if concentrate supplementation at pasture was required.

The use of a grass-fed diet during early life may impact the fatty acid composition, the antioxidants present in the meat and also the flavour of the cooked product. There are many studies that show that inclusion of grass at the finishing stage increases the proportion of n-3 fatty acids (e.g., Elmore et al., 2004; Fruet et al., 2018) and thus the health benefits of the meat as well as sensory and flavour quality (e.g., Melton, 1990). However, there is little information on whether any benefits due to grazing during the growing phase would be retained during the finishing stage. If the benefits of grazing are retained, this could enable NI farmers to sell an enhanced product.

The aim of this project was to determine if there is a residual benefit of early life grass-feeding on the sensory quality of beef sirloins, and to evaluate how this relates to fatty acid composition, flavour and antioxidant capacity. The hypothesis to be tested was: provision of a grass-based diet in early life (up to 9 months) will have a positive effect on meat quality and sensory characteristics of Holstein bulls slaughtered at 15.5 months.

3. Experimental

It was planned that trained panellists would score grilled steak from 64 animals against a list of agreed attributes. In parallel, a PhD student would conduct analyses on fatty acids, flavour volatiles and antioxidant capacity, as part of her first year. Multivariate statistical methods would be used to determine how these attributes are affected by fatty acids, flavour volatiles and antioxidant capacity.

These plans were interrupted by the COVID pandemic and both the sensory work and the student's analyses were delayed. Furthermore, some of the stored loins were found to be high pH and had to be excluded from the study.

3.1. Rearing of animals and sampling of meat

This study involved of total of 112 Holstein bulls in 2017/18. This formed part of a larger trial conducted over two years (2017/18 (n = 112) and 2018/19 (n = 112)). In each year a group of 56 autumn born (AB) and a group 56 spring born (SB) bull calves were selected. These bulls were assigned to one of four production system treatments, which differed during the grower period and had a homogenous finishing period, the durations of which are reported in Table 1 and explained further in Rutherford et al. (2020). Prior to trial the bulls were fed ad lib silage and 2kg concentrates/d from 3 weeks post-weaning until the start of the trial (grower period).

Table 1. Experimental design

Treatment	Grower regime (from May**)	Grower period	Housing	Finishing ration	Slaughter
Autumn born calves					
AB GN	Grazed, no conc*	From 6mo for 90 days**	From August for finishing	2kg for 1 week and then increased by 1kg per week until ad lib, with silage	17 Jan – 24 Apr
AB G2	Grazed, 2kg conc				17 Jan – 24 Apr
AB GA	Grazed, ad lib*** conc			ad lib conc, with silage	17 Jan – 24 Apr
AB HA	Housed, ad lib conc		Throughout		17 Jan – 24 Apr
Spring born calves					
SB GN	Grazed, no conc*	From 3mo for 138 days**	From October for finishing	2kg for 1 week and then increased by 1kg per week until ad lib, with silage	10 Apr – 12 Jun
SB G2	Grazed, 2kg conc				10 Apr – 12 Jun
SB GA	Grazed, ad lib conc			ad lib conc, with silage	10 Apr – 12 Jun
SB HA	Housed, ad lib conc		Throughout		10 Apr – 12 Jun

** Conc = concentrate*
*** Naomi Rutherford, personal communication, 15 Sep 2022*
**** ad libitum*

The four production system treatments included; (i) grazed with no concentrate supplementation (GN), (ii) grazed with 2kg concentrate supplementation per day (G2), (iii) grazed with ad libitum access to concentrates (GA) and (iv) housed with ad libitum access to concentrates and grass silage (HA). Each treatment group consisted of 14 animals and was balanced for live weight (LW) and age. All bulls were housed and finished on a diet of ad libitum concentrates and grass silage before being slaughtered at a mean age of 15.5 months. Tables 1 and 2 provide more detail on the different rearing regimes and the timelines involved, respectively.

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Table 2. Production timelines for autumn born (AB) and spring born (SB) bulls, 2017-18 only

Treatment	Age at start of grower period (d)*	Grower period duration (d)	Age at start of finishing period (d)*	Finisher period duration (d)	Age at slaughter (d)
AB GN	194	90	284	184	468
AB G2	184	90	274	195	469
AB GA	190	90	280	190	470
AB HA	192	90	282	185	467
Average AB	190	90	280	189	469
SB GN	106	138	244	237	481
SB G2	109	138	247	228	475
SB GA	100	138	239	238	477
SB HA	110	138	248	231	479
Average SB	106	138	245	233	478
Average	**148**	**114**	**262**	**211**	**473**

** Obtained by subtraction from slaughter date*

Two samples from the *longissimus dorsi* muscle were removed from the anterior fore-rib joint at three days post-slaughter. These meat samples were aged at 3 ^{o}C, one until day 7 and the other until day 14 post-slaughter.

The posterior *longissimus dorsi* (sirloin) was retained for sensory, fatty acid and other analyses. These loins were vacuum packed, aged for 7 days and frozen to -20^{o}C until needed for analysis. Due to the COVID-19 pandemic, the period of time frozen was longer than usual, up to 4 years. However, the samples were securely vacuum packed and there was no sign of burst packs. The freezer temperatures were monitored and were maintained at -20^{o}C throughout.

3.2. Determination of pH and other meat quality measurements

Determination of pH and colour was conducted on these samples as part of the main trial, as described previously (Rutherford et al., 2020, Murphy et al., 2017). These meat samples were aged at 3 ◦C, one until day 7 and the other until day 14 post-slaughter. At day 7 ultimate pH (pHu) and colour (L*(lightness), a*(redness) and b*(yellowness)) were assessed using a Jenway 370 pH meter and a Chroma Meter CR-400, respectively. Both instruments were calibrated prior to measurements being taken. The illuminant for the Chroma Meter was D65, while the angle of observation was 2 degrees.

Day 7 and day 14 samples were then vacuum-packed and frozen, where they were stored until further measurements were taken. Samples were left to thaw over a period of 24 h at 4 ◦C. Following this, they were removed from the packaging and left to bloom for 40 min. Samples were vacuum-packed and cooked in a water bath at 70 ◦C for 50 min. Samples were weighed pre- and post-cooking, and cooking loss was calculated as follows:

$$\text{Cooking loss \%} = 100 \times \frac{\text{(pre-cooking weight – post-cooking weight)}}{\text{pre-cooking weight}}$$

Warner Bratzler Shear Force (WBSF) measurements were completed on each sample using an Instron 3366 Universal Testing Instrument. Samples were cored parallel to the longitudinal orientation of the muscle fibres and ten cores (sub-samples) were taken from each meat sample. All sub-samples were of the same diameter (12.5 mm), and were sheared perpendicular to the muscle fibres. The mean maximum load of the 10 sub-samples was considered as the WBSF value for each meat sample.

3.3. Experimental plan

For this Agrisearch-funded project and the associated postgraduate studies, samples were selected as follows. Of the eight treatments detailed in Tables 1 and 2, two of the SB treatments gave a very high incidence of high pHu meat, such that there were insufficient samples with a normal pHu for sensory analyses. For this reason, sensory analyses were conducted on meat from only six treatments: AB G2, AB GA, AB HA, AB GN, SB G2, SB GA. At least six animals from each treatment were subjected to sensory analyses and associated fatty acid and flavour analyses.

3.4. Sensory analyses

Sensory profiling analyses were delayed due to COVID and the consequent difficulties in bringing trained panellists together to do the work. During COVID restrictions, a “home trial” was conducted, to make the most of the availability of colleagues at home. These assessments were conducted in 2020 and 2022.

Sirloins were subjected to analysis by home consumers and trained sensory panels for the acceptability and sensory attributes of the cooked meat. Each loin was cut into one inch thick slices and the slices were then blast frozen and stored at -18oC until required. Frozen

samples were sorted into presentation order for the consumer or profiling panels according to the Latin square designs created using FIZZ software.

3.4.1. Home sensory trials

During COVID lockdown (2020), a short trial was conducted using home consumers, with the aim to obtain a sensory assessment of the beef and to determine if this could be used as an alternative to conventional sensory methods.

Two people from each of 66 households, 132 people, were asked to assess two steaks from two different treatments. Assessments were made on 10cm line-scales for six attributes:

Attribute	Anchor 1	Anchor 2
Liking Of aroma	Dislike extremely	Like extremely
Texture	Not tender	Very tender
Juiciness	Not juicy	Very juicy
Intensity of flavour	Not intense	Very intense
Liking of flavour	Dislike extremely	Like extremely
Overall liking	Dislike extremely	Like extremely

3.4.2. Sensory profiling analyses

Due to COVID, this trial was delayed until 2022. The trained panel first attended several training sessions to develop a vocabulary and descriptions (Appendix 1).

Samples were presented to assessors according to a balanced latin square design. Each of the 8 assessors received 6 samples per session and there were 6 sessions.

At 24 hours prior to a session, the required sample packs were thawed at 4^{o}C in a refrigerator. The sensory profiling were conducted at the Sensory Research Unit at AFBI. The samples were cooked in Rational ovens using a meat grilling cooking programme with the following settings: option 1 (thickness) set at “Thin”; option 2 (browning) set at “Middle”; and option 3 (time) set at 8 minutes and the temperature of each sample was checked that it was ≥ 65^{o}C.

3.5. Analytical methods

3.5.1. Fatty acid analyses

Lean muscle, with an added internal standard, was subjected to alkaline hydrolysis and the free fatty acids generated esterified to fatty acid methyl esters (FAMEs) by acid catalysis in the presence of methanol. After extraction with hexane the FAMEs were quantified by gas chromatography using flame ionization detection. Samples were analysed in randomised batches in duplicate. For reporting purposes FAMEs are

converted to fatty acids (FAs) by applying a conversion factor for each FAME. FAs are reported as mg/g beef muscle. An in house reference material, procedural blanks and calibration verification were undertaken with each batch to ensure acceptable method performance.

3.5.2. Volatile analyses

Volatile compounds comprising the aroma of the grilled meat were analysed by SPME GC-MS (Agilent). The samples were defrosted overnight in a fridge at 4^{o}C. Steak portions (25mm thick) were cooked at 180^{o}C using a clam grill for 3 minutes and 30 seconds and until an internal temperature of 65^{o}C was reached. The cooked steak was then cored using a coring device (diameter = 5 mm). The cores were covered in liquid nitrogen to freeze, and once the liquid nitrogen evaporated, the cores were placed into a 20mL head space vial and weighed to 2g ± 0.1 g. The vials were sealed with a magnetic screw top cap with a PTFE/silicone septum and stored in an ice bath for a maximum of 90 minutes, until placed on the autosampler tray of the SPME GC-MS at 6^{o}C.

Collection of the volatiles was achieved using a DVB/CAR/PDMS SPME fibre. Agitation temperature was set to 65^{o}C, equilibration time was 15 minutes, collection time was 15 minutes, desorption temperature was 250^{o}C, desorption time was 5 minutes, with conditioning of fibre at 250^{o}C for 30 minutes between samples. Prior to commencement of a sample sequence the fibre was conditioned at 270^{o}C for 30 minutes. GC-MS was used in splitless mode with a purge to flow vent of 50ml after 5 minutes. The initial temperature was 30^{o}C for 5 minutes followed by 6^{o}C increase per minute until 270^{o}C is reached.

