final report

Project code:	B.SBP.0085
Prepared by:	L.M. Cafe, W.A. McKiernan, D.L. Robinson
	NSW Department of Primary Industries
Date published:	October 2014
ISBN:	9781925045536

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Using muscling selection line cows to inform maternal productivity modelling

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

This project studied the effect in an Angus cow herd of selection for divergent muscling on the maternal productivity of the cow herd under divergent nutrition. The herd comprised a Low muscle line (D muscle score average); a High muscle line (C muscle score average); and a High^{Het} line (B muscle score average) comprising High muscled cows with one copy of the 821del11 mutation in the *myostatin* gene. The herd was managed on Low or Medium/High nutrition over three reproductive cycles, generating 750 joining records. High muscle cows had similar calving rates and maternal performance to Low muscle cows under nutritional challenge (average cow pre-join liveweight 498-508kg, rib fat 2.7-4.4mm), but the High^{Het} cows showed a slight reduction in calving rates. Low and High muscle cows weaned 191kg of calf/cow joined.year while High^{Het} cows weaned 165kg. This project also reports on the ability of 3D RGBD (Red, Green, Blue, + Depth) camera systems to estimate cow body condition, and on differences in plasma metabonomics with change in cow condition. Industry can be confident that moderate increases in muscling in cows will not affect maternal productivity, but using the *myostatin* gene to further increase muscling could result in reduced productivity under nutritional challenge.

Executive summary

There is a perception in the Australian beef industry that market signals for enhanced carcase yield could have adverse impacts on maternal productivity traits through decreased fatness or increased muscling in breeding cows. Higher visible muscling increases dressing percentage and saleable meat yield at slaughter, and accordingly the value of animals with higher muscling is recognised in the marketplace. However, the Australian industry has been reluctant to increase cow herd muscularity and only 5%-10% of Australia's beef cattle population is classified as highly muscled (McKiernan 2002). The introduction of various highly-muscled European breeds into the Australian cattle population created negative perceptions towards increased muscling. These breeds brought with them the advantage of high muscling, but they also had higher calving difficulties, inferior fertility, low levels of fatness and large frame size (Morris et al. 1993) which limited their use in harsher pastoral conditions. These traits were attributed to high muscling, rather than being seen as being breed specific, and discouraged Australian cattle producers from selecting for increased muscling in their females under the belief that it would result in reduced maternal performance in the inevitable tough times experienced under pastoral conditions.

Selection for visual muscling over several generations in the NSW DPI Angus muscling selection line herd had resulted in a cow herd comprising a Low muscle line (D muscle score average), a High muscle line (C muscle score average), and a $High^{Het}$ line (B muscle score average) with one copy of the 821del11 mutation (Grobet et al. 1997) in the *myostatin* gene causing muscle hypertrophy. Inclusion of the heterozygous cows allowed an additional level of biological variation in muscling to be studied. Results previously reported on this herd showed no difference in maternal productivity between the lines under average to good nutritional conditions (Cafe et al. 2012, Cafe et al. *in press*). This project was designed to test the effect of selection for muscling on maternal productivity under nutritional challenge.

The three muscle lines grazed divergent (Low and Medium/High) nutritional treatments for three reproductive cycles. There was variation in the level of nutrition provided over time. The Low treatment was always the worst, but a short period of nutritional challenge also occurred in the Medium/High treatment. Under these conditions no differences were observed in pregnancy rate, calving rate, weaning rate or weaning weight of calves produced by cows from the Low and High muscle lines. Low and High cows had the same maternal productivity of 191 kg calf weaned per cow joined per year across the three years of the experiment. The $High^{Het}$ cows showed a trend towards reduced pregnancy rate and, consequently, reduced weaning rate, but no difference in weight of calves weaned. Their reduced weaning rates led to a maternal productivity of 165 kg calf weaned/cow joined per year across the three years of the experiment, 26kg (13.6%) less than the Low and High lines. This result indicates that the incorporation of the 821del11 *myostatin* mutation into the breeding herd should be treated with some care. If heterozygous *myostatin* females are to be kept for breeding they should be run under more favourable nutritional conditions, with the knowledge that they may show reduced productivity under nutritional stress.

A maternal performance decision support system was developed to prototype level as part of the Beef CRC for Genetic Technologies. Models which accurately predict the effect of management and selection decisions on herd productivity are required to provide the basis for a decision support system which can be used by industry with confidence. One of the objectives of this project was to produce a dataset of maternal performance in cows with a large divergence in muscling and fatness traits suitable for use in validating the maternal models. The herd was tested for its ability to be productive under nutritional restriction, and were pushed to the point of a drop in fertility, providing data that will be useful for validating the prototype models.

During the project the cows were also used to test 3D Red, Green, Blue, + Depth (RGBD) camera technology for its ability to assess cow body condition score. A method was developed to estimate condition score using data gathered from cattle using a pair of RGBD cameras, without the need for ultrasonic measurements or trained assessors. A data-driven, supervised-learning approach employing classification and regression techniques was used. The analysis showed 72.3% ± 4.9% and 77.6% ± 4.8% correct classification for condition score on two data sets. This method has some potential to be further developed into an objective automatic system to assess body condition as cows pass freely through a race, allowing more effective cow management and reducing the risk of poor welfare.

A metabonomics study was conducted on plasma samples from the cows to attempt to gain a better understanding of the differences in metabolism between cows of divergent body composition. Preliminary principal components analysis of Nuclear Magnetic Resonance (NMR) spectral data indicated a number of metabolites that differed between thin (≤ 2mm rib fat) and fat (≥ 7mm rib fat) cows. These included betaine (trimethylglicine), creatine, phosphatidylcholine, lipids and glucose. Betaine appeared to have the strongest relationship with body condition, and was also related to muscling. This result could be pursued further by determining accurate concentrations of the metabolites of interest, and conducting more direct analysis of the relationship between differences in body composition and maternal productivity.

Conclusions

1. Selection for moderate increases in muscling in cows will not affect maternal productivity, but using the 821del11 mutation in the *myostatin* gene to further increase muscling could result in reduced productivity under nutritional challenge.
2. A number of plasma metabolites were found to vary with body condition in the muscling cows, with betaine appearing to have the strongest relationship.
3. This study has provided a ‘proof of concept’ of using 3D camera images for estimating condition score in breeding cows.

Actions and recommendations for continuing and future research

1. Prepare a fact sheet summarising the results for the maternal productivity of the muscle lines under nutritional constraint to facilitate release of the findings to industry
2. Consider value of continuing to collect weight and scan data on the herd as the cows from Low nutrition return to good body condition, to assess any residual impacts of longer-term nutritional restriction on productivity and, if present, for any link to muscle line.
3. Measure the glucose (or lactate) concentration of some key plasma samples to enable accurate calculation of concentrations of betaine and other significant metabolites from the spectra of all samples. This would allow more direct analysis of the implication of variation in these metabolites with cow body composition traits, and their relationship with maternal performance.
4. The 3D RGBD camera method has the potential to be further developed into an automatic system for identifying cows that are changing body condition, allowing more effective cow management and reducing the risk of poor welfare.
5. There are a number of research questions that the muscling herd could be used to study in the future. These include: testing the usefulness of a whole body muscling EBV, studying the genetic control of muscling outside the extreme myostatin genes, testing the effect of muscling on the efficiency of cows at pasture and on cow maintenance requirements, and incorporating cross-breeding studies.

Table of Contents

1 Background

Two collaborating programs have been undertaken to evaluate the impacts of selection for increased progeny carcase yield on cow herd productivity. Firstly, the Beef CRC Maternal Productivity project examined the effect of divergence in rib and rump fat EBV on fertility and productivity of the cow herd. Secondly, the NSW DPI Angus muscling herd provides additional data for cows with significant variation in muscularity, which is not available for other purebred herds. Substantial improvement in carcase yield can be achieved by either decreased fatness or increased muscling, each of which has a different effect on over-all body composition and is likely to have different effects on the cow herd. Thus both of these components need to be examined to fully understand the effects. The combination of information generated by these programs allows exploration of the interaction between decisions based on both selection and management. It also offers the opportunity to amalgamate the acquired maternal productivity knowledge base into a modelling tool which can be used by industry to assess the potential effects of management and selection decisions on cow herd productivity.

The development of the NSW DPI Angus muscling herd, the performance of the lines over 12 years of average to good nutritional conditions, and the results after one year of divergent nutrition were presented in the final report of the previous project (BFG.0049: Additional measurements on muscle line cattle, February 2012) from which this project continues. In this report results are presented for full three years on divergent planes of nutrition. In addition, a metabonomic study was conducted to see if any differences in physiology between cows of divergent muscling in poor or good condition could be detected. A better understanding of any differences in metabolism between cows of divergent body composition could help further describe the effects of selection for muscling, as well as adding to the understanding of the metabolism of change in condition and how it affects maternal productivity.

The muscling herd under divergent nutrition also provided an ideal resource for validating live cattle 3D camera measurement systems as a method of detecting changes in cow body shape and body condition. This research was conducted largely by a collaborating project (B.BSC.0339: Initiative - Trait measurement from 3D images, McPhee 2013), the final report from which describes the success at predicting fatness and muscle score in live cattle using images from 3D RGBD cameras. In this project, the use of RGBD camera technology and data acquisition software systems to predict body condition score was tested.

This project was therefore designed to build on the data and understanding produced by the Maternal Productivity Research Project of the Beef CRC. Data collected from three muscle lines on divergent nutrition will enable the partitioning of the effects of selection for muscling (as opposed to fat) on maternal productivity to achieve overall herd improvement in meat yield, and allow a better understanding of any impact of selection for muscling on productivity.

2 Project objectives

1. Commit and maintain the muscle line cow herd on divergent nutritional treatments until May 2013 for the purposes of collection of reproductive and production data.
2. Female productivity will be assessed by:
    - Calving rate, days to calving, calving ease, and inter-calving interval following single sire natural mating
    - Calf growth to weaning including birth weight, weaning weight, and ultrasound scanning at weaning to describe body composition
    - Cow weight and body compositional changes through weighing and ultrasound scanning four times per year
3. Collect image analysis data (both laser and 3D camera data) and observed condition score (CS) in conjunction with ultrasound scanned body composition throughout 2012.
4. Develop laser and camera technology relationships with CS by August 2013.

3 Muscling herd nutrition experiment

Research Team: NSW Department of Primary Industries Armidale – Linda Cafe, Helen Smith, Dorothy Robinson; NSW DPI Glen Innes - Peter Newman, Peter Kamphorst, Phil Dawes; NSW DPI Grafton – David Bennett; NSW DPI beef extension officers - Jason Siddell, Bill Hoffman, Trevor Rose; AGBU – Matt Wolcott

3.1 Methods

3.1.1 Herd description

The muscling herd was originally established by NSW DPI in 1992 at its Menangle Research Station near Sydney. The herd began with unselected Hereford cows and heifers mated to high and low muscled Angus bulls. In 1997, the project was continued by selecting females produced in the earlier matings by visual muscle score into Low and High muscle lines. Continued divergence in the lines has been achieved by mating the cows to low and high muscled Angus bulls sourced from industry. In 2005, following the segregation of a *myostatin* mutation (nt821 del11, Grobet et al. 1997) in the High line, a third selection line of females carrying one copy of the *myostatin* mutation was established (Myostatin line). High muscled progeny are allocated to either the High or Myostatin line based on their *myostatin* genotype.

The current design is for low muscle bulls (D muscle score average) to be mated to Low cows and maiden heifers; high muscle bulls (B+ muscle score average) without the *myostatin* deletion mated to Myostatin cows and maiden heifers, and High maiden heifers; and Myostatin bulls (A muscle score average) carrying one copy of the *myostatin* mutation mated to High cows without the *myostatin* mutation. This strategy avoids the generation of animals homozygous for the mutation, which are less functional and undesirable.

3.1.2 Data collection methodology

3.1.2.1 Breeding herd management

The cows were single-sire mated naturally in Spring/Summer, to calve in Spring the following year. Calves were weighed and tagged at birth, and their birth date, sex, dam, and need for birth assistance recorded. All calves were bled to determine *myostatin* genotype testing prior to weaning. Weaning was carried out in late April to early May, the weaners were weighed, ultrasound scanned for body composition and muscle scored. Cows were allowed to remain in the herd if they didn't calve for one year, but were culled if they did not calve the following year.

Heifers joining the herd were first mated as yearlings but were not subjected to the nutritional treatments for their first lactation because the Low nutrition treatment would have been too much of an imposition on them. Heifers were provided with good nutrition while lactating with their first calf. Cows were culled for age at 10 to 11 years of age. The reason for all culling was recorded.

Bulls were used for 2-3 seasons, and mated to small groups of cows (15-40) to ensure a reasonable number of sires were represented each year.

The herd was run as a commercial beef herd in terms of the required treatments for parasites and disease to suit the location.

3.1.2.2 Nutritional treatments

The cow herd was managed under divergent pasture-based nutritional treatments from April 2010 (mid way through gestation of the 2010-born calf cohort) until May 2013 (weaning of the 2012-born calf cohort). Cows (including 2 year olds that had just weaned their first calves) were allocated to either Low or Medium/High treatments in 2010 and remained on the same treatment throughout. Young cows which had just weaned their first calf joined the treatments in May 2011 and June 2012, mid-way through gestation of their second calf. In contrast, older cows were on the treatments for the entire gestation of the 2011 and 2012-born cohorts. Bulls were managed as one group on medium nutrition outside the joining period, but were subjected to the nutritional treatments during joining.

Between April 2010 until June 2012 the cow herd was located at Grafton Primary Industries Institute. The Low or Medium/High pasture-based nutrition were driven by differences in soil type and fertility, and the amount of pasture improvement. The Low nutritional treatment was based on native and poor naturalised perennial grass species (ie carpet grass, blady grass, and bahia grass), on unfertilised duplex soils. The yield and quality of these pastures is generally low, with digestibility generally less than 63% and protein less than 6%. The Medium/High nutritional treatment was based on improved perennial species (ie kikyuyu, rhodes and setaria grasses, with some carpet and bahia grasses, and a small component of white clover) on heavy and red alluvial soils. The yield and quality of these pastures is generally moderate with digestibility of 60-70% and protein of 6-9%. High quality ryegrass pasture also formed part of the Medium/High treatment in late winter/early spring when conditions allowed. These treatments were located on opposite sides of the same research station, so that other environmental conditions were the same.

The 2010/2011 summer was challenging, with high buffalo fly numbers and many cows succumbing to three day sickness. Major flooding during this period also reduced pasture production and availability until Spring 2011. This resulted in the

need to supplement 40 cows on Low nutrition in late Winter 2011 to prevent poor welfare due to low body condition. Supplementation consisted of cottonseed meal and molasses, and some hay and ryegrass pasture for several weeks until the cows gained sufficient condition to be returned to the Low nutrition treatments. The 2011/2012 summer was less problematic and cows were able to gain condition through to weaning of the 2011-born calves in May. In June 2012 the herd was relocated to Glen Innes Research Station due to the closure of Grafton Primary Industries Institute to cattle research. To facilitate good welfare during the move the cows on Low nutrition were allowed to gain some condition for the period following weaning and prior to the move.

The nutritional treatments at Glen Innes were based on pasture availability, as the entire station has improved temperate pastures (predominantly phalaris, fescue, cocksfoot and clover) with good fertiliser history. The aim was to provide cows in the Medium/High nutritional treatment with sufficient pasture to maintain their fatness levels at or above recommended levels for a productive cow herd (8-12 mm rump fat) and to restrict the Low cows such that their fatness levels remained below recommended levels (2-4 mm). Divergence was achieved through managing the Low nutrition replicates at higher stocking rates and rotating them around frequently-grazed paddocks. Pastures were assessed between January and May 2013 when replicates entered or left a paddock, to give an indication of the divergence in feed quantity and quality available. Experienced assessors were calibrated through cutting and drying quadrats of pasture representing the range of feed on offer on several occasions during this period. Pluck samples were collected during the same time period for assessment of pasture quality. The pluck samples were oven dried at 70ºC to determine dry matter percentage, and assessed for ash, crude protein, neutral & acid detergent fibre, dry matter digestibility, and metabolisable energy content by the NSW DPI Feed Quality Service using Near Infrared (NIR) feed analysis.

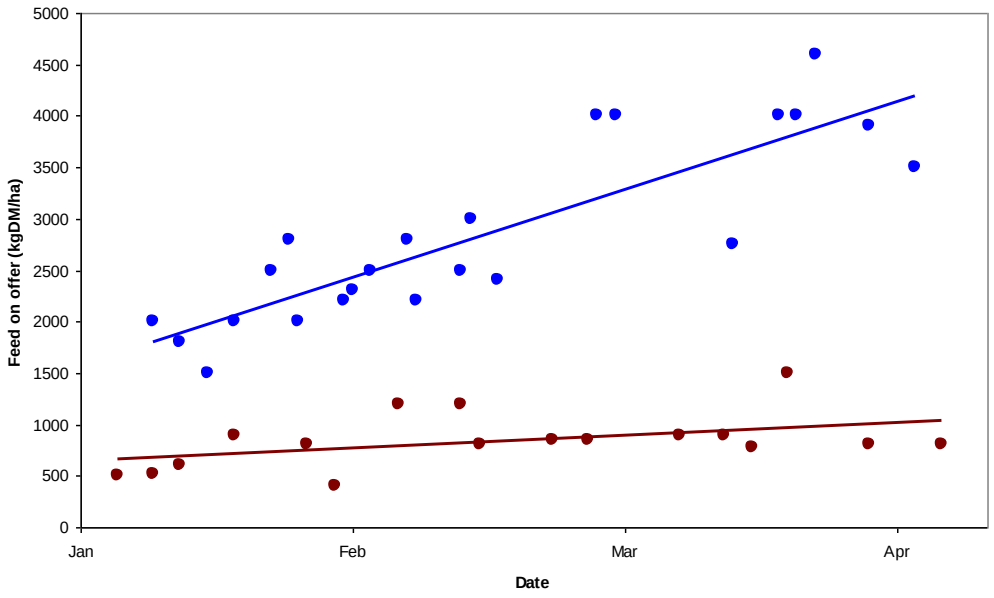

Figure 3.1. Estimated feed on offer (kgDM/ha) at the start of grazing fresh paddocks for Low (red) and Medium/High (blue) nutritional treatments at Glen Innes from the end of joining until weaning in 2013.