Separation was achieved on a DB5 column and helium was used as the carrier gas. Quantification was achieved using single ion monitoring (SIM) for duplicate samples with confirmation of identity using SCAN mode a further replicate.

Identification of compounds was achieved by comparison of the linear retention index and mas spectrum with those of authentic compounds or data reported in the literature.

Agilent's MassHunter Quantitation Software was used to analyse results. An ion peak area was determined for the quantitation ion selected for each compound. The ratio of additional ions was used to assist with confirmation of identity.

3.6. Statistics

Statistical analyses were conducted to determine the effect of rearing regime on the sensory and analytical data and associations between the sensory and analytical data. Analyses used included REML analysis, using Genstat 8. Analyses were conducted on the six treatments as a first order, 1x6 analysis, due to the absence of samples from the remaining two treatments.

4. Results and Discussion

The results of the two sensory studies funded by this project are presented together with the results of fatty acid analyses and flavour volatiles from an associated PhD study.

The results of pHu evaluation, conducted previously, are re-evaluated in terms of the incidence of dark cutting beef and how they impact on beef quality.

4.1. Sensory profiling

Table 3 shows the results of the trained sensory trials. **None of the attributes scored showed any significant differences between the six treatments[1].**

There is a considerable body of data reporting that diet can influence the sensory quality of beef. However, most of these papers focus on the impact of finishing diet not, as in this study, the growing diet. Melton et al. (1990) reviewed the effect of dietary ingredients on red meat flavour and concluded that, finishing on grain or corn or concentrates beef was preferred and had a stronger flavour than beef finished on grass. These studies were mainly American where grain-fed beef is the norm, but in countries where grass-fed beef is the norm local beef is usually preferred (Oliver et al., 2006). Revilla et al. (2021) reported that the higher the grass content, the lower the flavour quality. Other papers reported similar changes in sensory quality for different finishing diets (Chail et al., 2016; Vast et al, 2011; Elmore et al., 2006, 2004)

Few reports considered the role of growing diets rather than finishing diets. However, Mezgebo et al (2017) compared animals reared (in Ireland) on ad lib silage and 1.5kg concentrates for 120 days, followed by ad lib concentrates to finish (GSC), with those on ad lib concentrates and 1.5kg silage throughout (C). It is not clear how long the ad lib concentrates were fed to the GSC group, but it may have been ca. 100d, which would make this treatment roughly comparable with the GA/HA groups in terms of diet. In this study, the higher silage diet (GSC) gave meat with higher scores for flavour liking and overall liking, as assessed by a trained panel.

Liking was not assessed in our study as it is usually assumed that the training programme makes panellists unreliable assessors of hedonic liking. Also, much larger numbers of people are recommended for hedonic assessments. Nevertheless, it is known from past work that flavour liking is associated with “sweet flavour” and “roasted flavour”; there were no significant differences (nor trends) in these traits.

[1] The SED values are characteristically high for this type of study due to the numbers of trained assessors and the natural variation amongst human assessors. Nevertheless, sensory profiling often shows clear differences between treatments.

Table 3. Mean sensory scores for grilled beef sirloin from six rearing regimes

	AB GA	AB HA	AB G2	AB GN	SB G2	SB GN	avSED	Prob
AROMA								
Intensity of aroma	55.9	63.2	60.5	59.4	54.7	57.5	4.03	0.309
Roasted	39.2	43.0	37.3	40.4	37.3	37.1	4.89	0.803
Beefy	50.1	49.2	49.4	52.2	51.8	45.8	5.63	0.891
Chargrilled	27.1	26.4	26.7	25.9	24.5	22.8	4.11	0.907
Boiled meat	26.5	26.1	28.5	27.5	26.3	25.8	4.71	0.992
Fatty	19.4	21.8	24.6	21.6	26.5	20.2	3.46	0.289
Metallic/bloody	22.4	21.7	18.9	19.1	19.1	17.8	4.09	0.857
EXTERNAL APPEARANCE								
Evenness of colour	45.2	53.0	56.2	48.0	50.9	49.5	4.69	0.275
Charred	18.0	19.1	15.6	20.3	22.1	14.7	4.19	0.492
Bloody	34.1	31.5	27.6	27.1	27.7	23.2	6.39	0.621
Greasy/oily/fatty	22.5	18.6	23.1	17.4	23.3	19.4	3.19	0.272
TEXTURE ON CUTTING								
Tenderness	53.9	59.3	56.3	54.3	50.5	52.4	4.89	0.567
Fibrous/stringy	28.3	28.6	26.1	30.9	22.1	28.1	4.32	0.456
Sticky/clingy	23.1	22.0	19.5	20.7	20.7	23.1	3.56	0.887
INTERNAL APPEARANCE								
Juicy	37.3	38.7	37.5	35.8	29.8	36.1	4.71	0.494
Closely packed	45.1	48.8	45.4	43.9	51.5	48.8	4.78	0.605
FLAVOUR								
Intensity of flavour	58.6	59.1	61.5	61.7	59.1	58.2	3.82	0.906
Roasted	35.7	35.7	35.2	32.9	32.7	30.9	4.92	0.891
Beefy	54.0	52.6	52.6	53.7	53.8	48.9	5.38	0.934
Chargrilled	26.1	28.0	25.7	24.0	21.9	21.8	4.81	0.757
Metallic/bloody	34.0	32.6	31.8	32.7	33.7	26.2	3.80	0.331
Sour/acid	25.1	19.9	24.7	24.8	26.0	24.4	3.99	0.711
Bitter	11.6	11.6	15.4	14.2	13.9	12.2	2.70	0.625

	AB GA	AB HA	AB G2	AB GN	SB G2	SB GN	avSED	Prob
Sweet	11.5	13.3	10.6	9.8	9.2	11.4	2.52	0.644
Rancid	9.2	8.4	9.4	11.2	8.8	8.7	2.98	0.952
TEXTURE (MOUTH)								
Tenderness	41.4	44.4	45.7	46.0	42.1	42.5	4.32	0.837
Rubbery	32.3	30.0	33.9	31.8	34.3	31.8	4.32	0.929
Sticky/clingy	25.8	24.0	28.4	23.2	26.2	27.1	3.75	0.749
Stringy/clingy	26.1	26.0	28.3	24.0	26.5	30.2	4.39	0.790
Greasy/oily	26.4	24.5	24.5	20.0	24.2	25.0	4.11	0.732
AFTERTASTE								
Intensity of aftertaste	47.3	50.6	51.7	51.1	53.3	48.8	3.82	0.679
Roasted	21.6	23.0	25.7	19.8	24.2	23.5	3.75	0.693
Beefy	41.5	39.5	42.7	41.3	41.2	38.5	5.18	0.972
Metallic/bloody	35.5	36.6	34.3	38.3	32.5	32.7	4.10	0.693
Greasy/oily	26.4	25.7	26.2	20.7	24.4	26.9	4.15	0.689

4.2. Home sensory trials

The results of the home consumer panels are presented in Table 4. Some assessors' results were removed from the trial due to likely misinterpretation of instructions. Thus, each treatment was assessed by between 24 and 42 assessors. **Again, there was no significant effect of treatment on the scores given by home assessors.**

Table 4. Mean consumer scores from home trial of grilled sirloin

Identifier	AB GA	AB HA	AB G2	AB GN	SB G2	SB GN	avSED	Prob
Aroma Liking	71.3	70.5	71.8	75.2	67.9	70.4	4.41	0.711
Tenderness	68.6	70.3	71.5	77.3	69.0	67.5	4.27	0.221
Juiciness	69.7	70.6	73.8	74.2	68.4	69.7	4.86	0.775
Intensity of Flavour	65.3	68.9	70.3	71.3	71.7	68.9	4.54	0.765
Flavour Liking	71.1	75.8	75.8	76.3	75.4	70.7	4.22	0.589
Overall Liking	73.3	74.5	77.1	78.4	76.0	73.1	4.07	0.733

This method was investigated as a method of conducting sensory panels during the COVID lockdown period. Aspects worked well, in that there was good uptake and return of electronic questionnaires. However, quality assurance of the returns suggests that further work is required to ensure that this could provide a consistent and validated alternative to consumer panels conducted under controlled conditions.

4.3. Fatty acids

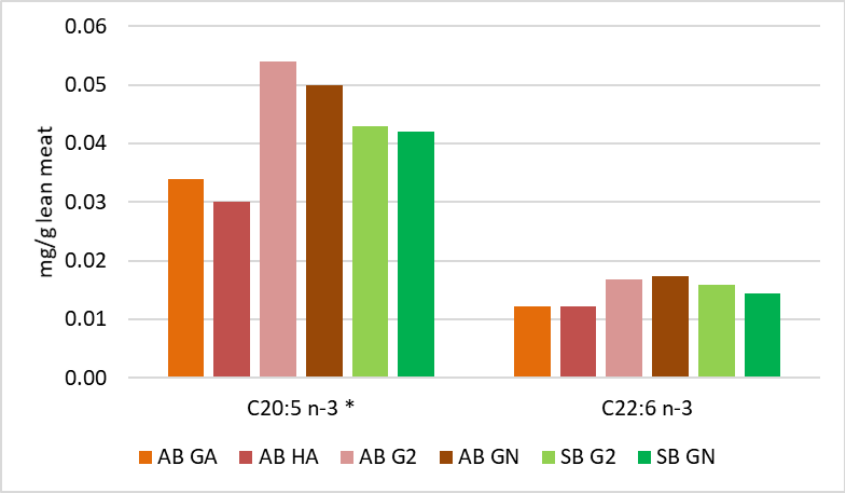
Fatty acid analysis of the sirloin beef showed only two significant differences (Table 5). The differences shown are small and need to be treated with caution as, in a large dataset, one in twenty would be expected to be significant at $P<0.05$ by chance, by definition. In the case of C15:1 such an effect may explain the significantly higher level of C15:1 in sirloin from autumn born animals restricted to 2kg concentrate per day as it is not mirrored in other fatty acids. However, the significant ($P<0.05$) result for eicosapentaenic acid (C20:5) is of some interest. Figure 1 shows that docosahexaenic acid (C22:6) shows a similar non-significant trend but the same does not apply to other n-3 fatty acids. The animals fed *ad lib* concentrates during the mid-part of their rearing regime have the lowest levels of these fatty acids, and those with higher levels of grass or silage have higher levels, as would be expected. However, these differences are very small, and the lack of significant effect suggests that the 6 months of finishing diet that followed these different grass-based regimes has largely eliminated any fatty acid differences.