Average estimates of the feed on offer at the start of grazing a fresh paddock from January to May 2013 were 2820 kgDM/ha for the Medium/High treatments and 909

kgDM/ha for the Low treatment. Average estimates of the feed remaining when cows were removed from the paddock were 1599 kgDM/ha for the Medium/High treatments and 373 kgDM/ha for the Low treatment (sed=154, P<0.001). See Figure 3.1 for the pattern of change in the feed on offer at the start of grazing a fresh paddock over this period. Plate 3.4 (Page 15) shows examples of a Low and a High paddock.

The quality of the feed on offer to the Low nutrition replicates tended to be slightly higher than that on offer to the Medium/Highs during this period. This was expected due to the heavily-grazed Low treatments containing a high proportion of short fresh regrowth. Quality at the start of grazing a fresh paddock for Low vs Medium/High were: metabolisable energy (11.8 vs 11.0 MJME/kgDM, P=0.082), crude protein (22.4 vs 17.4%, P=0.034) and dry matter digestibility (78.0 vs 73.8%, P=0.064).

3.1.2.3 Data and sample collection

The cows were weighed, ultrasound scanned for body composition [rump fat, rib fat and eye muscle area (EMA)] and muscle scored on 15 occasions between March 2010 and May 2013. All ultrasound scans were conducted by an accredited scanner using a 3.5 MHz/180-mm linear array animal science probe (Esoate Pie Medical, Maastricht, Netherlands). Muscle scoring was conducted by one experienced assessor until May 2011, who then trained a new assessor to carry on from that point. The 15 point, E- to A+ muscle scoring system was used (McKiernan 2007), and converted to a numeric scale of 1 to15 for analysis.

Three-dimensional RGBD camera images were collected at the time of ultrasound scanning on four occasions between Feb 2012 and Feb 2013. Condition score (1 to 9 scale, NRC 1996) was also assessed at five of the six scanning occasions between February 2012 and February 2013. Details of the methods and results for the 3D camera research are presented in Section 5: Estimating cow body condition using 3D RGBD cameras.

Blood samples were collected from the cow herd on 6 occasions throughout the experiment. Blood was collected from the tail vein into EDTA vacuum tubes, placed immediately in crushed ice, centrifuged at 1200Xg for 10 min within 60 min of collection, and plasma aliquots frozen at -20 until return to Armidale, where they were stored for the longer term at -80oC.

3.1.3 Statistical analyses

A number of methods and models, varying with data type, were used to analyse the data.

Maternal performance data, including calf birth weight and calf weaning traits (weight, scanned fat and EMA measurements, muscle score, pre-weaning ADG and age at weaning) were analysed using the REML methodology in ASREML-R (Butler et al. 2009). Models included fixed effects for dam muscle line, nutrition, year, calf sex, dam age group (2, 3, or ≥ 4 YO at joining) plus most of their 2-way interactions as well as a covariate for calf *myostatin* genotype. Random effects were fitted for dam's lactation status the previous year, dam age x year, and dam muscle line x nutrition x year.

Days to calving (DTC) was calculated from the first day of joining to the resulting calf birth date; cows that did not produce a calf were given a 21d penalty above that of

the longest successful calving interval for that calving year (Johnston and Bunter 1996). The analysis of DTC included fixed effects for dam muscle line, nutrition, year, dam muscle line x nutrition, dam muscle line x year, nutrition x year and cow age group, plus random terms for joining sire, lactation status the previous year, cow age x year, and dam muscle line x nutrition x year.

Calving and weaning rates for cows joined in 2009, 2010 and 2011 were analysed using the REML methodology in ASREML-R (Butler et al. 2009), fitting a logistic model (1 = calf born or weaned, 0 = otherwise) with binomial errors and fixed effects for dam muscle line, year, nutrition, dam muscle line x nutrition, and dam age group, plus random effects for cow, joining sire, lactation status the previous year, nutrition x year and dam muscle line x nutrition x year. Conditional Wald P values were used to assess the significance of the terms fitted in the model.

Pregnancy rates for cows joined in 2010, 2011 and 2012 were analysed using a similar binomial model to the calving and weaning rates. Covariates for liveweight, scanned rib fat and frame score of these cows prior to joining were also tested for effect in the binomial models, as well as a covariate to test the common perception that 7mm of rib fat is sufficient to promote good fertility. Cows with only 1 or 2 mm of fat were expected to have greater reductions in fertility than cows with 6 mm, so the covariate was calculated as the scanned rib fat measurement for all cows with rib fat less than 7 mm and a value of 7 mm for all other cows. The cow liveweight and scanned body composition traits prior to joining were also analysed using the same fixed and random effects within the REML methodology in ASREML-R. These analyses allowed results to be included for the 2012 joining, from which the resulting calves had not been born when this report was prepared. The analyses excluded the data from the 2 YO cows, so that all cows had grazed the nutritional treatments for a minimum of five months prior to joining. Data for the 10 YO cows were also included, though they exited the herd prior to calving hence were not included in the calving analyses.

Statistical analyses of lactating cow body composition were conducted by fitting Linear Mixed Models using the REML methodology (Robinson, 1987) in Genstat V16 (VSN International Ltd, Hemel Hempstead, UK). The model included fixed effects for nutrition, muscle line, date and their interactions, and a covariate for cow age.

Statistical significance was accepted at $P<0.05$, and a tendency at $P<0.1$. In the results tables, means followed by different letters denote significant differences ($P<0.05$).

3.2 Results and discussion

3.2.1 Nutritional treatments and changes in cow body composition

The divergence between Medium/High and Low nutrition was maintained over the three year study period, although the level of nutrition provided differed over time. These effects are demonstrated in Figure 3.2 where the change in weight and body composition of the lactating cows over the three years are displayed. The difficult winter/early spring in 2011 at Grafton resulted in the smallest divergence as supplementation of some lactating cows on the Low treatment was needed in July to prevent poor welfare, whilst the cows on the Medium/High treatments did not require supplementation. The Medium/High cows maintained weight reasonably well prior to calving in August/September, but dropped condition by the pre-joining weight and

scan measurements in October. They improved after this with the growth of spring pasture again providing good nutrition.

A further change in the nutritional treatments came with the herd being relocated from Grafton to Glen Innes in June 2012, where both nutritional treatments had a higher level of nutrition than at Grafton (as described in the materials and methods). The liveweight difference between cows on Low and Medium/High nutrition was maintained at Glen Innes, but the cows were heavier under both treatments. The environmental conditions at Glen Innes, to which *Bos taurus* cattle are more suited, would have also played a part in the weight gain of the cows.

In summary, the cows on Low nutrition were tested for their ability to cope with poor nutrition during the two years at Grafton, with the year at Glen Innes being less severe. The cows on Medium/High were effectively provided with medium nutrition whilst at Grafton, with a short period of lower nutrition in 2011, and received High nutrition at Glen Innes.

These treatments resulted in significant differences in weight and body composition for lactating cows over the three years (Table 3.1). The main effects of muscle line and nutrition were always significant (P<0.001), and the interaction between muscle line and nutrition were also usually significant. The results are presented for the interaction to give an indication of the differences.

Table 3.1 Weight, scanned fat and EMA, and muscle score of lactating cows from the three muscle lines across three reproductive cycles on divergent nutrition.

	n records	Weight (kg)	Rib fat (mm)	Rump fat (mm)	EMA (cm^2)	Muscle score (1-15)
Medium/high nutrition						
Low muscle	517	541d	7.6e	11.4d	50.8d	4.0b
High muscle	569	540d	4.8d	6.9c	54.2e	8.3d
High^{Het} muscle	339	528c	3.5c	5.2b	57.1f	10.1f
Low nutrition						
Low muscle	448	483a	3.9c	5.4b	40.6a	3.2a
High muscle	549	490b	2.9b	4.0a	44.7b	7.4c
High^{Het} muscle	291	487b	2.4a	3.6a	49.4c	9.0e
Nutrition signif.		***	***	***	***	***
Muscle line signif.		***	***	***	***	***
Nutrition x Muscle		**	***	***	**	ns

abc Letters denote means which are significantly different. †*P*<0.1, **P*<0.05, ***P*<0.01, ****P*<0.001, ns *P*>0.1

Overall, the nutritional treatments generated greater differences in cow liveweight, fatness and EMA than the differences due to muscle line, but the muscle score differences were greater due to muscle line. Cows from the Low muscle line had more fat and less muscle than the High or High^{Het} cows, with the muscle line fatness differences being better expressed on Medium/High nutrition (Table 3.1). Cows from the three lines showed reduced EMA and muscle score on Low nutrition, with the nutritional effects being proportionally greater in the Low muscled cows for EMA and muscle score. These results indicate that cows from the three muscle lines responded similarly in terms of liveweight change to the nutritional treatments, with each utilising both fat and muscle tissues to meet the nutritional challenges.

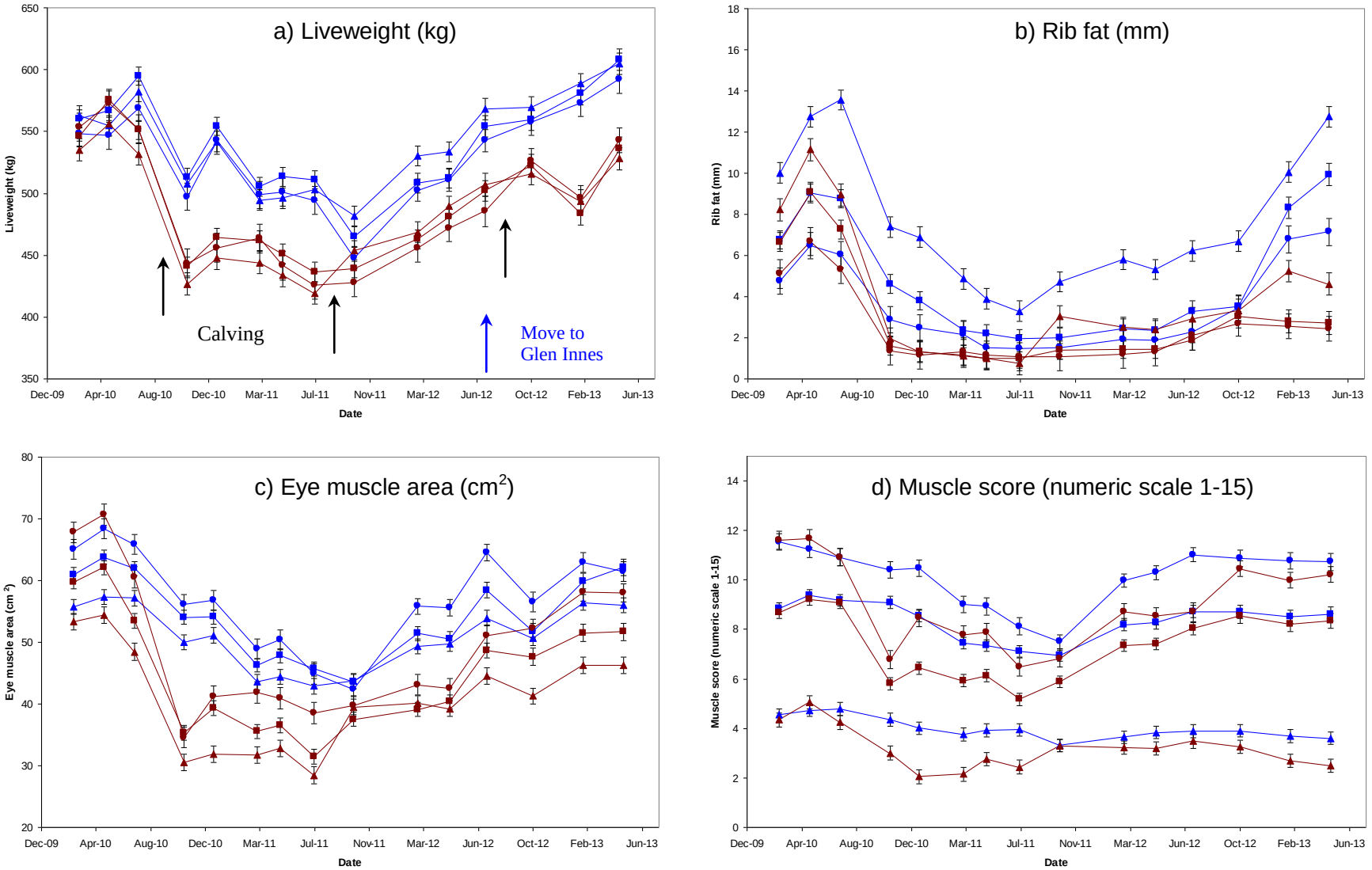

Figure 3.2. Change in liveweight, rib fat, eye muscle area and muscle score for lactating cows from the High (■), Myostatin (●) and Low (▲) muscling lines on Medium/High (blue) and Low (red) nutrition over three reproductive cycles. Error bars are se.

Plate 3.1 Cows from the Low muscle line with 1mm rump fat (above) and 14mm rump fat (below)

Plate 3.2 Cows from the High muscle line with 1mm fat (above) and 6mm fat (below)

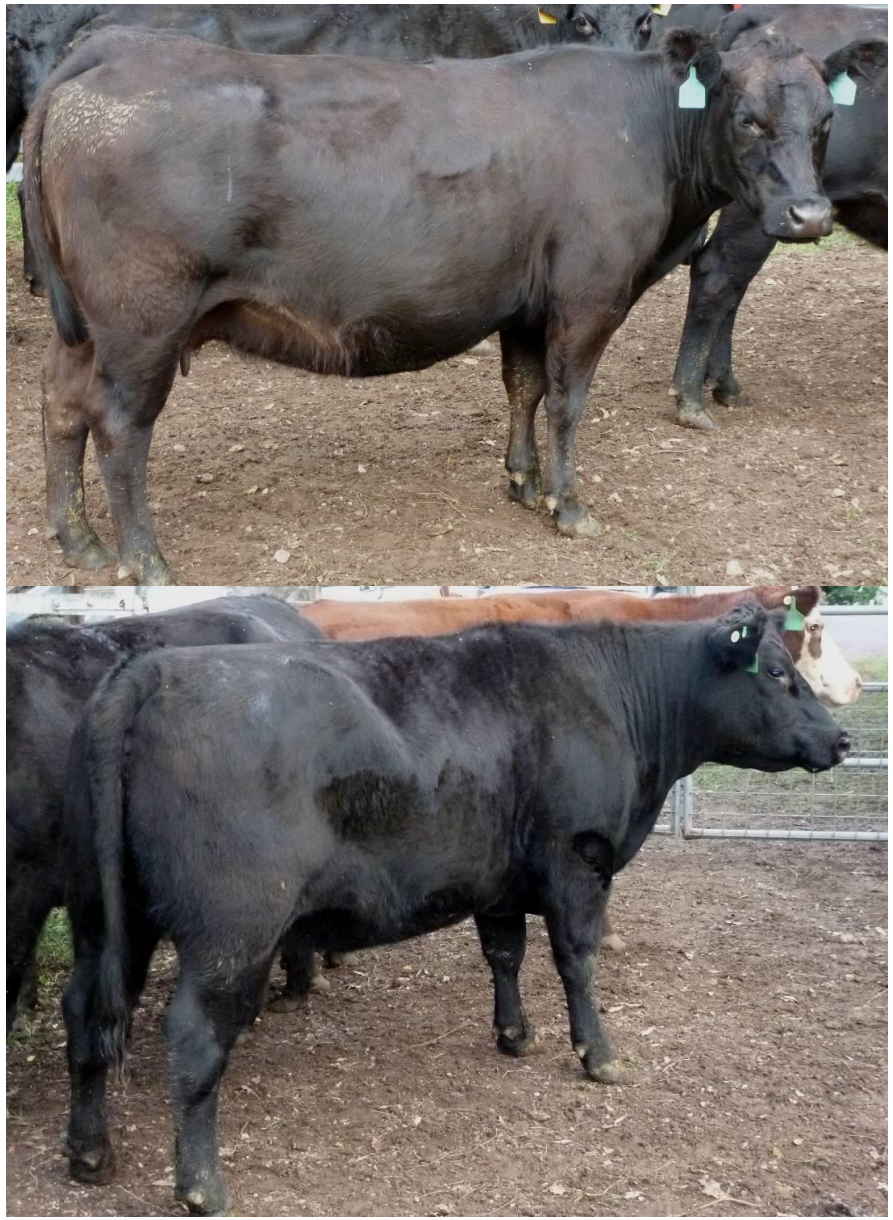

Plate 3.3 Cows from the High^{Het} line with 1mm rump fat (above) and 13 mm rump fat (below)

Plate 3.4 Cows on High nutrition (above) and Low nutrition (below) at Glen Innes in January 2013.

Cow liveweight and composition also varied significantly (P<0.001) with date of measurement. The weight, rib fat, EMA and muscle score for lactating cows at each of the 15 measurements are presented in Figure 3.2. Plates 3.1-3.3 provide a visual representation of cows from the three muscle lines with varying levels of fatness.

As intended, this project generated body compositional data from breeding cows with a large range in muscling and fatness, which will be a valuable data set to aid in the further development and validation of maternal productivity models.

3.2.2 Maternal performance

3.2.2.1 Pregnancy rates

Pregnancy rates after joining in 2010, 2011, and 2012 give the clearest indication of the effect of the nutritional treatments on the fertility of the cows, and any differences in fertility of the muscling lines under nutritional restriction. This analysis included data from the 2012 joining, the resulting calves from which were still *in utero* when this report was prepared. It does not include data from the 2YO heifers which began the nutritional treatments after joining, but does include the 10 year-old cows which were joined but culled prior to calving. No interaction was observed between cow muscle line and nutrition, so the main effects are presented (Table 3.2).

The difficult season leading up to joining in 2011 resulted in a significant decrease in pregnancy rates across the herd in that year. A pregnancy rate of 69.1% across the herd was an unusually poor result. Under better nutritional conditions, calving rates of more than 90% have been observed (Cafe et al. *in press*). This is consistent with the greater pregnancy rates observed in 2010 and 2012. It is not surprising that fertility was reduced in 2011 given the average modelled cow weight of 461 kg and rib fat of 2.7 mm prior to joining that year. This is well below the industry recommendation of 8-12 mm rump fat for breeding cows at joining (MLA 2004).

The 2011 data had an impact on results for the nutritional treatments because the cows on the Medium/ High treatment also showed a drop in fertility that year. That drop prevented the treatment difference in pregnancy rate from being significant, though there were differences in liveweight, fatness, EMA and muscle score at joining due to treatment across the three years.

The divergence in muscling and fatness traits between the muscle lines was as expected at joining, with muscling (muscle score and scanned EMA) increasing and fatness (scanned rump and rib fat) decreasing from Low to High to High^{Het} (Table 3.2). There was no significant difference in weight at joining between the muscle lines, which is consistent with longer term results for the herd (Cafe et al. 2012). However, the High^{Het} line were trending to be a little lighter under the more challenging conditions, with average weights 10-12 kg lighter than the Low and High cows. Similarly, while there was not a significant effect of muscle line on pregnancy rate based on the conditional Wald test, the difference on the underlying scale between High and High^{Het} cows was substantially greater than its standard error, suggesting that High^{Het} cows might have been somewhat less fertile. The reduction of 10% in pregnancy rate in the High^{Het} cows would be considered biologically significant.

The inclusion of cow liveweight, hip height and fatness at joining as covariates in the pregnancy models revealed that liveweight had the dominant effect on pregnancy result, and the quadratic fit implies a greater effect on cows on Low nutrition. Fatness

Table 3.2 Pregnancy rates of cows (4 YO and over) following joining in 2010, 2011 and 2012, and liveweight and body composition of cows at the commencement of joining. Pregnancy rates are presented as means and sed on the underlying logistic scale with back-transformed means in parentheses.

	n joined	Pregnancy rate	Liveweight (kg)	Rump fat (mm)	Rib fat (mm)	EMA (cm2)	Muscle score (1-15)
Joining year							
2010	248	2.16b (89.7%)	501a	7.1	3.8	53.4	7.3
2011	236	0.81a (69.1%)	461a	2.7	2.1	46.7	6.2
2012	234	2.36b (91.4%)	550b	8.4	4.1	57.4	7.8
sed		0.428	14.9	2.76	0.65	3.14	0.48
signif.		*	*	ns	ns	ns	ns
Nutritional treatment							
Medium/High	364	1.91(87.1%)	527b	5.7b	5.0b	56.7b	7.6b
Low	354	1.65 (83.8%)	481a	3.4a	3.0a	48.3a	6.5a
sed		0.319	5.0	0.23	0.29	0.87	0.17
signif.		Ns	***	***	***	***	***
Cow muscle line							
Low	262	2.19 (89.3%)	508	6.0c	5.7c	50.0a	3.9a
High	278	1.90 (87.0%)	506	4.3b	3.7b	52.7b	8.0b
High ^{Het}	178	1.24 (77.6%)	498	3.4a	2.7a	54.9c	9.3c
sed		0.456	6.4	0.30	0.38	1.11	0.26
signif.		ns	ns	***	***	*	***

abc Letters denote means which are significantly different. †P<0.1, *P<0.05, **P<0.01, ***P<0.001, ns P>0.1

didn't have a significant effect when included with liveweight as these two variables were closely correlated.

While the year differences increase the variation of the experimental results, they do provide a very large range of maternal performance data. An objective of this project was to produce such a dataset, which will be available to be used to validate maternal performance models in the future.

3.2.2.2 Calving results

Analysis of the calving and weaning performance of the herd was conducted using calves from joining in 2009, 2010 and 2011. No interaction was observed between cow muscle line and nutrition, so the main effects are presented (Table 3.3).

There was an effect of year on calving and weaning rates. As discussed above, the 2011 joining resulted in lower pregnancy rates and hence lower calving and weaning rates for the 2012 calving year. Calves were heavier at birth in 2012 due to the cows being on a higher level of nutrition at Glen Innes for the final trimester of pregnancy. The days to calving (DTC) did not differ with year.

Calves born from Low nutrition were 1.4 kg lighter than those from Medium/High nutrition. There was a tendency for a lower calving rate on Low nutrition, but this effect was not significant for live calving rate or weaning rate. Once again the results from the 2011 joining diluted the differences between the nutritional treatments because the cows on the Medium/ High treatment also showed a drop in fertility that year. There was no effect of nutrition on DTC.

Table 3.3 Calf birth weight, days to calving, and calving and weaning rates for cows joined in 2009, 2010 and 2011. Calving, live calving and weaning rates are all calculated as a proportion of cows joined, and presented as means and sed on the underlying logistic scale with back-transformed means in parentheses.

	n cows joined	Calf birth weight (kg)	Days to calving (d)	Calving rate	Live calving rate	Weaning rate
Calving year						
2010	251	33.8a	321	2.76b (94.0%)	2.64b (93.3%)	2.52b (92.6%)
2011	242	34.5a	314	2.30b (90.9%)	2.02b (88.2%)	1.85b (86.5%)
2012	253	38.8b	324	1.03a (73.7%)	0.84a (69.9%)	0.67a (66.1%)
sed		0.60	5.8	0.386	0.374	0.371
signif.		*	ns	*	**	*
Nutritional treatment						
Medium/High	377	36.4b	319	2.77b (90.7%)	2.01 (88.2%)	1.80 (85.8%)
Low	369	35.0a	321	1.79a (85.7%)	1.66 (84.0%)	1.57 (84.0%)
sed		0.40	3.5	0.269	0.265	0.267
signif.		†	ns	†	ns	ns
Cow muscle line						
Low	285	36.0	324	2.15 (89.6%)	2.02 (88.3%)	1.85 (86.4%)
High	274	36.0	320	2.42 (91.8%)	2.18 (89.8%)	2.01 (88.2%)
High ^{Het}	187	35.1	315	1.52 (82.1%)	1.31 (78.7%)	1.19 (76.6%)
sed		0.60	10.6	0.347	0.362	0.372
signif.		ns	ns	ns	ns	ns

abc Letters denote means which are significantly different. †P<0.1, *P<0.05, **P<0.01, ***P<0.001, ns P>0.1

There were no significant differences in calving performance between cows from the three muscle lines. While there was not a significant effect of muscle line on calving or weaning rates based on the conditional Wald test, the difference on the underlying scale between High and High^{Het} cows was more than twice the standard errors, suggesting that High^{Het} cows performed slightly worse. The reduction of 9-11% in birth and weaning rates in the High^{Het} cows would be considered biologically significant. The most appropriate conclusion would be that while there was clearly no difference in calving performance between the Low and High lines, the High^{Het} cows were on the borderline of a drop in performance.

The myostatin genotype of the calf had a significant effect on birth weight, as High^{Het} calves with one copy of the myostatin gene were 1.5 kg heavier (P = 0.003) than High muscled calves with no copies. This is consistent with longer term results for this herd.

Birth assistance data was not analysed as only five cows were assisted to calve over the three years (1xLow, 2xHigh and 2xHigh^{Het} cows). This was only 0.9% of cows giving birth, indicating that dystocia was not an issue in these cows which were all giving birth to their second or subsequent calves.

3.2.2.3 Weaning results

Weaning weights and body composition were calculated for the calves born in 2010, 2011 and 2012. No interaction was observed between muscle line and nutrition, so main effects are presented. There were significant effects of year born and nutritional treatment on the calves weaned (Table 3.4). Calves born in 2010 and 2011 (Grafton) were of similar age, liveweight, fatness and EMA at weaning, but those born in 2011 had slightly higher preweaning ADG and a slightly higher muscle score at weaning.

Calves born in 2012 (Glen Innes) were the same age at weaning as the 2010 and 2011-born calves but were heavier and fatter after much faster preweaning growth rates.

Table 3.4. Weaning weight, scanned fat and eye muscle area (EMA), muscle score, prewean growth rate (ADG) and calf age at weaning of calves from Low muscle, High muscle and High^{Het} cows under divergent nutritional treatments for three years.

	n	Weight (kg)	Rib fat (mm)	Rump fat (mm)	EMA (cm^2)	Muscle score (1-15)	Prewean ADG (g/d)	Age at weaning (d)
Calving year								
2010	184	191a	1.5a	1.8a	32.9a	5.6a	626a	250
2011	202	192a	1.6a	2.1a	33.3a	6.6b	660b	239
2012	164	269b	3.5b	4.9b	45.2b	8.1c	931c	247
sed		3.8	0.10	0.15	0.72	0.22	13.0	2.3
signif.		***	***	***	***	***	***	ns
Nutritional treatment								
Medium/high	286	238b	2.7b	3.6b	41.3b	7.2b	823b	245
Low	264	196a	1.7a	2.3a	33.0a	6.3a	655a	245
sed		2.5	0.08	0.11	0.53	0.14	8.5	1.5
signif.		***	***	***	***	***	***	ns
Cow muscle line								
Low	199	221	2.2	3.0	36.1a	4.6a	759	244
High	218	216	2.2	3.0	39.1b	8.1c	734	245
High ^{Het}	133	215	2.2	2.9	36.2a	7.5b	725	247
sed		3.7	0.12	0.16	0.79	0.21	12.8	2.3
signif.		ns	ns	ns	**	***	ns	ns
Calf myostatin genotype covariate								
Estimate		-1.89	-0.40	-0.55	3.50	1.56	-0.01	-1.86
signif.		ns	***	***	***	***	ns	ns

abc Letters denote means which are significantly different. *P*<0.1, *P<0.05, **P<0.01, ***P<0.001, ns P>0.1

There were no differences in weight, prewean ADG, age at weaning or fatness of calves weaned by the cows in any of the three muscle lines (Table 3.4). High cows produced weaners with slightly greater muscling than High^{Het} cows, but both were greater than Low cows.

The myostatin genotype of the calf had a significant effect on the fatness and muscling characteristics of the calves at weaning (Table 3.4). High^{Het} calves with one copy of the myostatin gene had lower fat, greater EMA and greater muscle score at weaning than High muscled calves with no copies. As observed previously in this herd (Cafe et al. 2012), there was no effect of the myostatin genotype on weight or growth rates.

A simple but informative measure of maternal productivity is the weight of calf weaned per cow joined per year. The weaning rates (Table 3.1) and weaning weights (Table 3.2) of calves produced over the three years of the experiment were used to calculate this for cows from the three muscle lines. This resulted in 190.9 kg for Low cows, 190.5 kg for High cows, and 164.7 kg for High^{Het} cows. This clearly indicates that there was no difference in maternal productivity between the Low and High cows, even under a period of challenging nutritional conditions. However there was a drop of 14% from the Low to the High^{Het} cows. This differs from earlier results presented for the herd under less challenging conditions, where Low cows weaned

218 kg calf/cow joined, High cows 225 kg and High^{Het} cows 216 kg (Cafe et al. *in press*).

3.3 Conclusions

The muscling herd was managed under divergent nutritional conditions over three reproductive cycles, allowing body composition to be measured across a large liveweight range. The annual variability in the nutritional treatments increased the variation in the experimental results, but also drove a larger divergence in cow weight and composition across the three years. One of the objectives of this project was to produce a dataset of maternal performance in cows with a large divergence in muscling and fatness traits suitable for validating maternal performance models in the future. The herd was tested for its ability to be productive under nutritional restriction, and were pushed to the point of a drop in fertility, providing data that should be useful for modelling exercises.

An important objective of the project was to test whether selection for increased muscling in Angus cows led to a reduced ability of more highly muscled cows to remain productive under nutritional challenge. There were no differences in pregnancy rate, calving rate, weaning rate or weaning weight of calves produced by cows from the Low and High muscle lines. Low and High cows had the same maternal productivity of 191 kg calf weaned per cow joined per year across the three years of the experiment. The High^{Het} cows, with one copy of the 821del11 *myostatin* mutation, showed a trend towards reduced pregnancy rate, calving rate and weaning rate, but no difference in weight of calves weaned compared to the Low and High cows. Their reduced weaning rates led to a maternal productivity of 165 kg calf weaned per cow joined per year across the three years of the experiment, 26kg (or 13.6%) less than the Low and High lines. This result indicates that the incorporation of the extreme 821del11 *myostatin* mutation into the breeding herd should be treated with some care. If heterozygous *myostatin* females are to be kept for breeding they should be run under more favourable nutritional conditions to maximise their advantage, with the awareness that they may show reduced productivity under nutritional stress.

4 Estimating cow body condition using 3D RGBD cameras

Research Team: University of Technology Sydney – Brad Skinner, Alen Alempijevic; NSW Department of Primary Industries – Malcolm McPhee, Brad Walmsley, John Wilkins, Jason Siddell, Linda Cafe

The assessment of cow body condition is an important tool for managing breeding herd productivity. Breeding herd management recommendations from MLA' s 'More Beef from Pastures' manual (MLA 2004) highlight the importance of assessing breeding cows for condition, not only liveweight, throughout the reproductive cycle.
It would be useful if condition scoring could be conducted objectively, and possibly even automated to occur as cows passed through a race.

In this experiment the 3D RGBD (Red, Green, Blue, + Depth) camera technology was tested for its ability to assess cow body condition score. The cows in the muscling herd have genetic divergence in muscling and fatness, with further phenotypic divergence in weight and fatness being created by the nutritional

treatments. As such it provided a wider phenotypic range for training and testing the cameras than would be found in most beef herds at a given point in time.

This work was conducted in collaboration with MLA project 'B.BSC.0339: Initiative - Trait measurement from 3D image'. The final report for that project details the camera methodology, and their success at estimating muscle score and rump fat in these cows and their progeny. In this report the same methods and analyses were used to assess the ability of the cameras to estimate cow body condition score.

4.1 Methods

Three-dimensional RGBD camera images were collected on the cow herd while cows stood in the race in February 2012, May 2012, October 2012 and February 2013. A laser unit was also used to take measurements of hip height. The use of the laser data to predict other characteristics of body composition was not pursued, as it became apparent that RGBD camera technology has a much greater scope for doing this than the laser technology.

At the time of RGBD image collection, the herd was also weighed, ultrasound scanned for rump fat, rib fat and eye muscle area, and visual assessments of muscle score (McKiernan 2007) and condition score (NRC 1996) were conducted. The condition score system used a 1-9 scale where 1 = emaciated and 9 = very fat, based on the visual assessment of muscle and fat cover over the skeleton. All condition score assessments were made by the one observer.

The RDGB camera and data acquisition methodology used is currently confidential due to a pending patent application. Briefly, a data-driven supervised learning approach, employing state of the art classification and regression techniques, was used. These methods are described in detail in the final report for MLA project B.BSC.0339: Initiative - Trait measurement from 3D image, and are not repeated here.

4.2 Results

With any data-driven machine-learning technique it is desirable to have a large sample size distributed equally over the estimated values for Condition Scores. It is important to realise, that the confidence of experimental results decreases with smaller sample sizes and unequal class or measurement distributions (Witten et al. 2011).

There exist significantly overrepresented classes and measurement bands in the cow data for February and May data sets. We have attempted to minimise the bias caused by the instance distribution by stratifying the training and testing sets through random sampling to help ensure that each class is properly represented in both training and testing sets.

State of the art machine learning (ML) algorithms were employed during the classification experimentation phase. Results from the best four performing algorithms are reported: 1) BayesNet (BN) algorithm (Cooper and Herskovits 1992) which is a probabilistic ML learning algorithm based on Bayes Theory, 2) the Sequential Minimal-Optimisation (SMO1) algorithm (Platt 1999) used to train function-based support vector classifier; 3) the LibSVM algorithm (Chang and Lin 2011), a very efficient, effective and reliable implementation of a support vector

classifier; and 4) the Gaussian Processes (GP) as the classifier mechanism (Rasmussen and Williams 2005).

4.2.1 Classification results - Condition Score for Cows Feb 2012

The frequency distribution of condition scores in the Feb 2012 cow data (n = 156) is illustrated in Figure 4.1. The data from figure 4.1 were used for the classification experiment with no cows with a condition score of class 1.

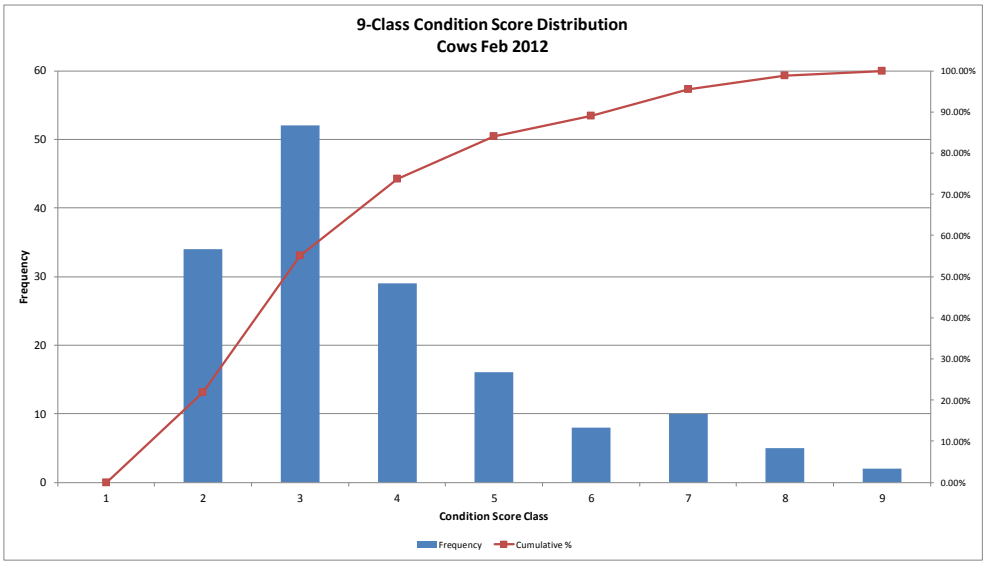

Figure 4.1 Frequency of Condition Scores (1 to 9) for Feb 2012 Cow data set used in Classification experiments

Figure 4.2 and 4.3 illustrate the classification accuracy for each of the machine learning algorithms for the condition score label (Feb 2012 dataset). The computed feature vectors used in the classification experiments contained only the concatenated signatures derived from the feature extraction methods. The highest average classification accuracy across 100 iterations was 77.6% ± 4.8% for the LibSVM6 machine learning algorithm.