Table 5. Fatty acid composition (mg/g wet weight lean meat) of beef sirloin from six rearing regimes

Fatty acid	AB GA	AB HA	AB G2	AB GN	SB G2	SB GN	avSED	Prob
C4:0	0.006	0.006	0.007	0.006	0.007	0.002	0.0029	0.422
C10:0	0.017	0.023	0.014	0.019	0.017	0.020	0.0052	0.708
C12:0	0.027	0.033	0.021	0.028	0.026	0.033	0.0084	0.690
C14:0	1.16	1.35	0.86	1.25	1.10	1.47	0.3880	0.672
C14:1	0.302	0.338	0.225	0.323	0.244	0.349	0.0931	0.669
C15:0	0.136	0.159	0.101	0.156	0.137	0.169	0.0419	0.611
C15:1	**0.021[a]**	**0.022[a]**	**0.025[b]**	**0.022[a]**	**0.020[a]**	**0.021[a]**	**0.0012**	**0.009**
C16:0	9.87	12.25	8.00	10.97	9.96	12.16	3.1040	0.726
C16:1	1.31	1.68	1.04	1.59	1.30	1.48	0.4080	0.667
C17:0	0.359	0.436	0.256	0.412	0.329	0.430	0.1200	0.620
C18:0	5.77	6.99	5.00	6.14	6.18	7.34	1.7800	0.791
C18:1t	1.10	1.14	0.85	1.37	0.90	1.26	0.3680	0.688
C18:1c9	14.12	19.10	11.44	16.68	14.49	16.42	4.6850	0.676
c18:1c11	0.553	0.744	0.459	0.683	0.530	0.580	0.1530	0.482
C19:0	0.047	0.046	0.034	0.065	0.036	0.060	0.0182	0.466
C18:2t	0.009	0.010	0.007	0.009	0.007	0.009	0.0016	0.485
C18:2 n-6	2.29	2.32	2.13	2.42	2.06	2.37	0.3390	0.874
C20:0	0.031	0.037	0.028	0.034	0.035	0.039	0.0084	0.807
C18:3 n-6	0.010	0.010	0.009	0.010	0.009	0.011	0.0013	0.524
C20:1 n-9	0.057	0.095	0.057	0.086	0.066	0.061	0.0225	0.447
C18:3 n-3	0.169	0.191	0.141	0.173	0.193	0.178	0.0381	0.753
CLA9,11	0.117	0.148	0.096	0.143	0.128	0.160	0.0511	0.815
C21:0	0.014	0.014	0.010	0.016	0.011	0.014	0.0038	0.607
CLA 10,12	0.005	0.011	0.004	0.007	0.003	0.004	0.0042	0.444
C20:2 n-6	0.024	0.028	0.024	0.029	0.023	0.025	0.0042	0.647
C22:0	0.006	0.007	0.006	0.007	0.007	0.007	0.0011	0.920
C20:3 n-6	0.107	0.106	0.119	0.114	0.101	0.121	0.0081	0.102
C22:1 n-9	0.007	0.008	0.010	0.009	0.008	0.005	0.0034	0.743

Fatty acid	AB GA	AB HA	AB G2	AB GN	SB G2	SB GN	avSED	Prob
C20:3 n-3	0.006	0.008	0.006	0.008	0.007	0.006	0.0019	0.889
C20:4 n-6	0.534	0.484	0.556	0.506	0.490	0.514	0.0260	0.077
C22:2 n-6	0.004	0.004	0.003	0.004	0.004	0.004	0.0015	0.972
C24:0	0.002	0.002	0.004	0.002	0.003	0.003	0.0010	0.662
C20:5 n-3	**0.034[a]**	**0.030[a]**	**0.054[b]**	**0.050[b]**	**0.043[ab]**	**0.042[ab]**	**0.0066**	**0.011**
C22:5 n-3	0.184	0.171	0.195	0.188	0.174	0.199	0.0236	0.813
C22:6 n-3	0.012	0.012	0.017	0.017	0.016	0.015	0.0022	0.131
Total ID FAMEs	38.41	48.02	31.80	43.55	38.68	45.61	11.4500	0.739
Total SAT	17.45	21.35	14.34	19.10	17.85	21.75	5.4350	0.744
Total MUFA	16.37	22.00	13.26	19.41	16.67	18.94	5.3470	0.671
Total PUFA	3.37	3.36	3.25	3.52	3.12	3.49	0.3770	0.897
Total n3	0.405	0.412	0.412	0.436	0.433	0.439	0.0437	0.948
Total n6	2.96	2.95	2.84	3.08	2.69	3.05	0.3480	0.870
Total n7	1.86	2.44	1.50	2.29	1.84	2.08	0.5640	0.614
Total n9	14.2	19.2	11.5	16.8	14.6	16.5	4.7070	0.676
Ratio n6/n3	7.40	7.17	6.97	7.09	6.34	6.88	0.6590	0.690

Figure 1. Two n-3 fatty acids (mg/g wet weight lean meat) in beef sirloin from six rearing regimes



Many studies have shown that a grass-based finishing diet will give elevated n-3 fatty acids, conjugated linoleic acid and lower n6/n3 ratio in the meat, thus improving nutritional benefits (e.g., Elmore et al., 2004; Revilla et al., 2021; Fruet et al., 2018; Moloney et al., 2001). Animals raised from 6 – 14 months (when slaughtered) on grass silage versus concentrates showed an increase in total n-3 fatty acids and a decrease in n-6 fatty acids compared with meat from animals fed a concentrate diet. However, little information is available on the role of the growing diets. Mezgebo et al (2017) compared animals reared (in Ireland) on ad lib silage and 1.5kg concentrates for 120 days, followed by ad lib concentrates to finish (GSC), with those on ad lib concentrates and 1.5kg silage throughout (C). It is not clear how long the ad lib concentrates were fed to the GSC group, but it may have been ca. 100d, which would make this treatment roughly comparable with the GA/HA groups in terms of diet. The higher silage diet gave meat with slightly higher ($P<0.05$) linolenic acid (n-3), but had no significant effect on C20:5 or C22:6. There was no significant difference in n-6/n-3 fatty acid ratio between these two treatments, though these were much lower than those reported herein (C=2.78, GSC=2.91).

Figure 2 shows that the n-6/n-3 ratio is between 6.3 and 7.4 for all these treatments, which is higher than the ideal ratio for human health of approx. 4:1. It should be noted, however, that typical western diets have n-6/n-3 ratios of 10 – 30:1. Table 6 compares the total n-3 and n-6 fatty acids and ratio between them from the animals of this trial with those reported by Elmore et al. (2004), Mezgebo et al. (2017) and McAfee et al. (2011). The meat from this study appears most similar to that from animals fed no silage at all (Elmore et al., 2004). These data highlight the fact that the concentrations and ratios observed in this study reflect the finishing diet rather than earlier grass-feeding. This has been reported previously (Daley et al., 2010).

Thus, any benefits towards a healthier balance of fatty acids achieved by a grass diet during the growing period is largely lost after 90/138 days on a concentrate-based finisher diet.

Figure 2. Fatty acid n-6/n-3 ratio of beef sirloin from six rearing regimes

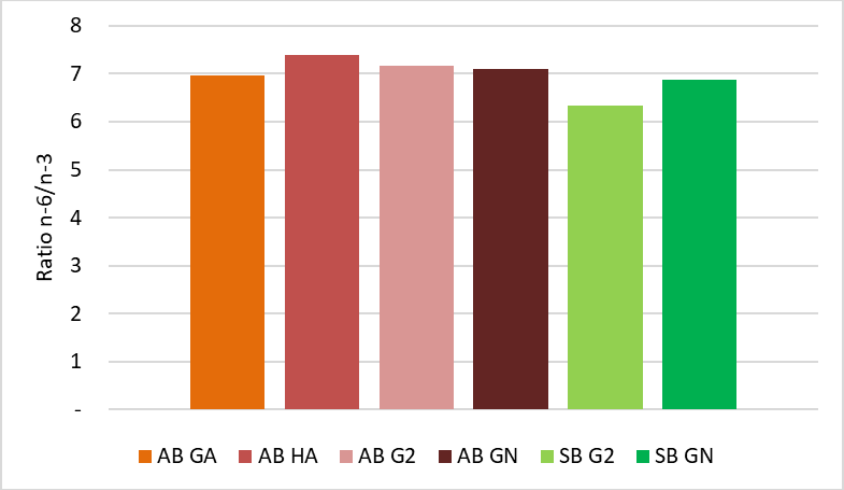


Table 6. Total n-3 and n-6 fatty acids and ratio between them from this study compared with selected literature reports.

Regime in brief	Total n-3 (mg/g)	Total n-6 (mg/g)	n-6/n-3 ratio	Reference
Grower – grazed only; Finisher - ad lib concentrates with silage	0.44	3.07	7.0	This study, Table 5, all GN
Grower – ad lib concentrates with grazing; Finisher - ad lib concentrates with silage	0.41	3.96	7.3	This study, Table 5, GA and HA
Diet 6-14mo – ad lib grass silage with sugar beet pulp shreds	0.81	1.07	1.3	Elmore et al. (2004), mean AA and Holstein – total lipid, M. longissimus lumborum
Diet 6-14mo – ad lib concentrates with chopped straw	0.24	2.16	8.9	
Ad lib silage and 1.5kg concentrates 120d, then ad lib concentrates to finish av. 94d (GSC)	0.41	1.19	2.9	Mezgebo et al (2017) – IMF from L. thoracis
Ad lib concentrates and 1.5kg silage throughout (C)	0.37	1.10	2.8	
Beef samples from commercial abattoirs over 12 mo in NI	0.42	1.37	3.3	McAfee et al., Agrisearch Booklet 20 – mean values

4.4. Volatile aroma compounds

Volatile compounds (n=105) were identified and quantified in the headspace from grilled beef sirloin and preliminary data is presented in Tables 6 and 7.

Analysis as a 6x1 factorial design (Table 6) shows that only six of these compounds were significantly different between sirloin from the six season/diet treatments while others approached significance ($P<0.10$). These were mainly compounds derived from lipid oxidation (aldehydes, ketones etc) and were due to higher levels identified in some AB diets.

Therefore, further analysis was conducted to evaluate the effect of season of birth more clearly; Table 7 shows the results of a 2x2 statistical analysis for AB versus SB and G2 versus GN. This stronger analysis emphasises a significant difference between AB and SB beef in terms of aroma volatiles from grilled beef, with higher levels of many lipid oxidation compounds in AB beef. However, there was very little effect on volatile compounds from cattle fed on 2kg concentrate during the grower period versus no concentrate/grazed beef.

The greater release of flavour volatiles, across all compound classes, by AB grilled sirloin, would be consistent with a lower concentration of lipid in this beef. However, Table 4.4 shows that there was no difference between AB and SB beef in total fatty acids. Total fatty acid levels provides a good indication of relative lipid content in the absence of an IMF determination, but will be lower. The greatest impact of season of birth was on the aldehydes, which arise from the oxidation of lipids. One might speculate that a greater formation could arise from lower levels of antioxidants in these samples. However, the reason why this could be so is unclear. AB animals had the shorter growing period on the specified diet (May to August), while SB cattle were on the grower diets from May until October (Table 1, 2). AB animals also had a shorter finishing period on concentrate and silage.

The scientific literature includes a number of reports on the effect of finishing diet on the volatiles from cooked meat. Whilst, the pattern of volatiles obtained from meat from animals fed concentrates rich in n-6 fatty acids differed from that from a grass based diet (Elmore et al., 2004; Vasta and Priolo, 2006; Vasta et al., 2011; Chail et al., 2017), there was also a tendency for concentrate or grain-fed animals to give meat with a higher level of volatiles derived from lipid oxidation (Elmore et al., 2004; Vasta and Priolo, 2006; Chail et al., 2016, 2017). It is of some interest that, despite the greater inherent propensity to oxidation of n-3 fatty acids, it was the grain-fed or concentrate-fed meat that showed the highest levels of TBARS and oxidation products (Legako, et al., 2018; Fruet et al., 2018; Revilla et al., 2021). This is likely due to the enhanced antioxidant status in grass-fed beef.