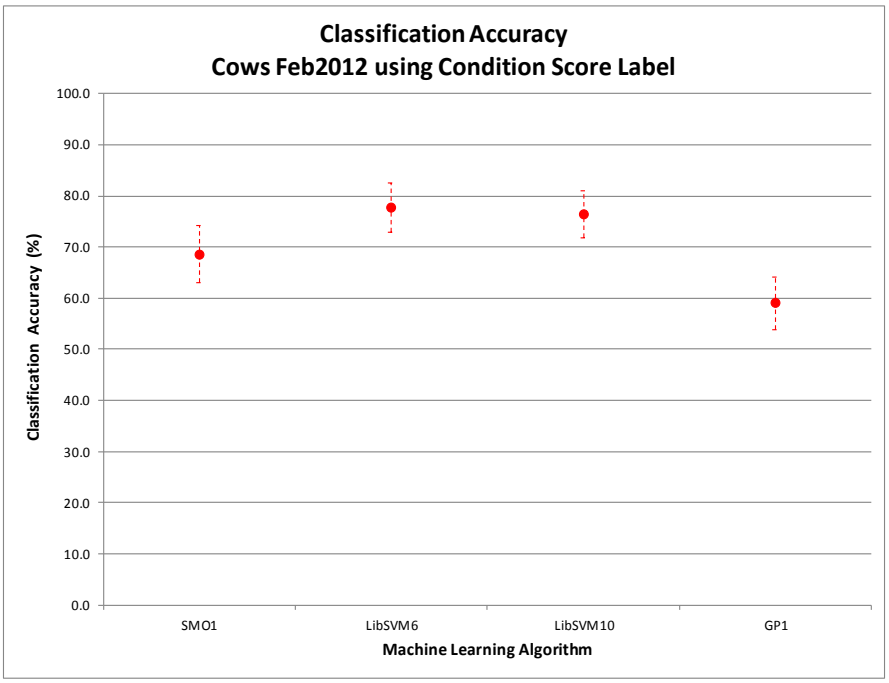

Figure 4.2 Mean ± SD classification accuracy of statistical-based feature vector with condition score label for low + high nutrition cows (Feb2012), (p=0.10, 90% confidence)

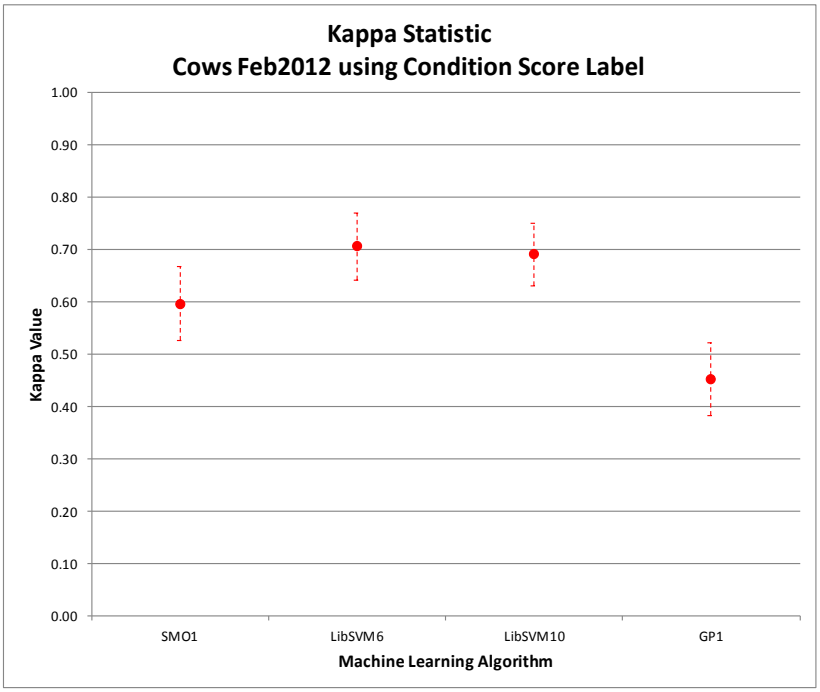

Figure 4.3 Mean ± SD maximum Kappa Statistic* of statistical-based feature vector with condition score label for low + high nutrition cows (Feb2012), (*p*=0.10, 90% confidence)

In multiclass classification experiments, the result on a single test set is typically analysed using the two-dimensional *confusion matrix* (Table 4.1) with a row and column allocated to each class or category. Each matrix element shows the number

* Generally used to evaluate categorical data where 1 is complete agreement and 0 is lack of agreement

of test examples for which the *observed* class is the row and the *predicted* class is the column. Good results correspond to large numbers running down the main diagonal and small, ideally zero, off-diagonal elements. The classification accuracies illustrated in Figure 4.2 do not provide information about the distribution of predicted class, rather a convenient result of overall classification accuracy. The Cohen Kappa Statistic (Figure 4.3) is used to measure the agreement between predicted and observed classes of a dataset, while correcting for an agreement that occurs by chance. It is a method to describe the distribution of predicted classes in the confusion matrix. The Kappa value ranges from [0 1] and a value of 1 indicates perfect prediction with all values lying on the central diagonal. The sample confusion matrix and error metrics for the LibSVM6 classification (highest kappa statistic) in the classification experiments are shown in Tables 4.1 and 4.2, where p=0.10, 90% confidence.

Table 4.1 Two-dimensional *confusion matrix* of cows (n=156) for each condition score classification using LibSVM6 algorithm for 1 single experimental fold. The data set does not contain cows with condition score of class 1

a	b	c	d	e	f	g	h	<-- classified as
26	6	2	0	0	0	0	0	|a = Condition Scr 2
2	47	2	1	0	0	0	0	|b = Condition Scr 3
0	4	25	0	0	0	0	0	|c = Condition Scr 4
0	2	2	12	0	0	0	0	|d = Condition Scr 5
0	3	1	2	2	0	0	0	|e = Condition Scr 6
0	1	0	2	1	6	0	0	|f = Condition Scr 7
0	2	2	0	0	0	1	0	|g = Condition Scr 8
0	0	0	1	0	0	0	1	|h = Condition Scr 9

Table 4.2 Stratified cross-validation of cows (n=156) for condition score using LibSVM6 algorithm for a single experimental fold

Item	**Value**
Correctly Classified Instances (%)	75.6%
Incorrectly Classified Instances (%)	24.4%
Statistic	
Kappa statistic*:	0.691
Mean absolute error :	0.061
Root mean squared error :	0.247

***Kappa [0,1] -** The Kappa Statistic is used to measure the agreement between predicted and observed classes of a dataset, while correcting for an agreement that occurs by chance in Table 4.1.

4.2.2 Classification results - Condition Score for Cows May 2012

The frequency distribution of condition scores in the May 2012 cow data (n = 148) is illustrated in Figure 5.4. The data from figure 5.4 were used for the classification experiment with no cows with a condition score of class 1.

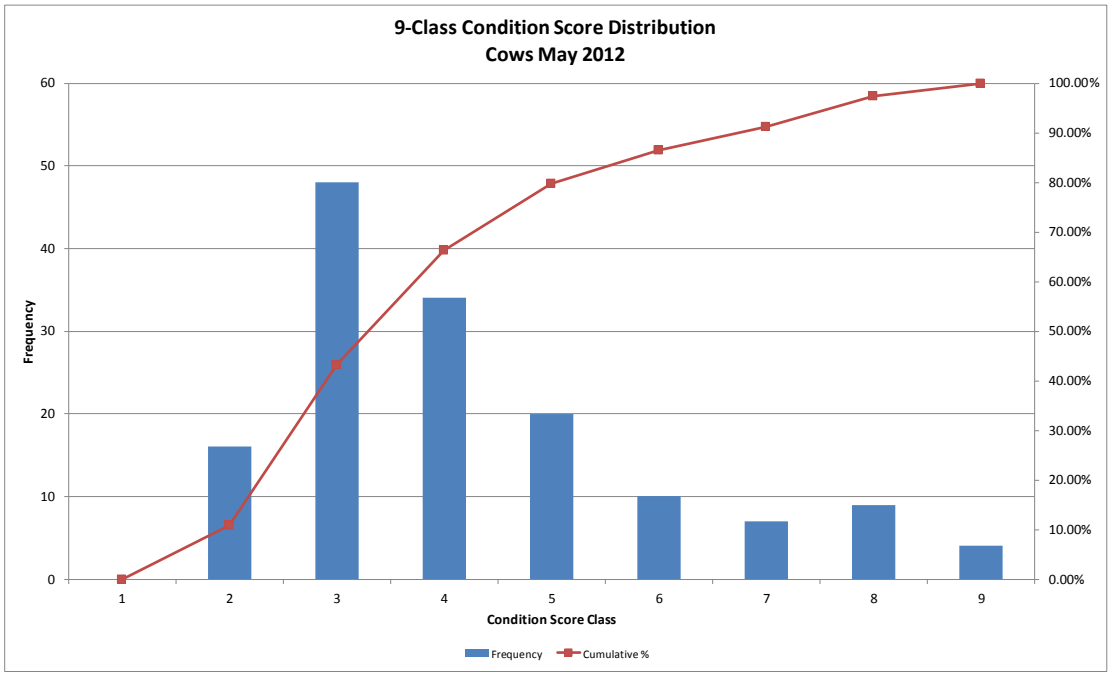

Figure 4.4 Frequency of condition scores (1 to 9) for May 2012 Cow data set used in classification experiments

Figures 4.5 and 4.6 illustrate the classification accuracy for each of the machine learning algorithms for the condition score label (May 2012 dataset). The computed feature vectors used in the classification experiments contained only the concatenated signatures derived from the feature extraction methods. The highest average classification accuracy across 100 iterations was 72.3% ± 4.9% for the LibSVM6 machine learning algorithm.

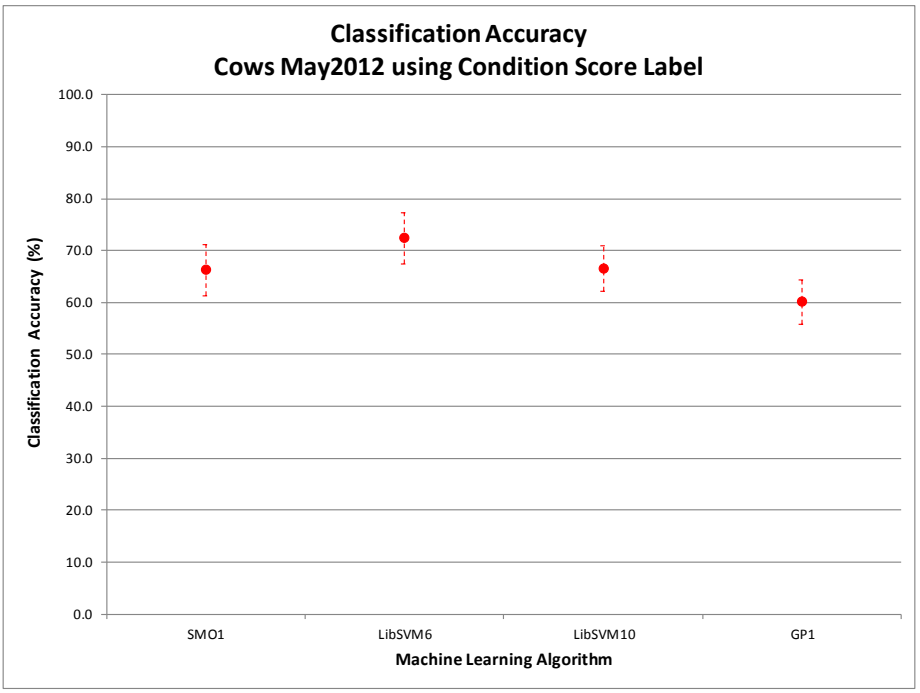

Figure 4.5 Mean ± SD classification accuracy of statistical-based feature vector with condition score label for low + high nutrition cows (May 2012), (p=0.10, 90% confidence)

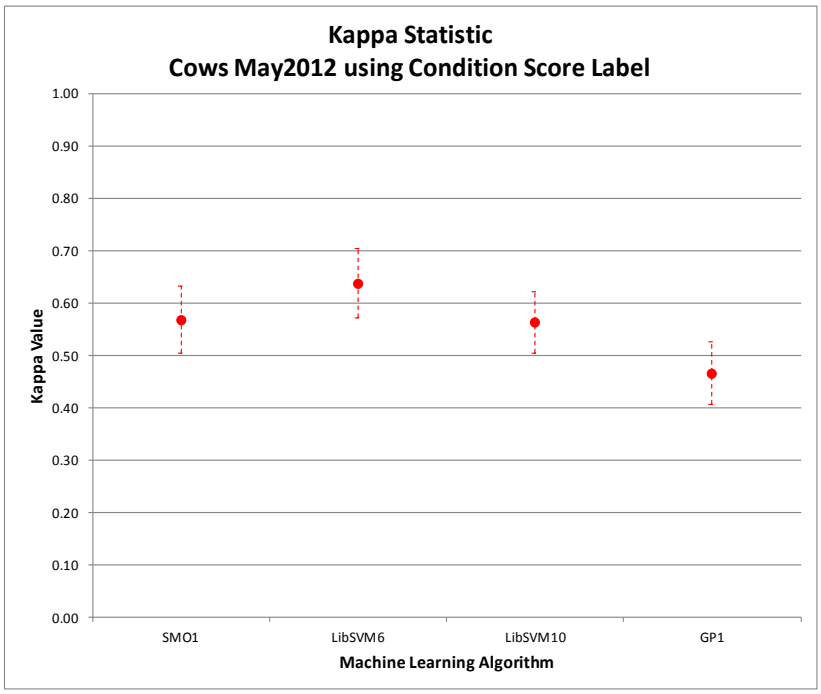

Figure 4.6 Mean ± SD maximum Kappa Statistic* of statistical-based feature vector with condition score label for low + high nutrition cows (May 2012), (*p*=0.10, 90% confidence)

* Generally used to evaluate categorical data where 1 is complete agreement and 0 is lack of agreement

The sample confusion matrix and error metrics for the LibSVM6 classification (highest kappa statistic) in the classification experiments are shown in Tables 4.3 and 4.4, where *p*=0.10, 90% confidence.

Table 4.3 Two-dimensional *confusion matrix* of cows (n=148) for each condition score classification using LibSVM6 algorithm for 1 single experimental fold

a	b	c	d	e	f	g	h	<-- classified as
7	7	2	0	0	0	0	0	|a = Condition Scr 2
0	47	1	0	0	0	0	0	|b = Condition Scr 3
0	2	32	0	0	0	0	0	|c = Condition Scr 4
0	2	4	13	0	0	0	0	|d = Condition Scr 5
0	1	1	1	6	0	1	0	|e = Condition Scr 6
0	0	1	2	1	2	1	0	|f = Condition Scr 7
0	1	4	1	2	0	1	0	|g = Condition Scr 8
0	1	0	1	0	0	1	1	|h = Condition Scr 9

Table 4.4 Stratified cross-validation of cows (n=148) for condition score using LibSVM6 algorithm for a single experimental fold

Item	**Value**
Correctly Classified Instances (%)	72.9%
Incorrectly Classified Instances (%)	27.1%
Statistic	
Kappa statistic*:	0.648
Mean absolute error :	0.067
Root mean squared error :	0.260

***Kappa [0,1] -** The Kappa Statistic is used to measure the agreement between predicted and observed classes of a dataset, while correcting for an agreement that occurs by chance in Table 4.3.

4.3 Conclusion

In this study, we have developed a method to estimate condition score using data gathered from cattle standing in the race using a pair of RGBD cameras, without the need for ultrasonic measurements or trained assessors. A data driven supervised learning approach employing state of the art classification and regression techniques was used for this purpose. Data obtained using cows show a 77.6% ± 4.8% correct classification for condition score using the Feb 2012 data set and 72.3% ± 4.9% correct classification for the May 2012 data set. While subjective scoring methods, such as body condition scoring, can be accurate when observers are trained regularly using good training material, repeatability can also be very variable. A recent study into the accuracy of trained assessors at body condition scoring dairy cows reported mean Kappa values for exact agreement between trainers and trainees at 0.35 to 0.59 (Vasseur et al. 2012). The Kappa values of 0.648 and 0.691 in this study were higher than that, showing substantial agreement accuracy between cameras and observer. With further development the cameras may provide a more objective method for condition scoring cows, and could possibly be developed to do so automatically while cows pass freely through a race.

5 Bibliography

Agenäs S, Heath MF, Nixon RM, Wilkinson JM, Phillips CJC (2006) Indicators of undernutrition in cattle. *Animal Welfare 15:* 149-160.

Ashraf M, Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59: 206-216.

Ball AJ, Oddy VH, Thompson JM (1997) Nutritional manipulation of body composition and efficiency in ruminants. In 'Recent advances in animal nutrition in Australia'. eds. J.L. Corbett, M. Choct, J.V. Nolan and J.B. Rowe pp. 192–208. (University of New England: Armidale)

Bines JA, Morant SV (2006) The effect of body condition on metabolic changes associated with intake of food by the cow. *British Journal of Nutrition 50:* 81-89.

Butler D, Cullis B, Gilmour A, Gogel B (2009) ASReml-R Reference Manual, release 3. Queensland Department of Primary Industries and Fisheries: Brisbane.

Cafe LM, Hennessy DW, Hearnshaw H, Morris SG and Greenwood PL (2006). Influences of nutrition during pregnancy and lactation on birth weights and growth to weaning of calves sired by Piedmontese or Wagyu bulls. *Australian Journal of Experimental Agriculture* 46: 245-255.

Cafe LM, McKiernan WA, Robinson DL, Walmsley BJ (2012) Additional measurements on muscle line cattle. MLA final report for project BFG.0049.

Cafe LM, Mckiernan WA, Robinson DL. Selection for increased muscling is not detrimental to maternal productivity traits in Angus cows. Animal Production Science, Special Issue: Maternal Productivity in Cattle, *in press*.

Camacho LE, Lemley CO, Van Emon ML, Caton JS, Swanson KC, Vonnahme KA (2014) Effects of maternal nutrient restriction followed by realimentation during early and midgestation on beef cows. I. Maternal performance and organ weights at different stages of gestation. *Journal of Animal Science* 92: 520-529.

Chang C-C, Lin C-J (2011) LIBSVM: A library for support vector machines. ACM Transactions on Intelligent Systems and Technology. 2(3): 27.

Chilliard Y, Ferlay A, Faulconnier Y, Bonnet M, Rouel J and Bocquier F (2000). Adipose tissue metabolism and its role in adaptations to undernutrition in ruminants. Proceedings of the Nutrition Society, 59, pp 127-134.

Cooper GF, Herskovits E (1992) A Bayesian Method for the Induction of Probabilistic Networks from Data. *Machine Learning.* 9(4): 309-47.

Grobet L, Martin LJ*,* Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Ménissier F, Massabanda J, Fries R, Hanset R, Georges M (1997) A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. *Nature Genetics* 17: 71-74.