Again, only one paper has been found looking at the volatiles from meat with variations in the grower diet (Metzgebo et al., 2017) and they reported few differences, but the diet with higher levels of silage in the growing phase gave significantly higher levels of some lipid oxidation products. No papers were found that examined the effect of season of birth on the volatiles from the cooked meat.

As was observed for the fatty acids, it may be assumed that any impact of grower period diet has largely disappeared by slaughter date. However, season of birth did affect formation of volatiles, with more of most compound classes detected from AB sirloin. Analysis of antioxidant capacity of the muscle may help deduce the reason for these differences in volatile compounds.

Table 6. Mean peak areas (x1/1000) for volatile aroma compounds collected from grilled sirloin from six treatments (P<0.10 only)

	Treatment							
Compound	**AB G2**	**AB GA**	**AB HA**	**AB GN**	**SB G2**	**SB GN**	**avSED**	**Prob**
Alcohols								
1-Octen-3-ol	17.0	17.2	10.5	19.1	7.9	9.3	4.15	0.050
n-aldehydes								
Hexanal	1,017	760	460	668	364	535	220.2	0.065
Tetradecanal	**8.3** ^{c}	**7.3** ^{bc}	**5.8** ^{abc}	**5.8** ^{abc}	**5.5** ^{ab}	**4.5** ^{a}	**1.23**	**0.048**
Pentadecanal-	14.1	12.8	10.4	9.8	9.2	8.1	2.28	0.097
Hexadecanal	**8.0** ^{b}	**5.5** ^{a}	**4.6** ^{a}	**5.0** ^{a}	**3.7** ^{a}	**3.3** ^{a}	**1.08**	**0.002**
Unsaturated aldehydes								
2-Heptenal, (E)-	7.5	6.5	5.3	8.3	4.2	4.6	1.52	0.073
2-Octenal, (E)-	**10.3** ^{a}	**8.5** ^{ab}	**6.9** ^{a}	**11.1** ^{b}	**5.8** ^{a}	**7.0** ^{a}	**1.55**	**0.017**
2-Nonenal, (E)-	**10.9** ^{a}	**11.0** ^{ab}	**10.5** ^{a}	**14.1** ^{b}	**8.2** ^{a}	**9.1** ^{a}	**1.45**	**0.015**
2,6-Nonadienal, (E,Z)-	**0.6**	**0.5**	**0.4**	**0.6**	**0.4**	**0.3**	**0.11**	**0.015**
Alkanes								
Ketones								
2-Dodecanone	**0.6** ^{ab}	**0.9** ^{bc}	**1.1** ^{c}	**0.5** ^{ab}	**0.6** ^{ab}	**0.5** ^{a}	**0.16**	**0.008**
Strecker aldehydes								
Acetaldehyde	437.4	365.9	364.4	325.7	284.6	301.6	52.85	0.074

Table 7. Mean peak areas (x1/1000) for volatile aroma compounds collected from grilled sirloin from two seasons x two diets (P<0.10 only)

	Season (S)				Diet (D)				S.D
Identifier	**AB**	**SB**	**avSED**	**Prob**	**G2**	**GN**	**avSED**	**Prob**	**Prob**
Alcohols									
1-Octanol	29.8	19.0	5.44	0.088	24.9	24.0	5.44	0.807	0.228
1-Octen-3-ol	**18.0**	**8.6**	**2.63**	**0.004**	12.5	14.1	2.63	0.535	0.924
n-Aldehydes									
Hexanal	**835**	**449**	**158.8**	**0.023**	690.8	593.1	158.81	0.608	0.113
Heptanal	**294**	**204**	**35.7**	**0.021**	247.9	250.4	35.69	0.932	0.777
Tridecanal	4.0	2.9	0.57	0.060	3.8	3.2	0.57	0.263	0.768
Tetradecanal	**7.0**	**5.0**	**0.94**	**0.034**	6.9	5.2	0.94	0.090	0.441
Pentadecanal-	12.0	8.6	1.81	0.067	11.6	9.0	1.81	0.171	0.383
Hexadecanal	**6.5**	**3.5**	**0.75**	**P<0.001**	**5.9**	**4.1**	**0.75**	**0.038**	0.106
Unsaturated aldehydes									
2-Heptenal, (E)-	**7.9**	**4.4**	**1.12**	**0.008**	5.9	6.5	1.12	0.602	0.865
2-Octenal, (E)-	**10.7**	**6.4**	**1.18**	**0.004**	8.0	9.1	1.18	0.390	0.879
2-Nonenal, (E)-	**12.6**	**8.7**	**1.06**	**0.001**	9.6	11.7	1.06	0.064	0.256
2,6-Nonadienal, (E,Z)-	**0.6**	**0.4**	**0.09**	**0.004**	0.5	0.5	0.09	0.744	0.836
2,4-Nonadienal, (E,E)-	**3.1**	**1.3**	**0.74**	**0.023**	2.3	2.1	0.74	0.802	0.534
Alkanes									
Tridecane	**38.4**	**30.6**	**2.57**	**0.006**	35.5	33.5	2.57	0.465	0.674
Pentadecane	**19.7**	**17.6**	**0.86**	**0.030**	18.9	18.4	0.86	0.598	0.837
Diketones / hydroxyketones									
2,3-Butanedione	15.5	12.4	2.06	0.119	15.5	12.5	2.06	0.220	**0.044**
2,3-Octanedione	**179**	**82**	**38.9**	**0.022**	141	120	38.9	0.637	0.230
Ketones									
2-Heptanone	**178**	**86**	**38.1**	**0.029**	122	141	38.1	0.650	0.519
2-Octanone	29.4	19.7	4.66	0.057	23.3	25.8	4.66	0.634	0.368
1-Nonen-3-one	23.6	17.3	3.48	0.091	22.1	18.8	3.48	0.324	0.206
Strecker aldehydes									

Identifier	Season (S) AB	SB	avSED	Prob	Diet (D) G2	GN	avSED	Prob	S.D Prob
Acetaldehyde	**380**	**293**	**37.3**	**0.025**	361	312	37.3	0.247	0.101
Benzaldehyde	**1,309**	**997**	**151.1**	**0.044**	1,249	1,057	151.1	0.239	0.347
Benzeneacetaldehyde	685	512	88.1	0.066	601	596	88.1	0.994	0.306
Furans / lactones									
Furan, 2-pentyl-	**164**	**92**	**21.3**	**0.015**	134	122	21.3	0.820	0.856
Terpenes									
D-Limonene	4.3	4.2	0.40	0.565	4.7	3.7	0.40	0.024	0.417

4.5. Observations on ultimate pH

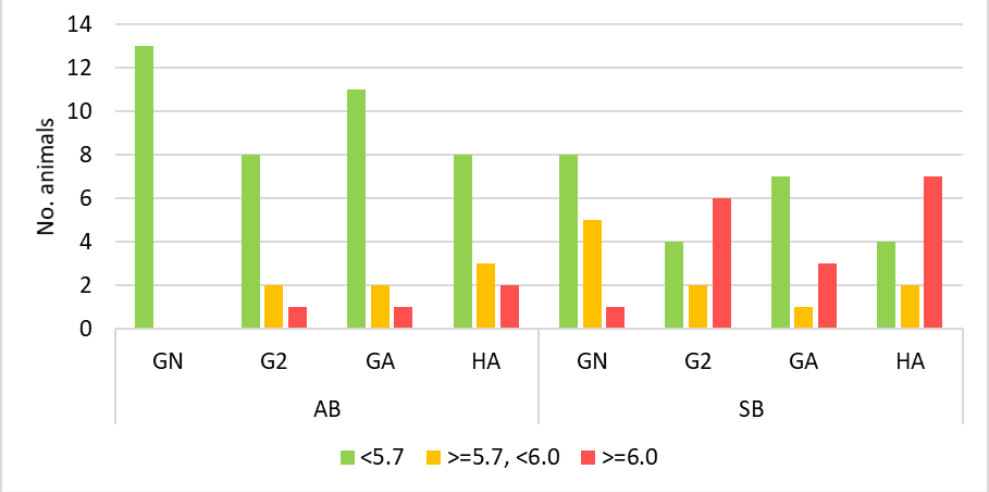
Beef with a pHu exceeding 6.0 is regarded as dark cutting or DFD and a few animals with these characteristics often arise due to stress or dietary issues. Studies in Australia have identified inconsistent quality with meat at pHu exceeding 5.7, and the Meat Standards Australia system excludes meat where the pHu of the *L. dorsi* exceeds this value.

Rutherford et al. (2020) have reported the mean pH value for the front section of the *L. dorsi* (ribeye) for the four treatments over two years (2017/18, 2018/19) and there were no significant differences in the mean values. However, there were some high pHu values amongst those slaughtered in 2018, as shown in Table 8 and Figure 3. These were largely associated with SB cattle, and with treatments SB G2 and SB HA. Tables 9 and 10 show that these higher pH values were also associated with two slaughter dates in May and June, but that even on these dates, they were mainly associated with the above two treatments. Animals not fed concentrates during the grower period (grazed only) had far fewer high pHu values, whether SB or AB.

Table 8. Mean pHu values and distribution of pHu values for ribeye from 101 animals by treatment, 2017-18

		Number of animals with ribeye pH:			
Treatment	**pHu**	**<5.7**	**>=5.7, <6.0**	**>=6.0**	**n**
AB G2	5.69	8	2	1	11
AB GA	5.65	11	2	1	14
AB HA	5.70	8	3	2	13
AB GN	5.58	13	0	0	13
Average/total AB	5.65	40	7	4	51
SB G2	6.02	4	2	6	12
SB GA	5.75	7	1	3	11
SB HA	6.05	4	2	7	13
SB GN	5.68	8	5	1	14
Average/total SB	5.87	23	10	17	50
Average	**5.76**	**63**	**17**	**21**	**101**

Figure 3. Distribution of pHu values for ribeye from 101 animals, 2017-18



For this reason, when selecting beef for sensory panels, it was necessary to omit treatments SB G2 and SB HA as there were insufficient sirloins without high pHu (>6.0) to conduct the panels. These treatments were also omitted from analyses for aroma volatiles and fatty acids. Likewise, individual animals giving high pHu from other treatments were not selected for further analysis, with only two exceeding pH 5.7 (both pHu 5.82). The mean pHu for the loins selected were between 5.57 and 5.64 for all treatments.

Reasons for high incidence of pHu

While not the primary aim of the project, this apparent effect of diet on the incidence of high pH justified further investigation. Two questions were considered:

a) Is the apparent impact of dietary treatment on incidence of high pHu confounded with slaughter date?

b) Is the same effect seen in the data for the second year of the trial?

a) Is the apparent impact of dietary treatment on incidence of high pHu confounded with slaughter date?