Johnston DJ, Bunter KL (1996) Days to calving in Angus cattle: genetic and environmental effects, and covariances with other traits. *Livestock Production Science* 45: 13-22.

Li J, Wijffels G, Yu Y-H, Nielsen LK, Niemeyer D, Fisher A, Ferguson D, Schirra, H J (2011) Altered fatty acid metabolism in long duration road transport: an NMR-based metabonomics study in sheep. *Journal of Proteome Research 10:* 1073-1087.

McKiernan WA (2002) Importance of various cattle traits in the national livestock reporting system. In 'Proceedings of the Australian Society of Animal Production' 24: 329.

McKiernan WA (2007). Muscle scoring beef cattle. NSW Department of Primary Industries Primefact No 328.

MLA (2004) More Beef from Pastures: The producer's manual. Meat and Livestock Australia.

McPhee M (2013) Initiative - Trait measurement from 3D images. MLA final report for project B.BSC.0339.

Morris CA, Baker RL, Hickey SM, Johnson DL, Cullen NG, Wilson JA (1993) Evidence of genotype by environment interaction for reproductive and maternal traits in beef cattle. *Animal Science* 56: 69-83.

Nicholson JK, Foxall PJD, Spraul M, Farrant RD, Lindon JC (1995) 750 MHz ^{1}H and ^{1}H-^{13}C NMR spectroscopy of human blood plasma. *Analytical Chemistry* 67: 793-811

NRC (1996). Nutrient Requirements of Beef Cattle. National Academy Press, Washington DC.

Platt JC (1999) Advances in Kernel Methods - Support Vector Learning. Schölkopf B, Burges C, A. Smola, editors: MIT Press; 185-208 pp.

Ragaller V, Hüther L, Lebzien, P (2009) Folic acid in ruminant nutrition: a review. *British Journal of Nutrition* 101: 153-164

Rasmussen CE, Williams CKI (2005) Gaussian Processes for Machine Learning (Adaptive Computation and Machine Learning): The MIT Press.

Robinson DL (1987) Estimation and use of variance components. *The Statistician* 36: 3-14.

Robinson DL, Cafe LM, Greenwood PL (2013) Developmental programming in cattle: Consequences for growth, efficiency, carcass, muscle, and beef quality characteristics. *Journal of Animal Science* 91: 1428–1442

Ueland PM, Holm PI, Hustad S (2005) Betaine: a key modulator of one-carbon metabolism and homocysteine status. *Clin. Chem. Lab. Med.* 43: 1069-75.

Vasseur E, Gibbons J, Rushen J, de Passille AM (2012) Development and implementation of a training program to ensure high repeatability of body condition scoring dairy cows . Journal of Dairy Science 96: 4725-4737.

Wagner JJ, Lusby KS, Oltjen JW, Rakestraw J, Wettemann RP, Walters LE (1988) Carcass Composition in Mature Hereford Cows: Estimation and Effect on Daily Metabolizable Energy Requirement During Winter. *Journal of Animal Science* 66: 603-612.

Witten I, Frank E, Hall M (2011) Data Mining – Practical Machine Learning Tools and Techniques, *Ed 3*, *Ch5*.

Appendix 1. Biological indicators of body condition

Research Team: NSW Department of Primary Industries – David Tucker, Linda Cafe, Helen Smith; University of Queensland – Ian Brereton, Horst Joachim Schirra, Greg Pierens

Variations in feed availability due to seasonal cycles are normal in extensive pastoral systems, and these lead to periods of catabolism or anabolism of fat and muscle tissue in lactating cows. During prolonged periods of undernutrition, such as during a drought, the losses in body mass can be significant and affect productivity, and may lead to poor welfare.

The cows in the muscling herd have genetic divergence in muscling and fatness, with further phenotypic divergence in weight and fatness being created by the nutritional treatments. As such the cows in this experiment provided an interesting test group to study the physiological differences between cows of divergent muscling in poor or good body condition. A better understanding of differences in metabolism between cows of divergent body composition could help further describe the effects of selection for muscling, as well as adding to the understanding of the metabolism of change in body condition, and its effects on reproductive performance.

A1.1 Methods

Plasma samples collected from cows at weaning in 2011, 2012 and 2013 were used in a study of the metabonomics of body condition. Inclusion of samples in the study was based on the individual scanned rib fat measurement at the time of sampling, with samples from cows with 2mm or less being chosen to represent thin cows, and those with 7mm or greater to represent fat cows. Only samples from cows which had been confirmed pregnant were included, but samples from both lactating and dry cows were included. Where possible, samples from individual cows in multiple years were utilised as significant individual variation in metabolic profile is known to occur.

A1.1.1 NMR methodology

^{1}H NMR spectra were acquired in 5 or 3 mm tubes on a Bruker Avance 900 NMR spectrometer with CryoProbe using a SampleJet (96 tube racks) for sample introduction. Standard Bruker pulse sequences were used (cpmgpr1d and noesypr1d).

NMR spectra were processed with Topspin 3.2 software, using exponential multiplication (LB 0.3) prior to Fourier transformation and manual phase correction. Spectra were referenced to the centre line of the β-glucose H-3 triplet (δ3.495).

Principal Component Analysis (PCA) was carried out using Bruker Amix software (version 3.9.14). The region of the NMR spectrum from δ4.5 to 0.5 was divided into bins of 0.01 ppm width. For the initial PCA all bins incorporating EDTA resonances were excluded, with the exception of part of the MgEDTA resonance centred at δ3.23, (*i.e.* δ3.65-3.58, 3.23 to 3.07, 2.73-2.68 and 2.60-2.54). For PCA emphasising betaine, exclusions were δ4.13 - 4.10, 3.73 - 3.72, 3.65 - 3.59, 3.43 - 3.42, 3.25 - 3.08, 3.05 - 3.03, 2.82 - 2.68, 2.58 - 2.54, 2.08 - 1.80 and 1.35 - 1.21. For PCA emphasising creatine, exclusions were δ4.13-4.10, 3.73-3.72, 3.65-3.59, 3.43-3.42, 3.27-3.08, 3.05-3.03, 2.82-2.68, 2.58-2.54, 2.08-1.80 and 1.35-1.21.

Principal component scores were assessed for their relationship to the body condition of the cows visually, using the Hotelling's T^2 distribution (95% confidence level) for the fat and the thin groups as a guide. The principal component scores were also analysed by fitting Linear Mixed Models using the REML methodology (Robinson, 1987) in Genstat V16 (VSN International Ltd, Hemel Hempstead, UK). The model included fixed effects for thin vs fat classification, muscle line, year and their interactions; and random terms for cow and lactation status.

A1.1.2 NMR method development

In a trial experiment, two plasma samples (Z128 and D22) were prepared for NMR spectroscopy in 4 different ways:

plasma + D_2O (9:1)	method A;
plasma + pH 7.4 phosphate buffer + D_2O (2:1:1)	method B;
plasma + pH 7.4 phosphate buffer + D_2O (5:4:1)	method C;
plasma + pH 7.4 phosphate buffer + D_2O (10:17:3)	method D.

^1H NMR spectra of sample Z128 that had been prepared by method C were acquired using both the CPMG pulse sequence (see Figure A1.1) and a 1D NOESY pulse sequence (see Figure A1.2) in 5 mm NMR tubes. Both pulse sequences used presaturation of the water resonance (to cause partial suppression of the water signal). The CPMG pulse sequence removes broad resonances due to proteins and other macromolecules, while the NOESY sequence shows all resonances. The spectra produced by the CPMG sequence were considered to be more suitable for a metabonomic investigation, giving a vastly better baseline and thus allowing more accurate quantification, despite the undesirable consequence of partial suppression of the moderately broad resonances due to lipids.

Spectra were then run on each of the four samples prepared from Z128, using the CPMG pulse sequence. The sample prepared by method A was found to give a spectrum with superior signal/noise ratio and water suppression with no loss of resolution, when compared to spectra from samples prepared by the other three methods.

Finally, a spectrum of the plasma sample (method A) was acquired in a 3 mm for comparison with that run in a 5 mm tube. The spectrum acquired in the 3 mm tube was superior in resolution, in water suppression and in suppression of signals from macromolecules.

This combination of preparation method and acquisition procedure was then checked with sample D22, again resulting in a good spectrum, and so was used for all subsequent data collection.

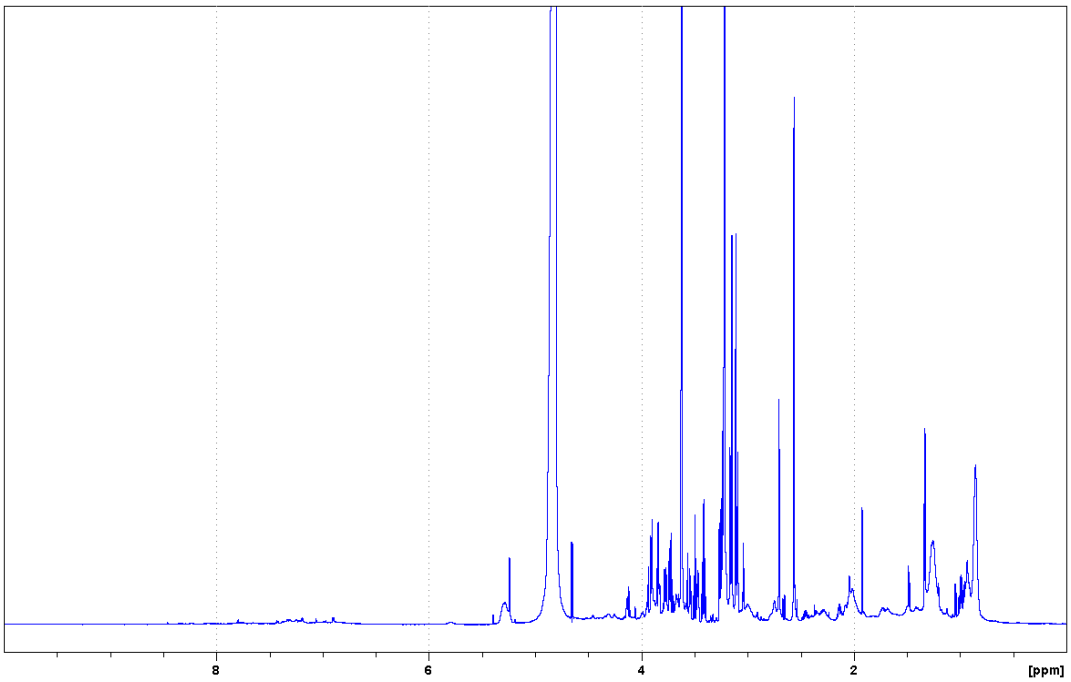

Figure A1.1 ^{1}H NMR spectrum of plasma + pH 7.4 buffer + D_2O (10:17:3) acquired with CPMG pulse sequence

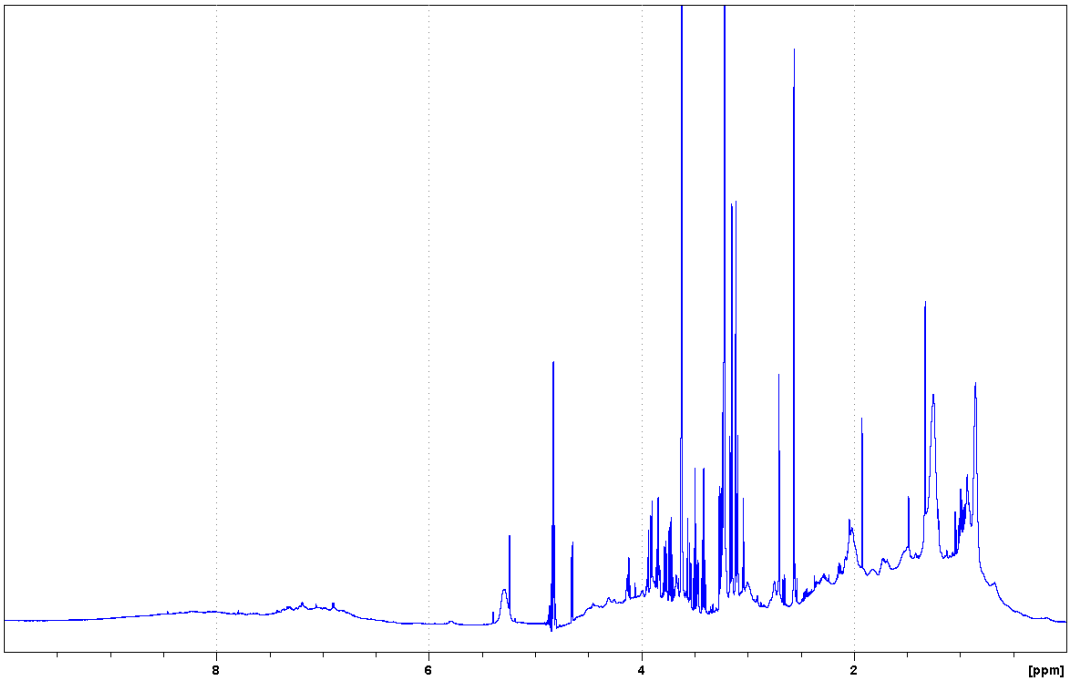

Figure A1.2 ^{1}H NMR spectrum of plasma + pH 7.4 buffer + D_2O (10:17:3) acquired with 1D NOESY pulse sequence

A1.2 Results and discussion

Unintentionally, the largest resonances in the ^{1}H NMR spectra of all plasma samples were due to the use of EDTA coated tubes to collect the plasma samples. This resulted in singlet resonances at δ3.61 and 3.20 (EDTA), a doublet of doublets at δ3.13 and a singlet at δ2.57 (CaEDTA) and a doublet of doublets at δ3.23 and a

singlet at δ2.70 (MgEDTA). Spectral regions containing EDTA resonances were excluded from analysis to remove this source of interference. There was also a considerable peak for residual water at δ4.80. A number of peaks due to metabolites were also apparent and these are annotated in Figures A1.3a and A1.3b, which show expansions of a typical plasma spectrum.

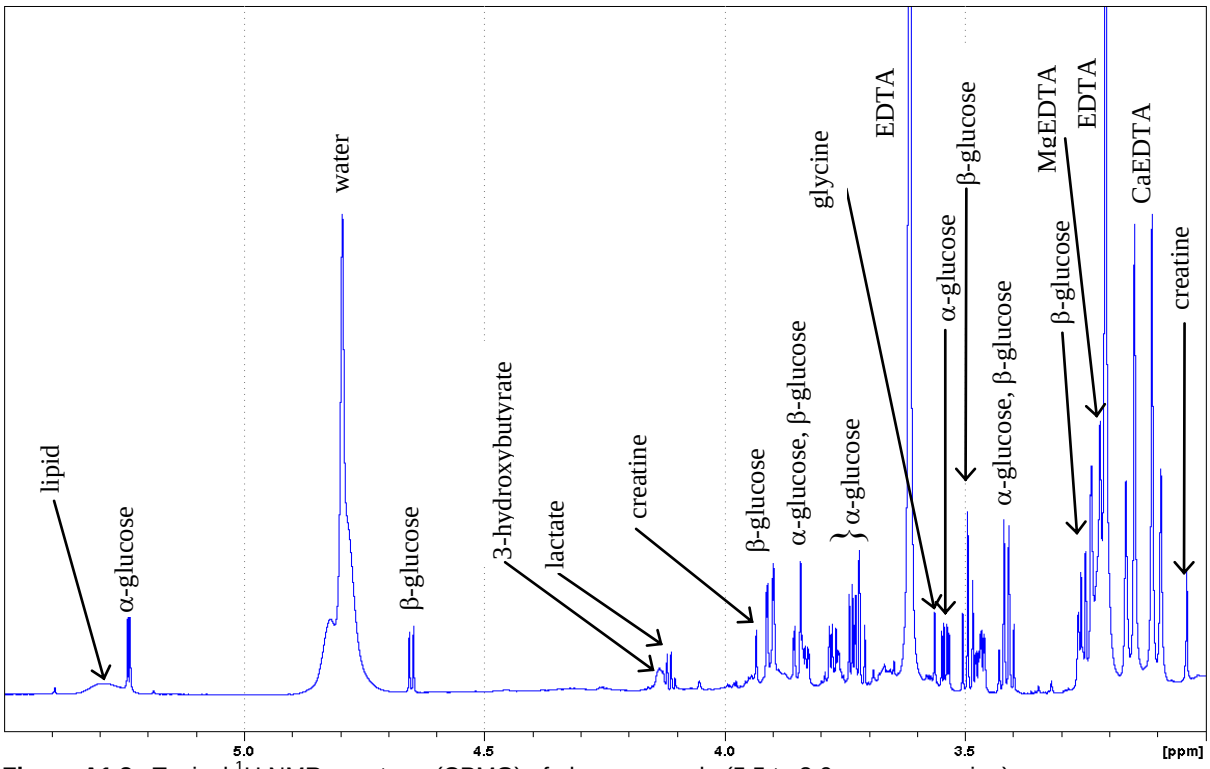

Figure A1.3a Typical ^{1}H NMR spectrum (CPMG) of plasma sample (5.5 to 3.0 ppm expansion)

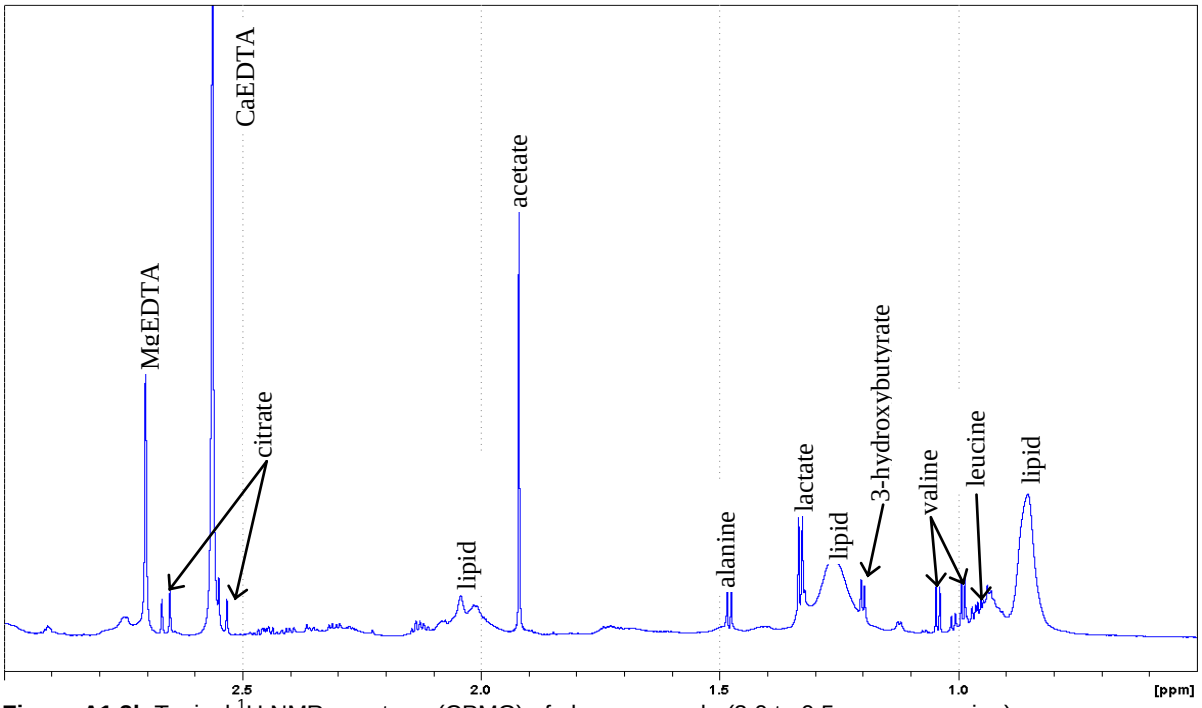

Figure A1.3b Typical ^{1}H NMR spectrum (CPMG) of plasma sample (3.0 to 0.5 ppm expansion)

A1.2.1 Principal components analysis

For principal component analysis, the NMR spectra were divided into bins of 0.01 ppm width. Only the region from δ4.5 to δ0.5 was included in the PCA and, additionally, all regions containing EDTA resonances were excluded, resulting in 370 bins being used. Figure 4.4 shows the scores plot for PC1 versus PC2 from the PCA of all plasma spectra. PC1 accounted for 55% of the variance in the data and PC2 accounted for 22%.

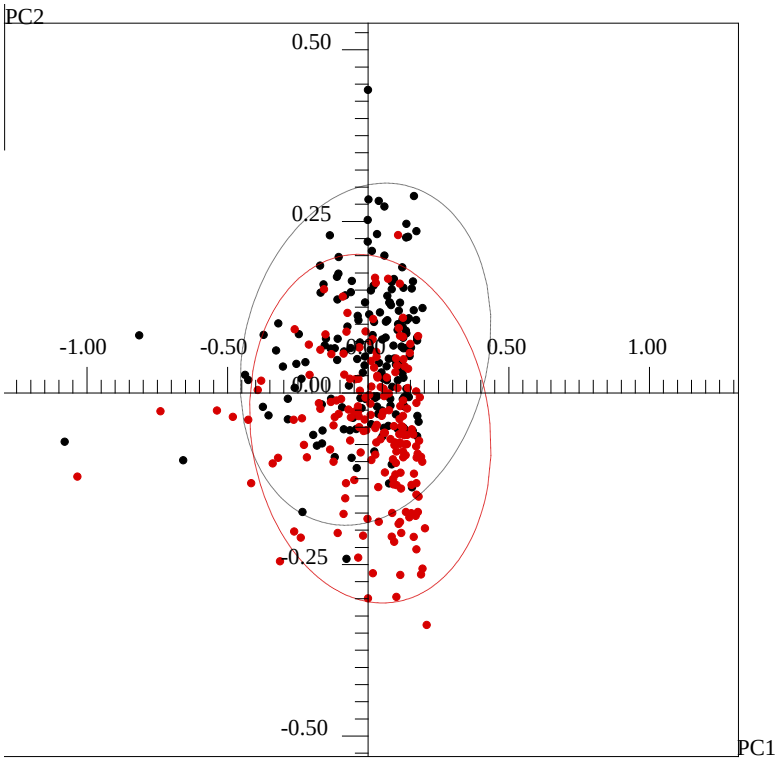

Figure A1.4 Scores plot from PCA of plasma ^{1}H NMR spectra (5.5 to 0.5 ppm). PC1 vs PC2. Black circles are cattle classified as fat; red circles are cattle classified as thin; dashed lines enclose the group Hotelling's T^2 distribution (95% confidence level)

The loadings plot for the PCA (see Figure A1.5) shows that the major loadings for PC1 (horizontal axis) are bins at 1.34 - 1.33 and 1.33 - 1.32 ppm. These correspond to the lactate methyl chemical shift, confirmed by the significant loadings at δ4.13 - 4.12 and 4.12 - 4.11 (lactate methyne proton). However, the scores plot suggests that there is no relationship between PC1 (and hence lactate concentration) and the fat/thin classification of the animal. Furthermore, the ten samples with the highest lactate concentrations appeared to be randomly distributed with respect to year of collection and muscling group as well. Two of these outlying samples came from one individual (in different years).

On the other hand, PC2 showed an appreciable separation between samples from fat and thin animals, with major loadings (in the negative direction) at 0.88 - 0.84 and 1.28 - 1.25, assigned as the methyl and methylene resonances (respectively) of the long-chain acyl groups of lipids. It is not possible to ascertain the nature of the lipid responsible for this effect based on those chemical shift values, the ^{1}H NMR shifts of the methylene and methyl resonances being very similar for phospholipids, triglycerides and free fatty acids, all of which can be present in plasma, aggregated with proteins as lipoproteins, as components of micelles or in a free or weakly bound

form. Reports of ^{1}H NMR spectroscopy of plasma typically assign these resonances simply as LDL / VLDL (e.g. Nicholson et al. (1995)). However, the influential loading at 3.24 - 3.23 provides further information on the possible nature of the lipid. This region is very crowded in all of the plasma spectra, containing part of the β-glucose H-2 resonance, a quartet due to MgEDTA and possibly a shoulder of the EDTA peak. The methyl resonance of choline (and choline containing compounds) also occurs around δ3.23. Observation of this region in two representative plasma spectra, one from a fat and one from a thin cow, shows an additional broad peak between (and overlapping) the two central lines of the MgEDTA resonance in the spectrum from a thin cow (see Figure A1.8). While it is possible that this additional peak is due to free choline, the apparent broad nature of the resonance (and the co-occurrence of lipid and choline resonances in the loadings plot) makes the phosphatidylcholine component of lipoproteins a more likely assignment.

Positive loadings in PC2 were of much lower magnitude, with the largest being 3.73 - 3.72 corresponding to part of the overlapping H3 and H6 resonances of β-glucose. Thus thin cattle would appear to have a higher ratio of lipid (most likely phosphatidylcholine) to glucose in their plasma. Bines and Morant (1983) found a significantly higher level of glucose in the blood of fat cattle compared to that of thin cattle immediately after feeding when fed *ad libitum* or at all stages when given restricted feed, suggesting that the separation of scores for fat and thin cattle in our study may be due to lower glucose levels in thin cattle. However, in a meta-analysis of existing research, Agenäs et al. (2006) found no correlation between blood glucose levels and body condition score (BCS). The greater magnitude of the loadings assigned to lipid and the apparent greater intensity of the choline methyl signals in most spectra from thin cows suggest that an increase in plasma lipid concentration is a more important factor than a decrease in glucose levels in causing the distinction observed between fat and thin cows in the PCA.

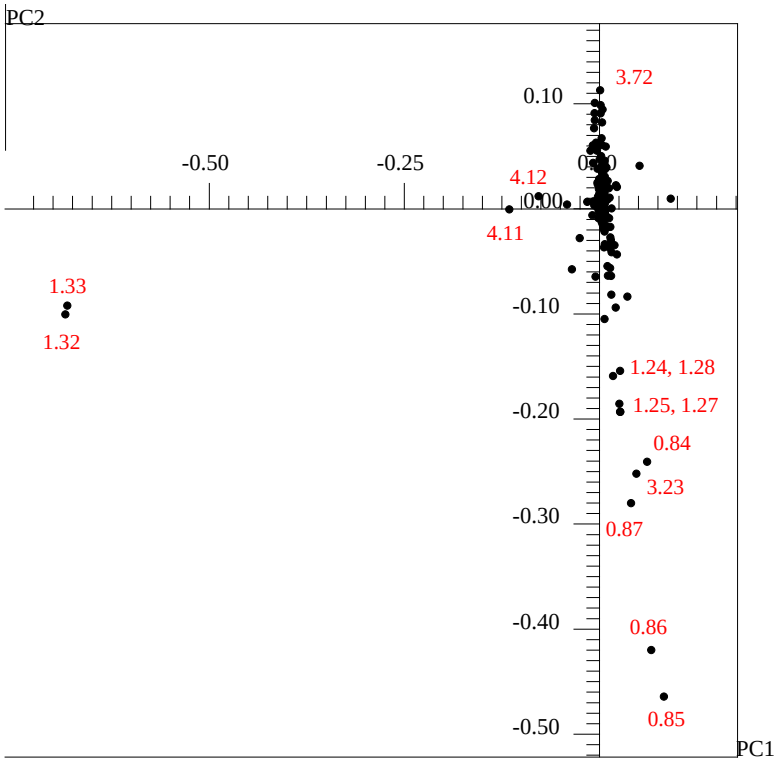

Figure A1.5 Loadings plot from PCA of plasma ^{1}H NMR spectra (5.5 to 0.5 ppm). PC1 vs PC2.Important loadings only are labelled (in red). The label refers to the upfield limit of the bin.

Observation of the scores plot of PC3 versus PC4 (not shown) showed little or no separation based on body condition and the loadings revealed that PC3 was dominated by bins at 1.93 - 1.92 (negative) and 1.92 - 1.91 (positive). These bins correspond to the acetate methyl resonance and observation of the original NMR spectra revealed that the chemical shift of this peak was somewhat variable (ranging from δ1.921 to δ1.919, a substantially larger variation than in any other metabolite resonance), causing it to fall into either bin. The major loadings in PC4 were 1.93 - 1.92 (positive) and 3.24 - 3.23 (negative). In this case there were no other lipid resonances with significant loadings and observation of the spectra of samples with large negative scores in PC4 indicated that the high loading of 3.23 was not due to an increase in phosphatidylcholine but to a downfield shift of the EDTA peak into this region. The variable chemical shift of the acetate and EDTA peaks may reflect small pH differences between samples, suggesting some buffering of the NMR samples might be desirable, despite the trial experiment showing that the spectral quality was better without the addition of buffer.

PC5 also showed no distinction between fat and thin cows (see Figure A1.6), the major loadings being 1.93 - 1.92 and 1.92 - 1.91 in the positive direction with no important loadings in the negative direction (see Figure A1.7). This suggests that body condition does not influence plasma acetate concentration.

PC6 did show some separation of fat and thin cows (Figure A1.6). The important positive loadings in PC6 were 3.27 - 3.26, assigned as the methyl resonance of betaine (trimethylglycine) and 2.05 - 2.04, assigned as the allylic methylene resonances of unsaturated lipid, and to a lesser extent 3.05 - 3.04 (creatine), while the major negative loadings were all glucose resonances. As lipid concentration has already been shown to reflect body condition (from PC2), it was not obvious whether the separation seen in PC6 was due to betaine (and creatine) concentration or simply the effect of this lipid.

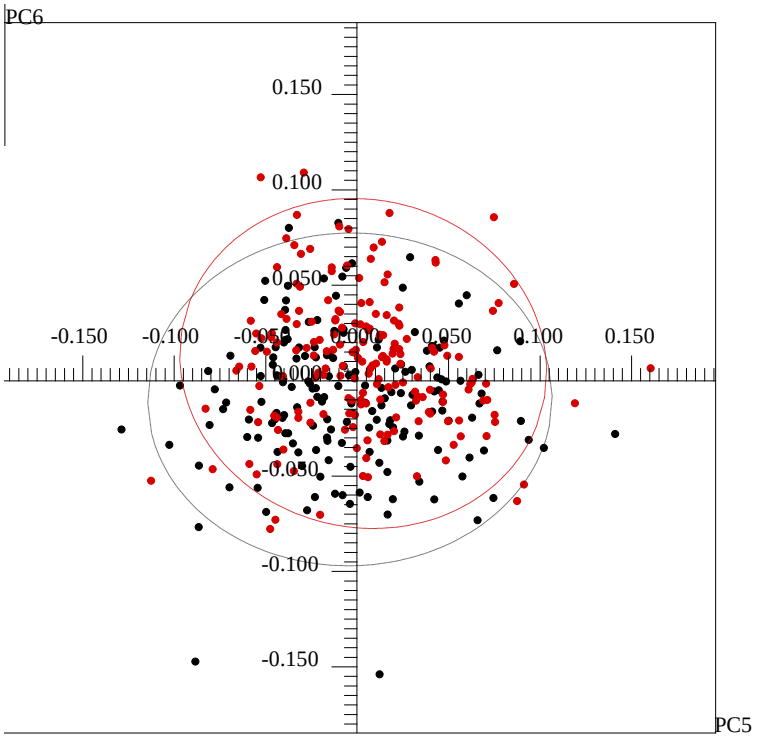

Figure A1.6 Scores plot from PCA of plasma ^{1}H NMR spectra (5.5 to 0.5 ppm). PC5 vs PC6. Black circles are cattle classified as fat; red circles are cattle classified as thin; dashed lines enclose the group Hotelling's T^2 distribution (95% confidence level)

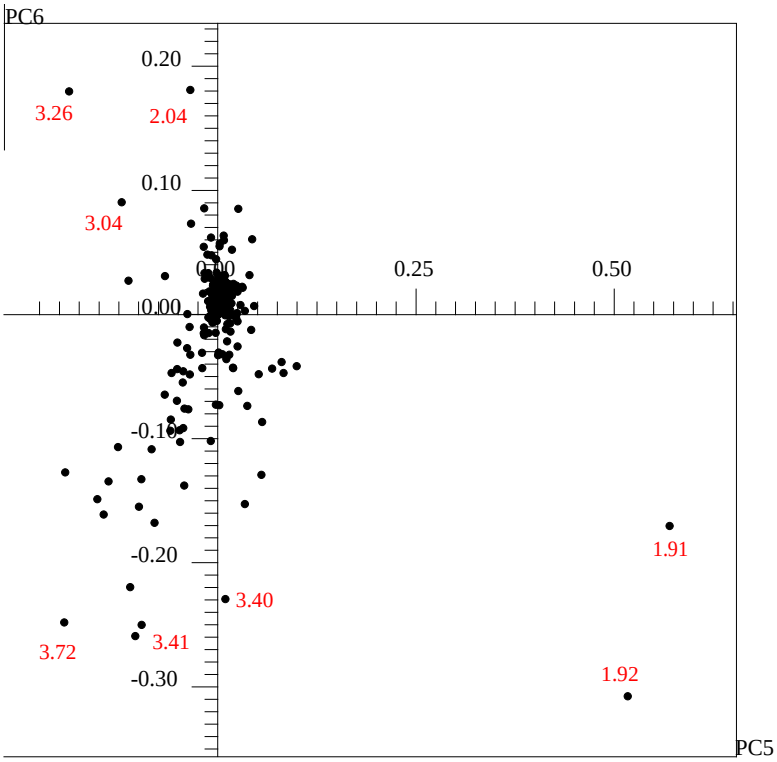

Figure A1.7 Loadings plot from PCA of plasma ^{1}H NMR spectra (5.5 to 0.5 ppm). PC5 vs PC6. Important loadings only are labelled (in red). The label refers to the upfield limit of the bin.

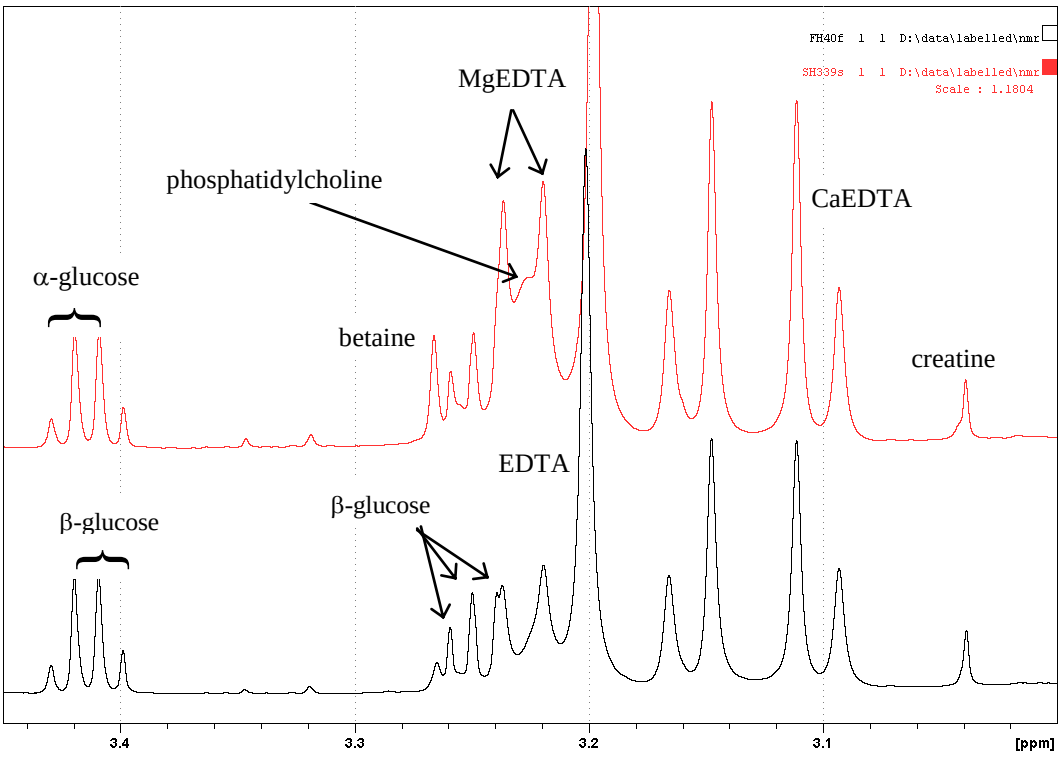

Figure A1.8 Typical ^{1}H NMR spectra of plasma samples from fat (black) and thin (red) cows (4.45 to 3.0 ppm expansion)

This question was resolved by comparison of spectra from fat and thin cows, which revealed that the betaine resonance is generally larger in the spectra of plasma from thin cows (see Figure A1.8).