Table 4.1b and c show the distribution of pHu by treatment and kill date. Most of the dark cutting beef occurred on the last three slaughter dates. Both AB and SB cattle were slaughtered on two of these dates. However, there were few dark cutters amongst the AB cattle. **Thus, the problem seems to be higher in SB cattle on the concentrate diets.**

Table 9. Mean pHu values and distribution of pHu values for ribeye from 101 animals by kill date and treatment, 2017-18

Kill Date	No. animals	AB G2	AB GA	AB HA	AB GN	SB G2	SB GA	SB HA	SB GN	Average
17/01/2018	7	5.60	5.70	5.69	5.57					5.67
30/01/2018	8	5.87		5.62	5.55					5.66
13/02/2018	11	5.74	5.62	5.90	5.57					5.67
27/02/2018	7	5.56	5.63	5.90	5.59					5.69
14/03/2018	3	5.50	5.50	5.45						5.48
29/03/2018	5	5.65	5.58		5.62					5.62
11/04/2018	6			5.63		5.71	5.61	5.72		5.68
01/05/2018	13	5.68	5.70	5.61	5.56	6.27		6.24	5.53	5.73
12/06/2018	41					6.06	5.77	6.10	5.71	5.90
Total/Average	**101**	**5.69**	**5.65**	**5.70**	**5.58**	**6.02**	**5.75**	**6.05**	**5.68**	**5.76**

Table 10. Ultimate pH values ribeye from individual animals killed on 11 April, 1 May and 12 June only, in pHu order

AB G2	AB GA	AB HA	AB GN	SB G2	SB GA	SB HA	SB GN
5.49	5.46	5.53	5.56	5.43	5.45	5.59	5.47
5.55	5.52	5.63		5.58	5.46	5.60	5.52
5.99	6.13	5.68		5.64	5.48	5.65	5.52
				5.69	5.49	5.69	5.54
				5.72	5.54	5.72	5.55
				5.99	5.55	5.72	5.56
				6.14	5.61	6.02	5.58
				6.27	5.98	6.24	5.59
				6.27	6.10	6.33	5.73
				6.32	6.18	6.38	5.82
				6.49	6.46	6.52	5.82
				6.70		6.60	5.87
						6.64	5.95

b) Is the same effect seen in the data for the second year of the trial?

Tables 11 and 12 show the mean pH values for animals slaughtered in the second year of the trial (2019), for comparison with the data for 2018 (Tables 8, 9). The overall incidence of high pHu carcases was high both in 2018 and 2019, with 21% and 13% (respectively) exceeding pHu 6.0 and 36% and 27% (respectively) exceeding pHu 5.7.

Table 11. Effect of treatment on mean pH values and distribution of pH values for ribeye from 103 animals, 2019

		Number of ribeye with pH			
Treatment	**pHu**	**<5.7**	**>=5.7, <6.0**	**>=6.0**	**n**
AB G2	5.81	9	1	3	13
AB GA	5.62	11	0	1	12
AB HA	5.68	10	0	2	12
AB GN	5.69	10	2	1	13
Average/total AB	5.70	40	3	7	50
SB G2	5.61	10	3	0	13
SB GA	5.70	10	2	2	14
SB HA	5.74	8	3	2	13
SB GN	5.78	7	4	2	13
Average/total SB	5.71	35	12	6	53
Average	**5.70**	**75**	**15**	**13**	**103**

Table 12. Effect of kill date on mean pH values and distribution of pH values for ribeye from 103 animals, 2019

			Number of ribeye with pH			
Kill date	**Season**	**pHu**	**<5.7**	**>=5.7, <6.0**	**>=6.0**	**n**
08/01/2019	AB	5.58	18	1	0	19
29/01/2019		5.74	14	1	3	18
12/02/2019		5.83	8	1	4	13
16/04/2019	SB	5.55	12	2	0	14
05/05/2019		5.90	7	5	5	17
21/05/2019		5.70	10	4	1	15
11/06/2019		5.57	6	1	0	7
Average/Total		**5.70**	**75**	**15**	**13**	**103**

In 2019, SB cattle again tend to give more high pHu meat than AB cattle, with 20% of AB carcases and 34% of SB carcases giving loins with a pHu > 5.7. In 2018, these values were 22% and 51%, respectively. However, there were fewer severely DFD carcases (pHu > 6.0). There was also a clear association with kill date with high proportions of high pHu meat slaughtered on 12 February, 5 May and 21 May.

The factors affecting the incidence of DFD meat have been recently reviewed by Gagaoua et al. (2021) and Ponnampalan et al. (2017). The underlying cause of high pHu meat is a shortage of hydrogen ions, which are formed by glycolysis and also ATP hydrolysis. Thus,

DFD meat is often associated with low levels of glycogen. The pre-slaughter factors that cause DFD can be grouped into nutrition, season and stress. Risk factors include a low plane of nutrition, heat stress, mixing of cattle and time in lairage (Gagaoua et al., 2021). It has been concluded that a combination of these factors is usually responsible for DFD meat (Ponnampalan et al., 2017). There are also many detailed individual studies on the impact of diet and other factors on the occurrence of high pHu beef (DRD or dark cutting). Loudon et al (2018) identified low pasture magnesium concentrations, occurrence of mycotoxins, gender (castrates compared with females), limited access to water and absence of feed supplementation in the last 7 days as risk factors for dark cutting beef on Australian farms.

The higher incidence of dark cutting beef in the 2018 study may have been associated with a period of drought and poor grass growth, which occurred in 2018 but not 2019. However, the higher DFD occurrence also occurred in the housed concentrate/silage-fed animals. The apparent effect of kill date could be linked to lairage factors on those dates; even one distressed animal can raise the stress levels in all animals present (Thompson, J., personal communication). There is no apparent reason why season of birth should affect DFD beef except insofar as this affects the period of slaughter and the dietary and climate conditions. All the 2017-18 SB animals were slaughtered in April – June 2018, with ~~unsually~~unusually high incidence of DFD (Table 9). The AB animals were slaughtered between January and May 2018 but even the late slaughtered animals did not show the same high incidence of DFD as the SB animals (Tables 9 and 10). This effect would justify further investigation.

Both season of birth and kill date appear to influence the incidence of high pHu in these experiments, with higher incidence in SB cattle and on certain dates. The apparent effect of concentrate diet observed in 2018 was not observed in 2019.

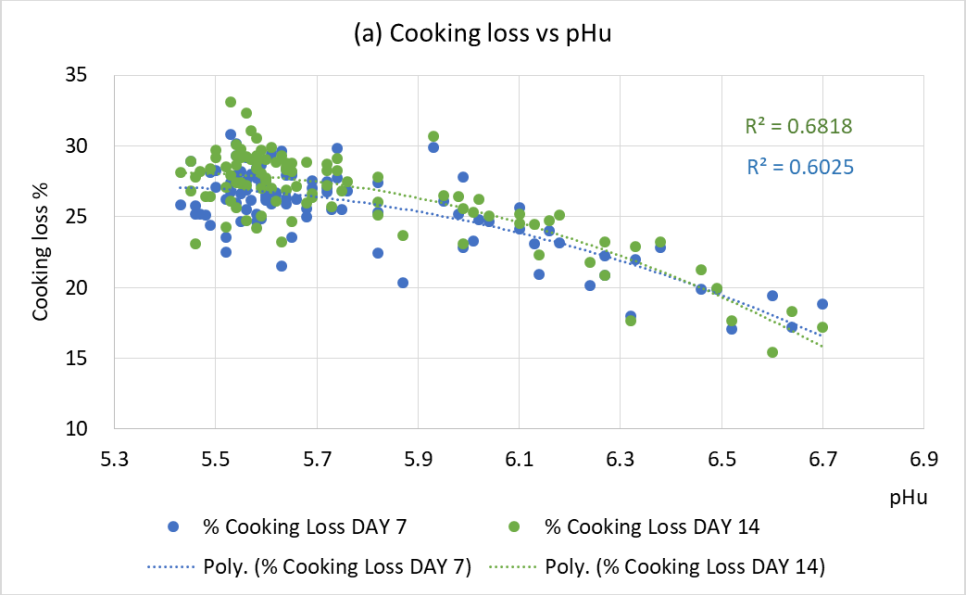
4.6. Interrelationships

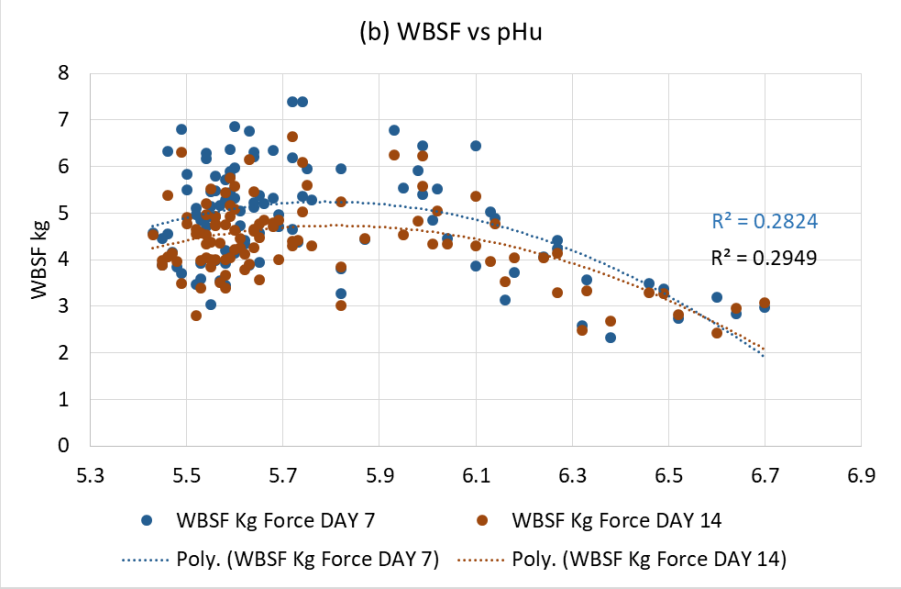
The large number of analyses conducted on the *longissimus dorsi* of the same animals allows the impact of various factors on aspects of meat quality to be considered.

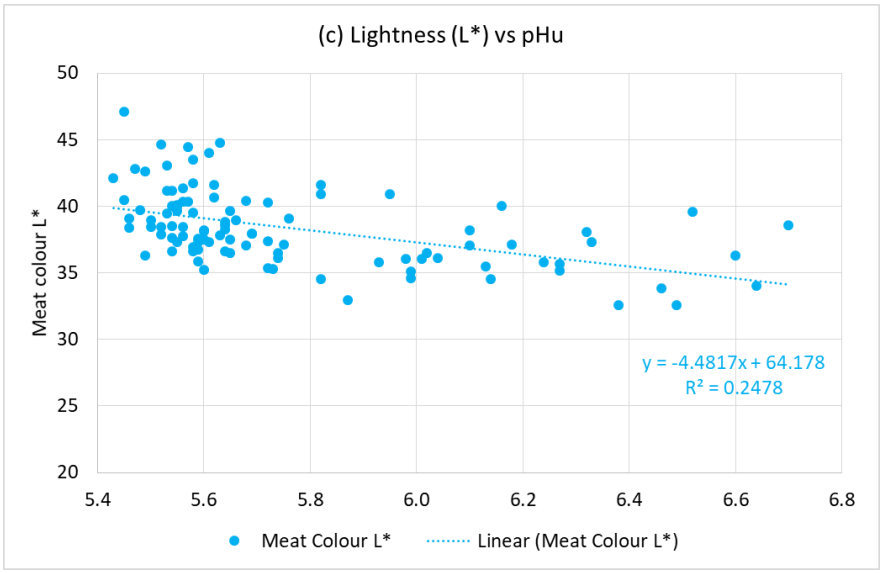
4.6.1. Impact of pHu

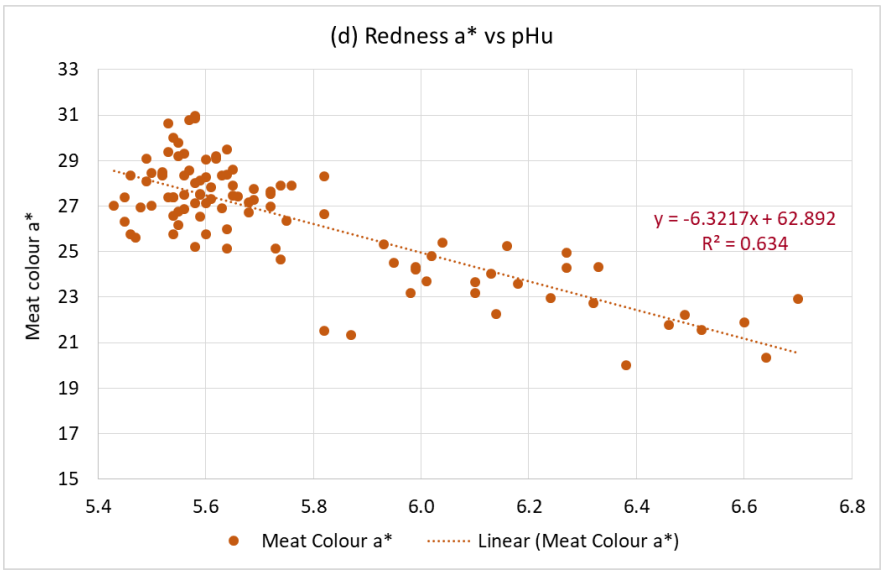
The wide range of pHu values detected proves an opportunity to examine the impact of this on beef quality measurements. The pHu, cooking loss, Warner Bratzler Shear Force (WBSF) and L*a*b* colour measurements were conducted on the ribeye section of the *longissimus dorsi* for all 103 animals. Figures 4 (a-e) shows the correlations between the pHu and these other meat quality parameters. All were very highly significant (P<0.001).

*Figure 4. Relationship between pHu and (a) Cooking loss, (b) WBSF, (c-e) L*a*b**






(c) Lightness (L*) vs pHu
Meat colour L*
50
45
40
35
30
25
20
5.4
5.6
5.8
6.0
6.2
6.4
6.6
6.8
y = -4.4817x + 64.178
R² = 0.2478
Meat Colour L*
Linear (Meat Colour L*)


(d) Redness a* vs pHu
Meat colour a*
33
31
29
27
25
23
21
19
17
15
5.4
5.6
5.8
6.0
6.2
6.4
6.6
6.8
y = -6.3217x + 62.892
R² = 0.634
Meat Colour a*
Linear (Meat Colour a*)

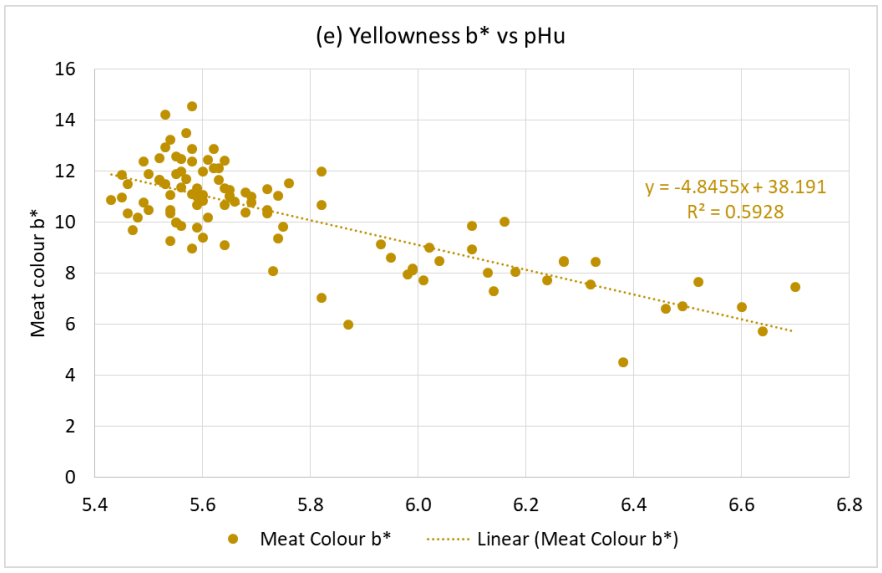

(e) Yellowness b* vs pHu
Meat colour b*
16
14
12
10
8
6
4
2
0
5.4
5.6
5.8
6.0
6.2
6.4
6.6
6.8
y = -4.8455x + 38.191
R² = 0.5928
Meat Colour b*
Linear (Meat Colour b*)

Figure 4 confirms relationships reported elsewhere, that higher pHu meat has reduced cooking loss, due to increased water holding capacity, reduced WBSF and is darker (less light), less red and yellow than beef in the normal pHu range (Lawrie, 1985; Holdstock et al., 2014; Mahmood et al., 2017). These data also indicate that, while the effects on cooking loss and WBSF appear to start at pHu = 6.0, the traditional threshold for dark cutting beef, the impact on colour appears to start nearer pHu = 5.7, the threshold used by Meat Standards Australia (Watson et al., 2008).

The remaining analyses were conducted on a reduced subset of sirloins from which high pHu carcases were deliberately excluded, so that the effect of treatment on sensory quality could be assessed. However, some effects were observed, even over a narrow pHu range. Figure 5 compares the pHu in the ribeye section of the *longissimus dorsi* and in the sirloin of these selected animals. Despite the narrow range (pHu<5.83), the relationship is strong with R^2=0.84, thus explaining 84% of the variation (P<0.001).

Figure 5. Correlation between pHu measured in sirloin and ribeye

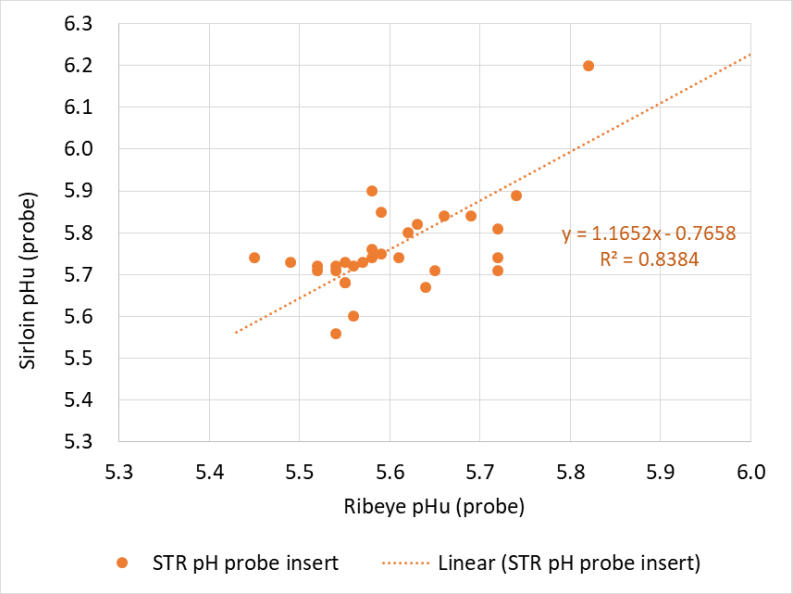
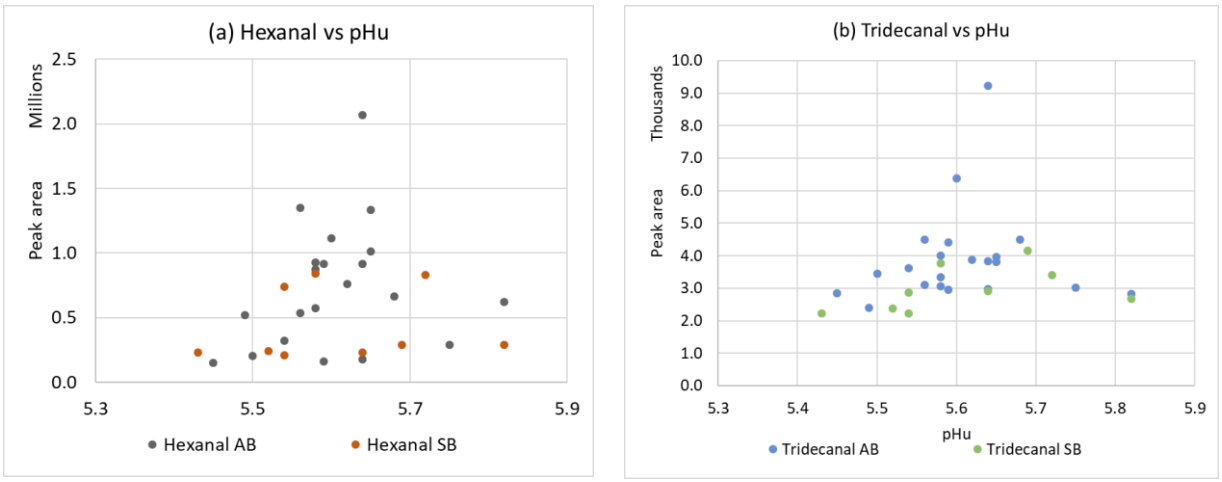


Figure 6 indicates a possible relationship between pHu and the formation of aldehydes, such as hexanal and tridecanal (not significant). There is a difference in the response of beef from AB and SB animals, with AB giving more of these compounds (as discussed previously). As discussed previously, this may be related to a difference in antioxidant levels. There is also a possible effect of increasing pHu but this requires further investigation.

Figure 6. Relationship between pHu and (a) hexanal and (b) tridecanal collected from the aroma of grilled beef



It is known that high pHu beef has very low levels of glucose (Ijaz et al., 2020) and this might be expected to affect the formation of products of the Maillard reaction between sugars and amino acids such as pyrazines and Strecker aldehydes. However, no such relationship was observed (data not shown). This was probably due to the narrow pHu range, with all meat with pHu between 5.50 and 5.83.

Likewise there were few relationship with sensory attributes, again likely due to the narrow range of pHu. However, there was a trend for rubbery texture to reduce with increasing pHu (R^2 = 26%).

4.6.2. Impact of marbling and fat content

Marbling is widely regarded as being important for tenderness and other aspects of eating quality. Marbling was recorded using the Meat Standards Australia scale and a number of meat quality measurements were correlated with these scores.

Cooking loss and WBSF were inversely correlated with marbling; despite high significance ($P<0.001$), the R^2 values are low and only 14-22% of the variation is explained. Both relationships are stronger after 7d ageing than at 14d ageing. Presumably, these parameters are influenced by additional factors, such as additional proteolysis occurring after 14d ageing.