Statistical analysis of the scores for PC1-6 from the PCA of all plasma spectra were conducted to asses their relationship with other aspects of the experiment. These revealed significant ($P<0.05$) relationships between body condition and PC2, PC3, PC4 and PC6. Consistent with the discussion above, PC2 and PC6 appeared to have the strongest relationship with body condition. These analyses also revealed differences with year for PC 1-6, and muscle line for PC3. The cow random term was significant for PC1-6, indicating that there were individual differences. Lactation status was related to PC2 and PC4.

A1.2.2 PCA excluding signals from lactate, acetate and lipids

As further evidence, the PCA was repeated with all lipid resonances excluded from the calculation. Additionally, as lactate and acetate were found to give no discrimination based on body condition but dominated PC1 and PC5 respectively, they were excluded as well. This resulted in reasonable separation of fat and thin cows in PC1, with betaine as the major loading, and to a lesser extent in PC2, with creatine (3.04-3.03) as the major positive loading. The major negative loading in PC2 was betaine.

To disentangle these two effects, the PCA was repeated twice more, once with bins corresponding to creatine removed in addition to the previous exclusions and a second time with betaine removed instead of creatine.

Both analyses achieve reasonable separation of samples from fat and thin cows in PC1 (see Figure A1.9a and A1.10a) with the major loadings being 3.27 - 3.26 (betaine) (see Figure A1.9b) in the former PCA and 3.05 - 3.04 (creatine), 0.95 – 0.94 and 0.94 – 0.93 (see Figure A1.10b) in the latter. It is difficult to assign the compounds responsible for the loadings from 0.95 to 0.93 as the region has many overlapping resonances. Compounds which have lines in this part of the spectrum include isoleucine and ω-3 fatty acids. However these bins do not appear to be responsible for any separation based on condition as removing them from the PCA produced little change in the scores plot (not shown).

The samples which do not group as expected (based on their body condition) in the PCA emphasising betaine also group unexpectedly in the PCA emphasising creatine and of the ten samples with the most negative scores in PC1 in the betaine PCA, five also appear among the ten samples with the most negative scores in the creatine PCA. This suggests that the betaine and creatine plasma levels are influenced by the same factor, namely body condition. Of the twelve samples with the most negative scores in PC1 in the betaine PCA, 10 had rib fat depth of 1 mm and two had scores of 2 mm. Of the twelve samples with the most negative scores in PC1 in the creatine PCA, 6 had a rib fat thickness of 1 mm, 4 had 2 mm and one (sample 92, one of the unexpectedly grouping samples) had a thickness of 12 mm. This indicates that a relatively high plasma betaine or creatine concentration is a good indicator of low body fat, although betaine does appear to be a slightly more accurate predictor than creatine. As PC1 in the creatine PCA had a large contribution from bins at 0.95 - 0.93, it seemed possible that the discrepancies observed between the betaine PCA and the creatine PCA were due to the contribution of this compound. However removing those bins from the PCA resulted in the same twelve samples with the most negative score in PC1 as before and it seems likely that differences in the results based on betaine and on creatine derive from inaccuracies in quantifying the

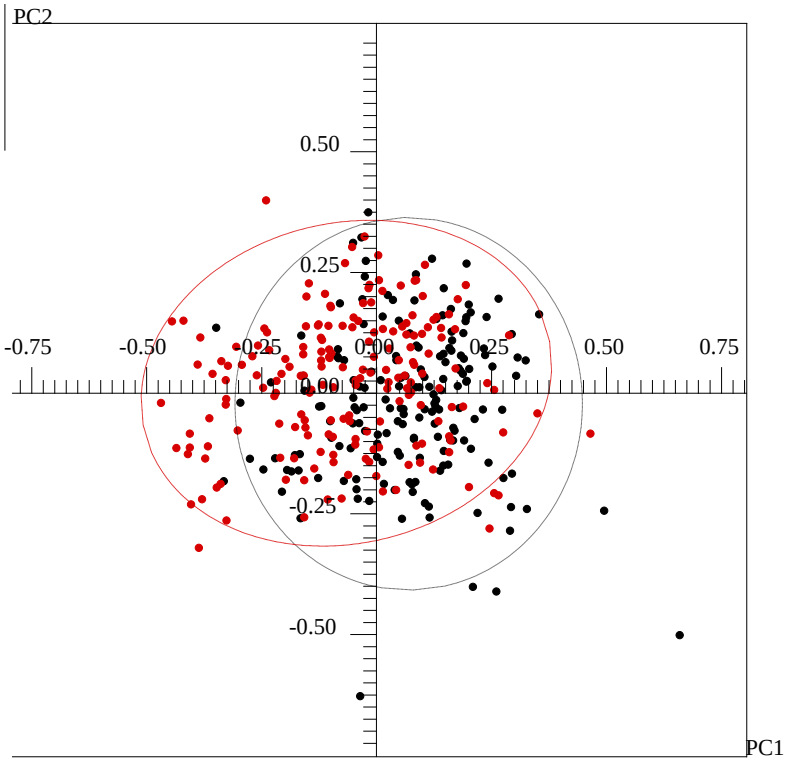

Figure A1.9a Scores plot from PCA of plasma ^{1}H NMR spectra (5.5 to 0.5 ppm) with binning to exclude lactate, lipid and creatine resonances (called betaine PCA) PC1 vs PC2. Black circles are cattle classified as fat; red circles are cattle classified as thin, dashed lines enclose the group Hotelling's T^2 distribution (95% confidence level)

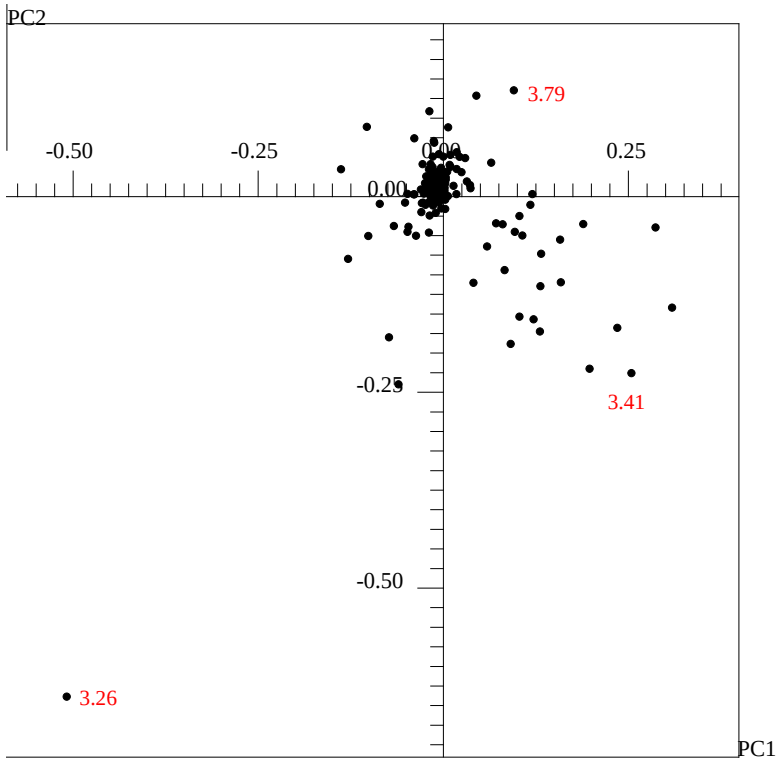

Figure A1.9b Loadings plot from PCA of plasma ^{1}H NMR spectra (5.5 to 0.5 ppm) with binning to exclude lactate, lipid and creatine resonances (called betaine PCA) PC1 vs PC2. Important loadings only are labelled (in red). The label refers to the upfield limit of the bin.

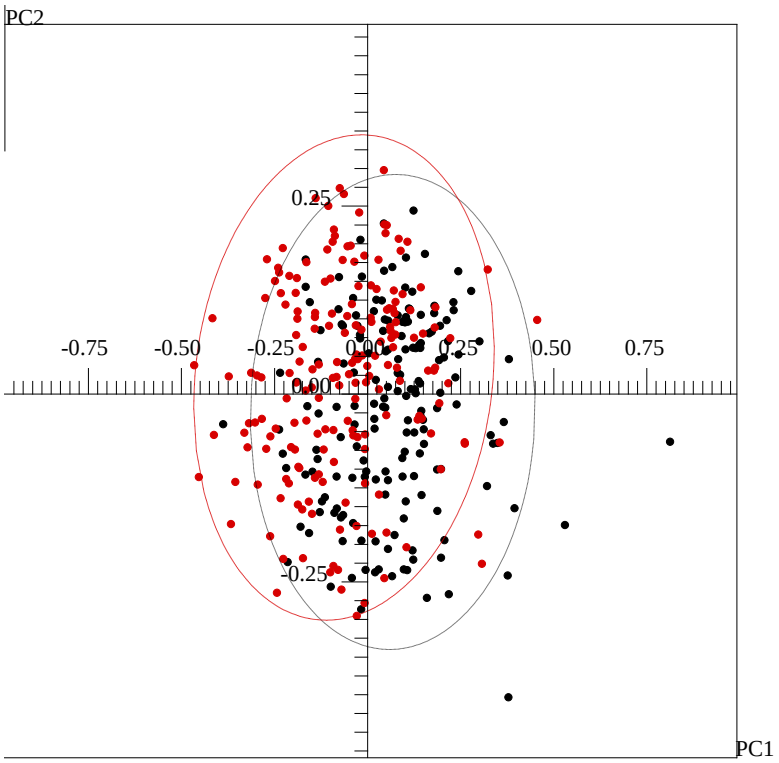

Figure A1.10a Scores plot from PCA of plasma ^{1}H NMR spectra (5.5 to 0.5 ppm) with binning to exclude lactate, lipid and betaine resonances (called creatine PCA) PC1 vs PC2. Black circles are cattle classified as fat; red circles are cattle classified as thin, dashed lines enclose the group Hotelling's T^2 distribution (95% confidence level)

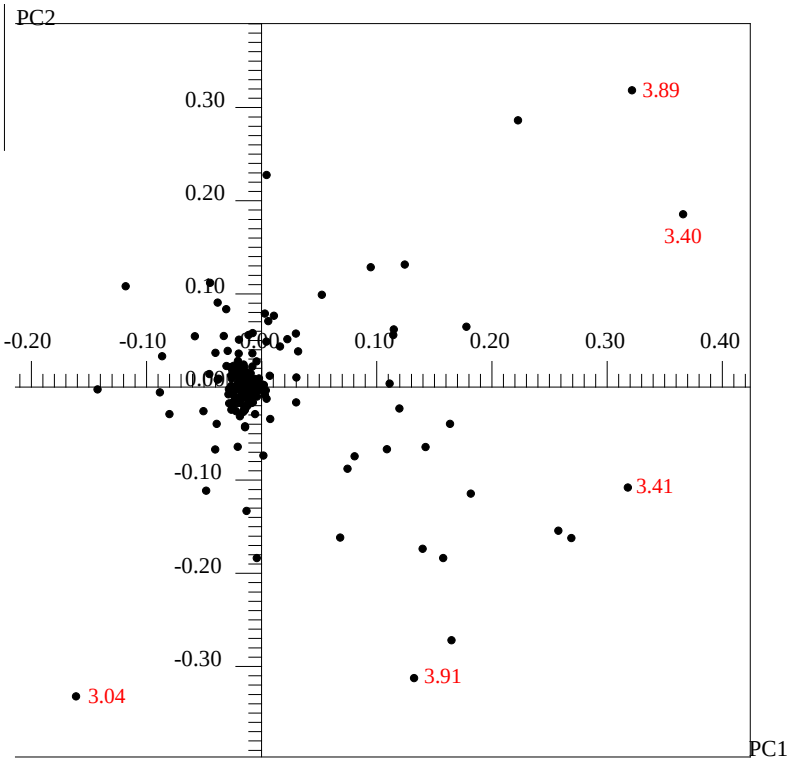

Figure A1.10b Loadings plot from PCA of plasma ^{1}H NMR spectra (5.5 to 0.5 ppm) with binning to exclude lactate, lipid and betaine resonances (called creatine PCA) PC1 vs PC2 Important loadings only are labelled (in red). The label refers to the upfield limit of the bin.

bins containing those compounds, due to overlap of the larger betaine resonance with other resonances and the small size of the creatine peak, combined with the fact that it falls across two bins and overlaps the creatinine resonance. The peak is assigned as being mainly due to creatine due to the relative intensities of the other creatine and creatinine resonances (δ3.94 and δ4.06 resp).

Statistical analysis of the scores from the betaine and creatine PCA were conducted to assess their relationship with other aspects of the experiment. These revealed significant ($P<0.05$) relationships between body condition and betaine PC1 and 2, and creatine PC1 and 2. These analyses also revealed differences with year for both betaine and creatine PC1 and PC2, and relationships with muscle line for betaine PC2 and creatine PC2. The cow random term was significant for betaine PC1 and PC2, and for creatine PC1 and PC2, indicating that there were individual differences. Lactation status was not related to any of the betaine or creatine PC.

A1.3 Conclusions and future research

Plasma betaine concentration has been shown in this research to be an indicator of body condition in cattle, and increased serum levels of both betaine and creatine in sheep subjected to long duration road transport have been previously reported by Li et al. (2011). Betaine is present in greater levels in plants that are subject to stress and there is considerable debate about whether this is an adaptive response to stress or a product of stress (Ashraf and Foolad, 2007). In mammals betaine is produced by the oxidation of choline, derived from phosphatidylcholine (Euland et al. 2005). While betaine could conceivably be produced and accumulated as a by-product of the catabolism of phospholipids to liberate (ultimately) fatty acids, this is not a typical response to undernutrition, triglycerides being the normal source of fatty acids. More likely is that the formation of betaine is a protective mechanism, possibly to conserve folate. Remethylation of homocysteine to methionine can occur through two mechanisms, via folate-dependent methionine synthase or via betaine and betaine homocysteine methyltransferase (BHMT), and the proportion contributed by each pathway depends on the relative availability of folate and betaine (Euland et al. 2005). It has been suggested that dietary folate levels may not be sufficient to achieve maximum efficiency in dairy cows (Ragaller et al. 2009). While lactating beef cows may have a lower folate requirement than dairy cows, it seems possible that during periods of undernutrition, a greater utilisation of the BHMT pathway occurs either due to unavailability of folate or to preserve it for other requirements. Thus mobilisation and degradation of phosphatidylcholine to produce betaine could account for the higher levels of lipid, choline and betaine observed in thin cows in this work.

It would be desirable to quantify the amount of betaine in the samples used in this analysis in order to allow a more accurate analysis of how this was related to body composition and other elements of the experimental design. This is not possible from NMR spectra without the addition of an internal standard. However by determining the glucose (or lactate) concentration of some key plasma samples by another method (e.g. a Radiometer ABL800 Flex analyser), the relative integrals of the glucose and betaine NMR resonances can be used to determine the betaine concentration of those samples. If a linear relationship between the betaine concentration and the PC1 score in the betaine PCA can be established, it may then be possible to calculate the concentration of betaine and other metabolites of interest in all samples.

The NMR data for this part of the project was acquired in mid June, so these results should be considered somewhat preliminary. Further investigation of the NMR data by PCA may reveal further metabolites which change concentration with body condition. In particular the levels of ketone bodies should be investigated. The quantities of acetone and acetoacetate present are very low in all spectra but there are appreciable amounts of β-hydroxybutyrate and PCA with binning chosen to emphasise this compound should be undertaken.

While ^{1}H NMR spectroscopy does not allow an easy characterisation of the lipid or the choline derivatives that we have found by principal component analysis to reflect body condition, liquid chromatography / mass spectrometry (LC/MS) should provide further information, even to the extent of determining if the fatty acid profile of the lipids changes as a result of undernutrition, and could be considered as a future avenue of investigation.

Although this research has shown that a metabonomic investigation by 1H NMR spectroscopy of plasma collected in EDTA tubes can yield valuable results, any further blood collections for NMR purposes should be done in heparin tubes. This would remove many interfering resonances, allowing the assignment and investigation of resonances from compounds present at much lower concentrations in the plasma.

Appendix 2. Publications and extension activities

Journal papers

Selection for increased muscling is not detrimental to maternal productivity traits in Angus cows. LM Cafe, WA Mckiernan, DL Robinson. Animal Production Science, Special Issue: Maternal Productivity in Cattle, *in press*.

Selection for increased muscling improves feed efficiency and carcase yield without reducing meat quality in Angus steers. LM Cafe, WA McKiernan, DL Robinson. Submit to Animal Production Science approximately December 2013.

Maternal productivity of Angus cows selected for increased muscling under divergent nutritional conditions. LM Cafe, WA McKiernan, DL Robinson. Submit to Animal Production Science approximately June 2014.

Popular press and other

In the past 12 months, articles presenting research results from the herd have been prepared for MLA Feedback Magazine, Agriculture Today, Beef CRC's final Beef Bulletin, The Stock Journal, and Town and Country Magazine.

Presentations

In the past 12 months, research results have been presented by Linda Cafe at Yalgoo Poll Hereford Open Day, and Tanholm Limousin Open and Field Day. In addition, Jason Siddell has remained involved with the herd, and has included current results in discussions with producers through his past role as DPI Beef Extension Officer, and current role as Senior Land Services Officer (Livestock) for Northern Tablelands Local Land Services.

Extension material

A fact sheet summarising the results for the maternal productivity of the muscle lines under nutritional constraint will be prepared to facilitate release of the findings to industry (by December 2013).

Appendix 3. Follow-up to B.SBP.0085 Final Report 31/8/2014

Three actions and recommendations for continuing and future research from the B.SBP.0085 Project '**Using muscling selection line cows to inform maternal productivity modelling'** were followed-up on during the 12 months after submission of the report. These were:

1. Prepare a fact sheet summarising the results for the maternal productivity of the muscle lines under nutritional constraint to facilitate release of the findings to industry
2. Consider value of continuing to collect weight and scan data on the herd as the cows from Low nutrition return to good body condition, to assess any residual impacts of longer-term nutritional restriction on productivity and, if present, for any link to muscle line.
3. Measure the glucose (or lactate) concentration of some key plasma samples to enable accurate calculation of concentrations of betaine and other significant metabolites from the spectra of all samples. This would allow more direct analysis of the implication of variation in these metabolites with cow body composition traits, and their relationship with maternal performance.

A 3.1 Muscling and maternal productivity fact sheet

A 3-page NSW DPI Primefact summarising the important industry outcomes for maternal productivity from this project was prepared and published in January 2014.