Figure 7. Relationship between marbling score and cooking loss after 7d and 14d ageing

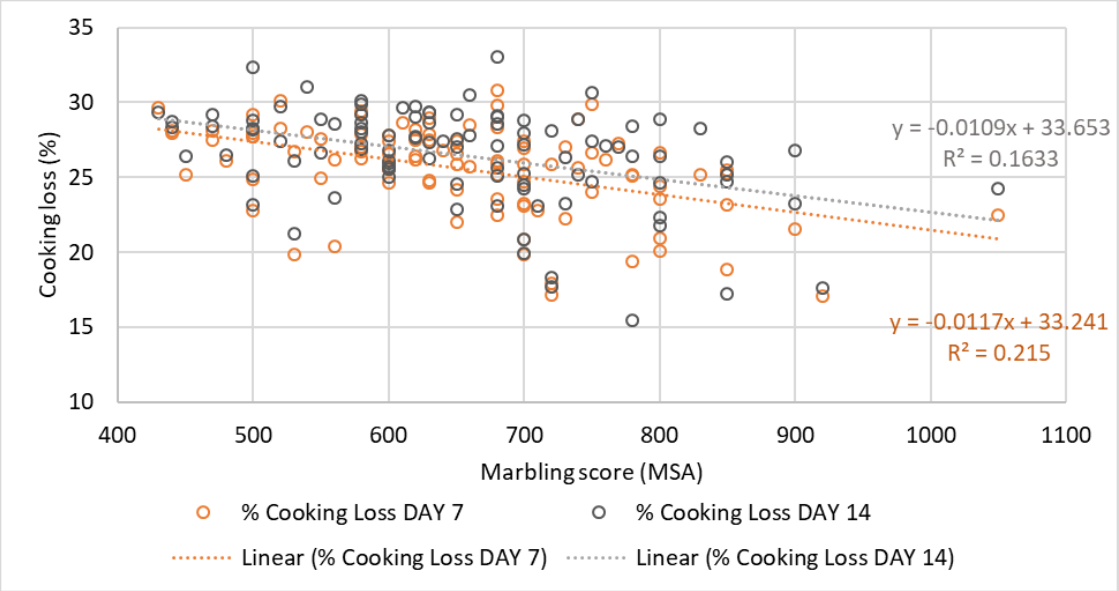
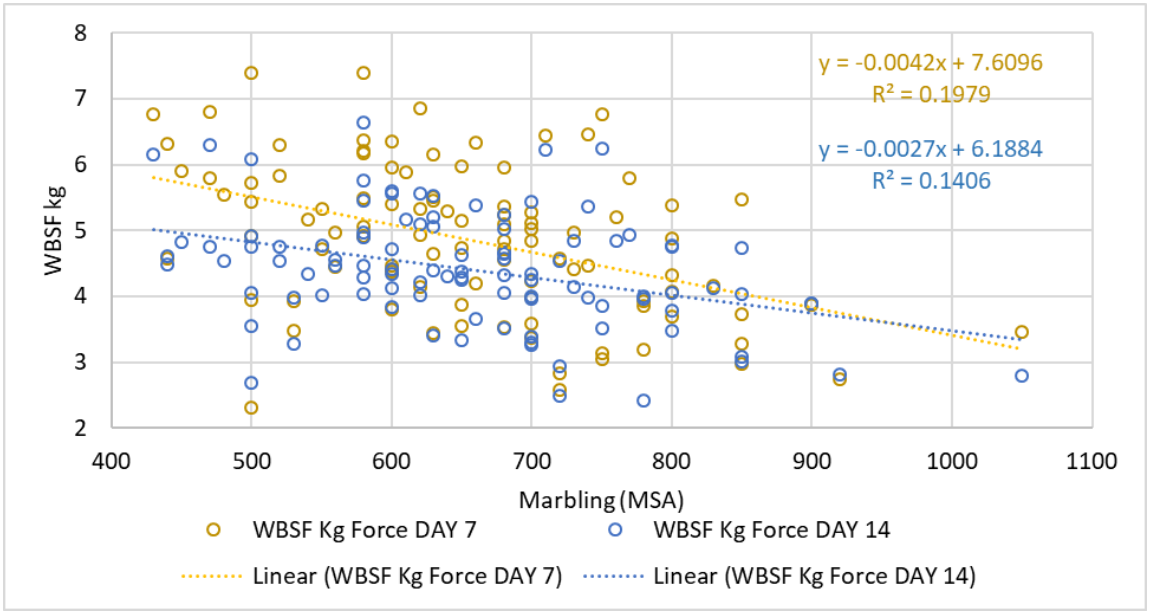
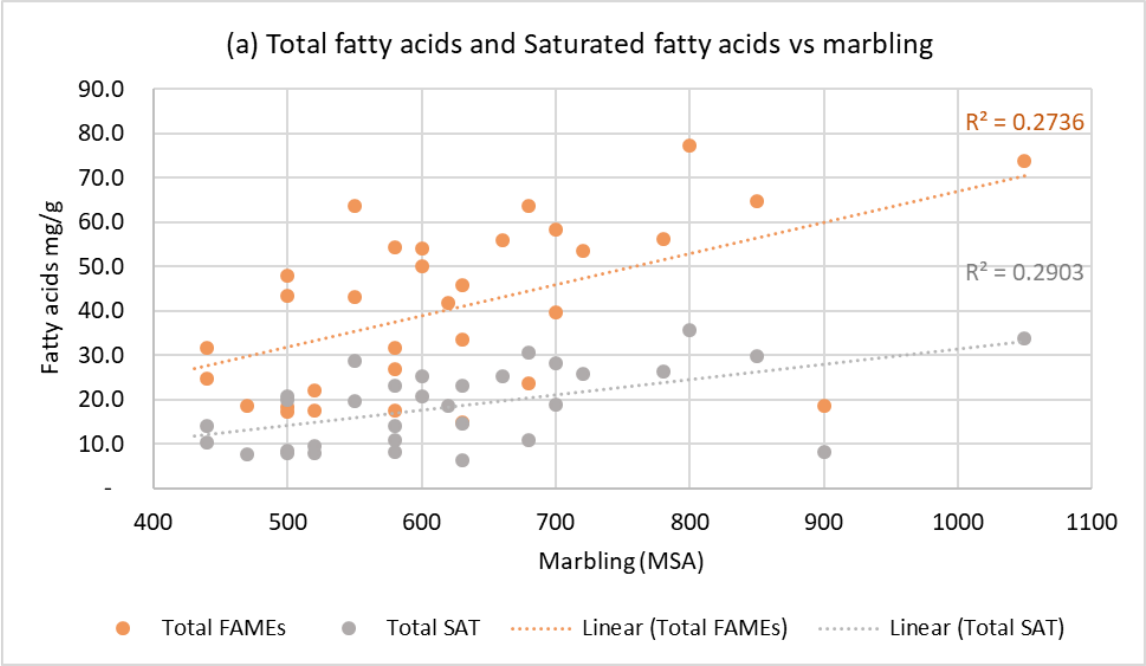


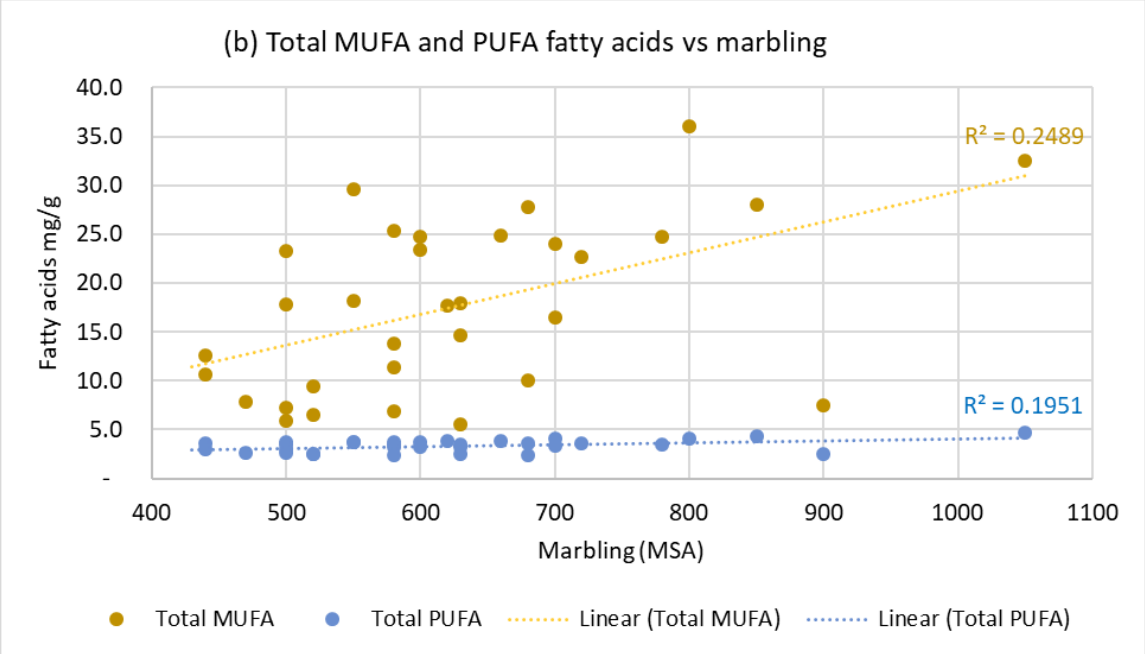
Figure 8. Relationship between marbling score and WBSF after 7d and 14d ageing



In the absence of direct measurement of intramuscular fat, the total fatty acids (as methyl esters) gives an indication of total fat. Figure 9 shows the relationship between total fatty acids and the main fatty acid groups and marbling ($P<0.01$, except for PUFA ($P<0.05$)). Higher levels of marbling show higher levels of total fatty acids, with the strongest relationship with saturated fatty acids, which are generally highest in the marbling fat. However, visible marbling only explains 29% and 27%, respectively, of the variation in these fatty acid measurements, so marbling is not the main driver of lipid content.

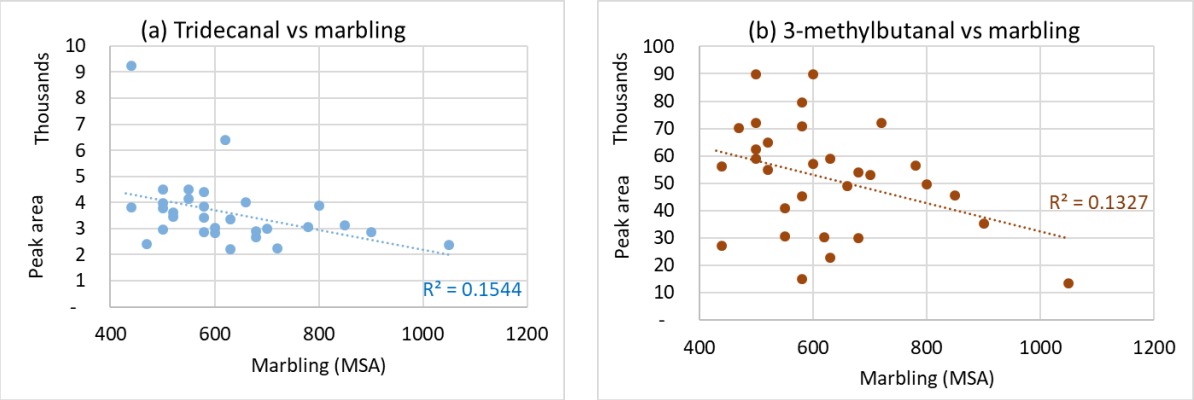
Figure 9. Relationship between marbling score and (a) total fatty acids and saturated fatty acids, (b) MUFAs and PUFAs





Marbling often reduces the volatile aroma compounds detected from cooked meat. This is due to the fact that these compounds dissolve in the fat and form a reservoir of flavour that is released gradually during eating. This is the main reason why high fat levels are perceived as desirable. This effect occurs for the data reported here and is illustrated for two compounds in Figure 10 ($P<0.05$), one from lipid oxidation and one from the Maillard reaction. There is considerable variation and this effect only explains 15% and 13% of the variation in these compounds, so other factors are also involved such as presence of precursors and antioxidants and conditions of pH and water activity.

Figure 10. Relationship between marbling score and (a) tridecanal and (b) 3-methylbutanal



5. Conclusions

The aim of this project was to determine if there is a residual benefit of early life grass-feeding on the sensory quality of beef sirloins. In an associated postgraduate study analyses were conducted to determine fatty acid composition and flavour volatiles. The results showed that:

1) **Differences in dietary regime during the "grower" period had little effect on the sensory quality, fatty acid content or flavour volatiles.** This suggests that the subsequent finishing period was sufficient to overwrite any impact of grass versus concentrate diets at the grower stage. Only a modest increase in some long chain n-3 fatty acids, significant only in the case of C20:5, carried through to slaughter date. It is unsurprising, therefore, that there were no consistent effects of dietary regime on flavour volatiles and no significant effects on sensory quality.

However, two significant effects were observed that are worthy of consideration.

2) **The flavour volatiles were significantly affected by season of birth, with more volatiles from grilled sirloin from AB meat**. This was reflected in increased scores for intensity of flavour from both the home panel and the trained panel but neither of these results was statistically significant. This increase in volatiles was not explained by fatty acid analyses nor fat content.

3) **Ribeye pHu varied considerably with a higher than expected incidence of high pHu beef across the experiment.** Both season of birth and kill date appear to influence the incidence of high pHu in these experiments, with higher incidence in SB cattle and on certain dates.

4) **The results over a wide pHu range confirm clear relationships between pHu and WBSF, cooking loss and colour.** These data also indicate that, while the effects on cooking loss and WBSF appear to start at pHu = 6.0, the traditional

threshold for dark cutting beef, the impact on colour appears to start nearer pHu = 5.7, the threshold used by Meat Standards Australia. There are also likely effects on volatile compounds over a narrower pHu range which appeared greater for AB than SB beef.

6. Applications and Impact

1) **If an improvement in fatty acid composition and the associated health benefits and/or sensory quality is required, dietary treatments need to be continued into the finishing phase.** It is apparent that the diet fed at the "grower stage" has only a minor impact on the quality of the final meat and is largely annulled by the effects of the finisher period.
2) **The high incidence of high pHu meat and its association with SB cattle and certain dates justifies further consideration.** The impact of date is likely associated with animal handling, whether due to transport or conditions at the abattoir, emphasising the need to keep animals calm and unstressed as far as possible.
3) **Season of birth has an impact on both the incidence of high pHu beef and on flavour volatiles.** Further investigation of the mechanisms by which these effects occur may help to optimise flavour quality.

7. References

Chail, A., Legako, J. F., Pitcher, L. R., Griggs, T. C., Ward, R. E., Martini, S., MacAdam, J. W. (2016). Legume finishing provides beef with positive human dietary fatty acid ratios and consumer preference comparable with grain-finished beef. **Journal of Animal Science**, 94, 2184-2197.

Chail, A., Legako, J. F., Pitcher, L. R., Ward, R. E., Martini, S., MacAdam, J. W. (2017). Consumer sensory evaluation and chemical composition of beef gluteus medius and triceps brachii steaks from cattle finished on forage or concentrate diets. **Journal of Animal Science**, 95, 1553-1564.

Daley, C.A., Abbott, A., Doyle, P.S., Nader, G.A., Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. **Nutrition Journal** 9(10). Published online 2010 Mar 10. doi: 10.1186/1475-2891-9-10

Elmore, J.S., Warren, H.E., Mottram, D.S., Scollan, N.D., Enser, M., Richardson, R.I., Wood, J.D. (2004). A comparison of the aroma volatiles and fatty acid compositions of grilled beef muscle from Aberdeen Angus and Holstein-Friesian steers fed diets based on silage or concentrates. **Meat Science**, 68, 27-33.

Fruet, A. P. B., Trombetta, F., Stefanello, F. S., Speroni, C. S., Donadel, J. Z., De Souza, A. N. M., Rosado Junior, A., Tonetto, C. J., Wagner, R., De Mello, A., Nornberg, J. L. (2018). Effects of feeding legume-grass pasture and different concentrate levels on fatty acid profile, volatile compounds, and off-flavor of the M. longissimus thoracis. **Meat Science**, 140, 112-118.

Gagaoua, M., Warner, R.D., Purslow, P., Ramanathan, R., Mullen, A.M., López-Pedrouso, M., Franco, D., Lorenzo, J.M., Tomasevic, I., Picard, B., Troy, D., Terlouw, E.M.C. (2021) Dark-cutting beef: A brief review and an integromics meta-analysis at the proteome level to decipher the underlying pathways. **Meat Science** 181, 108611

Holdstock. J., Aalhus, J.L, Uttaro, B. A., Lopez- Campos, O., Larsen, I. L. and Bruce, H.L. (2014). The impact of ultimate pH on muscle characteristics and sensory attributes of the *longissimus thoracis* within the dark cutting (Canada B4) beef carcass grade. **Meat Science** 98, 842- 849

Lawrie, R.A., Ledward, D.A., “Lawrie’s meat science” 7th Edition, CRC Press, Boca Raton, New York, Washington DC (1985).

Legako, J.F., Cramer, T., Yardley, K., Murphy, T.J., Gardner, T., Chail, A., Pitcher, L.R., MacAdam, J.W. (2018). Retail stability of three beef muscles from grass-, legume-, and feedlot-finished cattle. **Journal of Animal Science**, 96, 2238-2248.

Mahmood, S., Roy, B.C., Larsen, I.L., Aalhus, J.L., Dixon, W.T. and Bruce, H.L. (2017). Understanding the quality of typical and atypical dark cutting beef from heifers and steers. **Meat Science** 133, 75-85.

McAfee, A.J., McSorley, E.M., Wallace, J.M.W., Bonham, M.B., Strain, J.J., Cuskelly, G.J., Tempest, W.W., Fearon, A.M., Moss, B.W. (2011). Contribution of meat (beef and lamb) from grassfed ruminants to the total human dietary intake of long chain n-3 polyunsaturated fatty acids. Booklet 20, Agrisearch, Northern Ireland.

Melton, S.L. (1990). Effects of Feeds on Flavor of Red Meat - A Review. **Journal of Animal Science**, 68, 4421-4435.

Mezgebo, G.B., Monahan, F.J., McGee, M., O'Riordan, E.G., Richardson, I.R., Brunton, N.P., Moloney, A.P. (2017) Fatty acid, volatile and sensory characteristics of beef as affected by grass silage or pasture in the bovine diet. **Food Chemistry**, 235, 86-97.

Moloney, A.P., Mooney, M.T., Kerry, J.P., Troy, D.J. (2001). Producing tender and flavoursome beef with enhanced nutritional characteristics. **Proceedings of the Nutrition Society**, 60, 221-229.

Muawuz Ijaz, Xin Li, Dequan Zhang, Zubair Hussain, Chi Ren, Yuqiang Bai, Xiaochum Zheng, (2020). Association between meat colour of DFD beef and other quality attributes. **Meat Science** 161,107954

Murphy, V.S.; Lowe, D.E.; Lively, F.O.; Gordon, A.W. (2017). The effect of floor type on the performance, cleanliness, carcass characteristics and meat quality of dairy origin bulls. **Animal** 12, 1102–1110

Ponnampalam, EN; Hopkins, DL; Bruce, H; Li, D; Baldi, G; El-din Bekhit, A (2017) Causes and Contributing Factors to "Dark Cutting" Meat: Current Trends and Future Directions: A Review. **Comprehensive reviews in food science and food safety** 16 (3), pp.400-430

Revilla, I., Plaza, J., Palacios, C. (2021) The Effect of Grazing Level and Ageing Time on the Physicochemical and Sensory Characteristics of Beef Meat in Organic and Conventional Production. **Animals**, 11, *http://dx.doi.org/10.3390/ani11030635*

Rutherford, N. H., Gordon, A. W., Arnott, G., & Lively, F. O. (2020). The Effect of Beef Production System on the Health, Performance, Carcass Characteristics, and Meat Quality of Holstein Bulls. **Animals**, 10(10), [1922]. https://doi.org/10.3390/ani10101922

Vasta, V., Luciano, G., Dimauro, C., Rohrle, F., Priolo, A., Monahan, F.J., Moloney, A.P. (2011). The volatile profile of longissimus dorsi muscle of heifers fed pasture, pasture silage or cereal concentrate: Implication for dietary discrimination, **Meat Science**, 87, 282-289.

Vasta, V., Priolo, A. (2006). Ruminant fat volatiles as affected by diet. A review. **Meat Science**, 73, 218-228.

Watson, R.; Polkinghorne, R.; Thompson, J.M. (2008). Development of the Meat Standards Australia (MSA) prediction model for beef palatability. **Australian Journal of Experimental Agriculture**, 2008, 48, 1368–1379.

Appendices

Appendix 1. List of sensory attributes for trained sensory profiling panel and definitions

ATTRIBUTE ABBREVIATION	ATTRIBUTE
Int AR	Intensity of aroma
Roast AR	Roasted aroma
Beef AR	Beefy aroma
Charg AR	Chargrilled aroma
Boiled meat AR	Boiled meat aroma
Fatty AR	Fatty aroma
Bloody AR	Metallic/bloody aroma
Even APE	Evenness of colour – external appearance
Char APE	Charred external appearance
Bloody APE	Bloody external appearance
Greasy APE	Greasy/oily/fatty external appearance
Tender TX	Tenderness
Fibrous TX	Fibrous/stringy texture
Sticky TX	Sticky/clingy texture
Juicy API	Juicy internal appearance
Closep API	Closely packed internal appearance
Int FL	Intensity of flavour
Roast FL	Roasted flavour
Beefy FL	Beefy flavour
Charg FL	Chargrilled flavour
Metallic FL	Metallic/bloody flavour
Sour FL	Sour/acid flavour
Bitter FL	Bitter flavour
Sweet FL	Sweet flavour
Rancid FL	Rancid flavour
Tender TXM	Tenderness in mouth
Rubbery TXM	Rubbery in mouth
Sticky TXM	Sticky/clingy in mouth
Stringy TXM	Stringy/clingy in mouth
Greasy TXM	Greasy/oily in mouth
IntAT	Intensity of aftertaste
Roast AT	Roasted aftertaste
Beefy AT	Beefy aftertaste
Metallic AT	Metallic/bloody aftertaste
Greasy AT	Greasy/oily aftertaste