Cafe LM (2014) Cow muscularity and maternal productivity. Primefact 1332, NSW Department of Primary Industries, Orange, NSW.

A 3.2 Assessing residual impacts of divergent nutrition on cow body composition and maternal performance

At the end of the three years of nutritional divergence described in this report, the herd was returned to normal pasture-based nutrition. Maternal performance and cow body composition were measured for a period of 12 months to assess any residual impacts of longer-term nutritional restriction on productivity and, if present, for any relationship with muscle line.

A3.2.1 Methods

A3.2.1.1 Herd management

The cow herd remained at Glen Innes Research Station after the completion of the divergent nutritional treatments. General cow herd management was identical to that described for the previous three years. The only change was that all were run on the same level of pasture-based nutrition though frequent rotation of the management groups around the station.

A3.2.1.2. Seasonal conditions

The 2013/2014 summer rainfall was the lowest received at the station over 103 years of weather records. Well below average rainfall was received from December 2013 through to mid-March 2014 (Figure A3.1), which is usually the peak of pasture growth for the station. Temperatures were also higher than normal over spring and summer, leading to a high level of evaporation and little pasture growth. Hand feeding of lucerne and cereal hay was conducted through winter (August to November), which is normal practice at the station. Feeding commenced again in February due to low feed availability. Weaning was conducted in late March, 4-6 weeks earlier than usual, as a drought management strategy. Hand feeding of the cows continued until May, when pastures had recovered sufficiently after the rainfall in March to once again sustain the herd

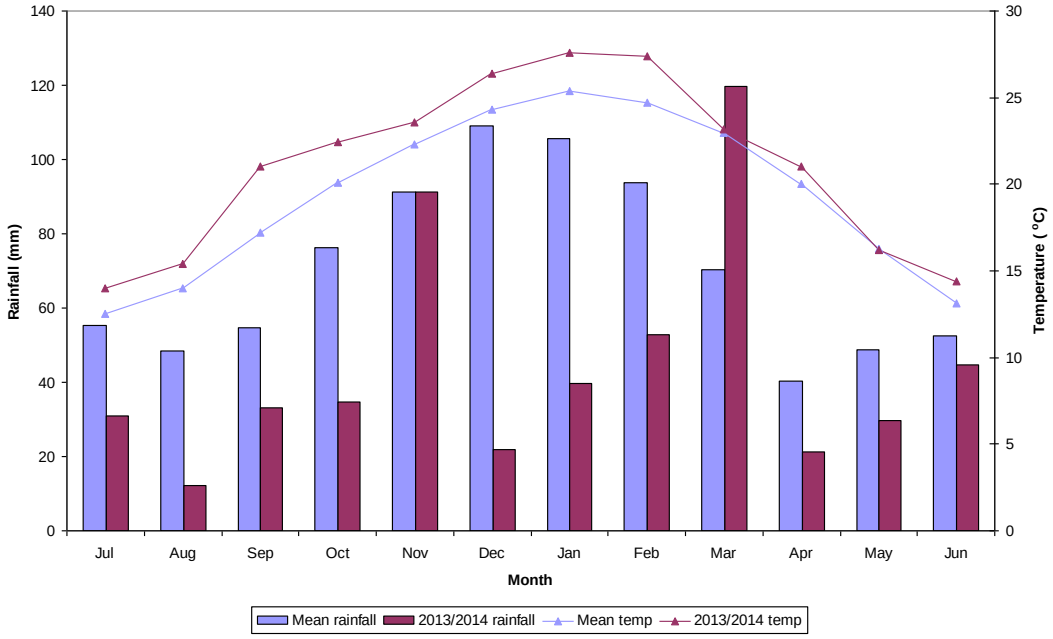

Figure A3.1. Monthly rainfall and mean maximum temperature recorded at Glen Innes Research Station for the 2013/2014 season, compared to the long term (103 year) averages

A3.2.1.3 Data collection

The cows were weighed, ultrasound scanned for body composition [rump fat, rib fat and eye muscle area (EMA)] and visually assessed for muscle score and body condition score at the end of calving in September 2013, and again at weaning in March 2014. All ultrasound scans were conducted by an accredited scanner using a 3.5 MHz/180-mm linear array animal science probe (Esoate Pie Medical, Maastricht, Netherlands). Muscle scoring was conducted by one experienced assessor. The 15 point, E- to A+ muscle scoring system was used (McKiernan 2007), and converted to a numeric scale of 1 to15 for analysis. Body condition was scored by one assessor from 1 = emaciated to 9 = obese, with a combination of muscling and fat cover taken into consideration (Wagner et al. 1988). Cows were also weighed in May 2014 to assess their rate of recovery after weaning.

Calves were weighed and tagged at birth, and their birth date, sex, dam, and need for birth assistance recorded. All calves were bled to determine *myostatin* genotype prior to weaning. Weaning was carried out in late March, at which time the weaners were weighed, ultrasound scanned for body composition and muscle scored.

A3.2.1.4. Statistical analyses

Maternal performance data, including calf birth weight and calf weaning traits (weight, scanned fat and EMA measurements, muscle score, pre-weaning ADG and age at weaning) were analysed by fitting Linear Mixed Models using the REML methodology (Robinson, 1987) in Genstat V16 (VSN International Ltd, Hemel Hempstead, UK). Models included fixed effects for dam muscle line, prior nutrition, calf sex, plus their interactions as well as a covariate for calf *myostatin* genotype. Random effects were fitted for dam's lactation status the previous year. Weaning traits are presented adjusted for age at weaning.

Days to calving (DTC) was calculated from the first day of joining to the resulting calf birth date; cows that did not produce a calf were given a 21d penalty above that of the longest successful calving interval (Johnston and Bunter 1996). The analysis of DTC included fixed effects for dam muscle line, nutrition, and their interaction, plus random terms for joining sire and dam's lactation status the previous year.

Calving and weaning rates were analysed using the REML methodology in ASREML-R (Butler et al. 2009), fitting a logistic model (1 = calf born or weaned, 0 = otherwise) with binomial errors and fixed effects for dam muscle line, prior nutrition, dam muscle line x nutrition, plus random effects for joining sire, lactation status the previous year. Conditional Wald P values were used to assess the significance of the terms fitted in the model.

Statistical analyses of lactating cow body composition were conducted by fitting Linear Mixed Models using the REML methodology in Genstat V16. The model included fixed effects for prior nutrition, muscle line, date and their interactions, and a random term for lactation status the previous year.

All cows were aged 4 years or over at calving in 2013. Earlier analysis on the herd indicated that age effects were observed for the 2 and 3 year old cows, but not for more mature cows. After checking that there were no significant effects of cow age in the models, it was omitted from the analyses. Four cows gave birth to twin calves. These were included in calculation of calving and weaning rates but the data from twin calves was excluded from further analyses.

A3.2.2 Results and discussion

A3.2.2.1. Cow liveweight and body composition

When the cow herd was returned to normal nutrition in May 2013, cows from Low nutrition (NL) gained weight, but remained lighter at the end of calving in September 2013 (Figure A3.2a) than cows from Medium/High nutrition (NMH) (NL 584 vs NMH 608, P = 0.004). The gain of approximately 40kg for the NL cows whilst the NMH cows maintained their body weight is typical of the compensatory gain response in cattle after a period of restricted nutrition. The liveweight comparison between May and September contains some error due to conceptus weight accounting for a proportion in the cow liveweight in May, where cows were 4-6 months pregnant. However the weight of the gravid uterus at that stage was unlikely to have varied a great deal due to prior nutritional treatment (Camancho et al. 2014). The compensatory gain occurred over the winter feed gap, while the herd was supplemented with hay to maintain condition, and it is likely that the NL cows may have compensated further had there been more feed on offer, or a longer recovery period.

The effect of the prior nutritional treatments on body composition were in keeping with the differences in liveweight at September 2013. Cows from Medium/High nutrition remained fatter (rib fat NMH 10.4 vs NL 6.6, P <0.001; rump fat NMH 16.5 vs NL 9.9, P < 0.001), had greater EMA (NMH 63.1 vs NL 55.5, P <0.001) and a higher condition score (CS; NMH 6.7 vs NL 5.4, P < 0.001) but similar muscle score (MS; NMH 7.9 vs NL 7.7, P = 0.3) than cows from Low nutrition. Adjusting the body composition traits for liveweight reduced the sed, but did not remove the differences in fatness, EMA and CS. These differences in body composition provide further evidence that the NL cows had not finished compensating (Ball et al. 1997) by the start of lactation.

All cows lost weight whilst lactating, and there was no difference in weight due to previous nutritional treatment at weaning in March (NL 503 vs NMH 508, P = 0.4) nor in May (NL 552 vs NMH 559, P = 0.3). Cows from both previous nutritional treatments utilised fat reserves during lactation (Figure A3.2b) and whilst the differences were reduced, NMH cows remained fatter at weaning than NL cows (Table A3.1.) There was also a tendency for the NMH cows to have slightly higher EMA, MS and CS than NL cows. Hence, the NMH cows began lactation in better condition than the NL cows, and remained a slightly better at the end of lactation.

There was no difference in liveweight between cows from the three muscle lines (Figure A3.2a, Table A3.1) in Sept 2013, Mar or May 2014 (all P > 0.2), and no interaction between nutrition and muscle line for liveweight (all P > 0.4). The usual difference in body composition observed between the three lines was maintained (Figure A3.2 b,c,d; and Table A3.1). In both September and March the Low muscle cows had smaller EMA and MS than $High^{Het}$ cows, with High muscle cows intermediate; whilst Low cows had greater fatness than $High^{Het}$ cows with High cows intermediate. There were no interactions between muscle line and prior nutrition for the fatness or muscling traits. There was no indication of any effect of selection for muscling on the residual impacts of longer-term low nutrition on cow liveweight or body composition.

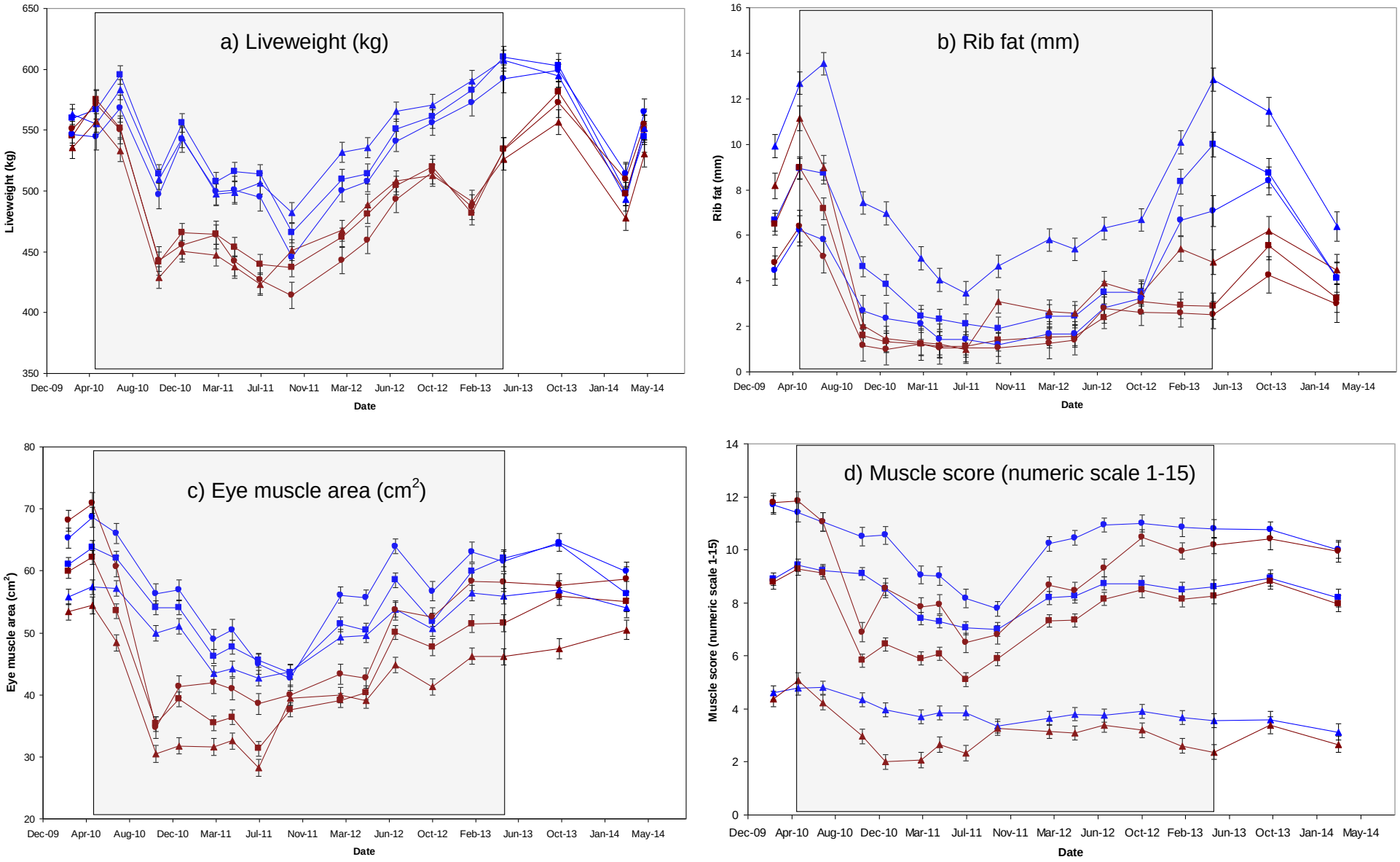

Figure A3.2. Change in liveweight, rib fat, eye muscle area and muscle score for lactating cows from the High (■), High^{Het} (●) and Low (▲) muscling lines from Medium/High (blue) and Low (red) nutrition over four reproductive cycles. Error bars show se. Grey box depicts the period of nutritional divergence

Table A3.1. Liveweight, scanned fat and eye muscle area (EMA), and muscle score of lactating cows from the three muscle lines at weaning in March 2014, 10 months after the end of the divergent nutritional treatments

	Liveweight	Rump fat (mm)	Rib fat (mm)	EMA (cm2)	Muscle score (1-15)	Condition score (1-9)
Prior nutritional treatment						
Medium/High	508	7.0b	5.4b	58.0b	7.2b	4.4
Low	503	5.8a	4.2a	56.2a	7.0a	4.0
sed	8.1	0.62	0.41	1.33	0.27	0.22
signif.	ns	**	**	†	†	†
Cow muscle line						
Low	497	8.7b	6.3b	54.3a	3.1a	4.3
High	506	5.4a	4.3a	57.1ab	8.2b	4.1
High ^{Het}	515	5.2a	3.8a	59.9b	10c	4.2
sed	10	0.76	0.51	1.64	0.34	0.27
signif.	ns	***	***	**	***	ns
Nutrition * Muscle	ns	ns	ns	ns	ns	

abc Letters denote means which are significantly different. †P<0.1, *P<0.05, **P<0.01, ***P<0.001, ns P>0.1

A3.2.2.2. Calving and weaning performance

Prior nutritional treatment significantly affected calf birth weight, with calves from cows previously on Low nutrition being born 1.6kg lighter than those from Medium/High nutrition (Table A3.2). The divergent nutritional treatments ended in May 2013, when these calves were approximately 4-6 months through gestation, and given the differences in the liveweight of the dams discussed above, this was not an unexpected result (Robinson *et al.* 2013). The impact of nutritional treatments on birth weight can be variable, but it is likely that a more severe or longer term restriction will have a greater effect. In this experiment the NL cows experienced varying severity of nutritional restriction over a three year period, which was enough to affect birth weight. There was no difference in birth weight of calves produced by cows from the three muscle lines (Table A3.1), and no interaction between muscle line and prior nutrition (P = 0.9) for calf birth weight. This is consistent with birth weight results reported for the three years of divergent nutrition.

Four cows (2% of herd) were assisted during calving, providing insufficient data to analyse calving assistance. One assisted cow was from prior Low nutrition, two were from the High muscle line, and two were from High^{Het}, with the calves being sired by three different bulls. This is a slightly higher level of dystocia than that seen over the three years of nutritional treatments (0.9%), but calf birth weights were also higher than during the previous period. The average weight of the four assisted calves was 45.6 kg (range 40.5 – 56.2kg), weights at which some birth assistance may be expected in Angus cows.

A binomial analysis showed no differences due to prior nutrition or cow muscle line on either calving or weaning rates (all P > 0.05). There were also no significant interactions between prior nutrition and cow muscle line on calving or weaning rates. Across the herd the calving rate was 93.6% of joined cows, and the weaning rate was 85.8% of joined cows. DTC was not affected by prior nutritional treatment, and did not differ between cow muscle lines (Table A3.2.).

Male calves were heavier at birth (F 38.9kg vs M 41.5kg, P < 0.001), grew faster than heifer calves and were heavier at weaning (Table A3.3.). Steers had a greater MS and EMA than heifers, but heifers were fatter at the rib and rump than steers at weaning. These results are consistent with the sex effect usually observed for calves within the herd.

Table A3.2. Calf birth weight and days to calving for Low muscle, High muscle and High^{Het} cows following divergent nutritional treatments

	n cows joined	Calf birth weight (kg)	Days to calving (d)
Prior nutritional treatment			
Medium/High	106	41.0b	298
Low	98	39.4a	296
sed		0.75	1.7
signif.		*	ns
Cow muscle line			
Low	67	40.5	298
High	76	40.6	298
High ^{Het}	61	39.6	293
sed		0.92	2.1
signif.		ns	ns

abc Letters denote means which are significantly different. †*P*<0.1, **P*<0.05, ***P*<0.01, ****P*<0.001, ns *P*>0.1

Table A3.3. Weaning weight, scanned fat and eye muscle area (EMA), muscle score, and prewean growth rate (ADG) of calves from Low muscle, High muscle and High^{Het} lines born after divergent nutritional treatments. Adjusted for age at weaning (average 213d)

	n	Weight (kg)	Rib fat (mm)	Rump fat (mm)	EMA (cm^2)	Muscle score (1-15)	Prewean ADG (g/d)
Prior nutritional treatment							
Medium/high	82	228	2.8b	3.5b	41.5	8.4	880
Low	84	221	2.5a	3.0a	41.1	8.1	857
sed		3.2	0.11	0.11	0.66	0.21	14.3
signif.		ns	**	**	ns	ns	ns
Calf muscle line							
Low	59	226	2.9b	3.3ab	39.4a	4.7	874
High	49	226	2.8b	3.5b	40.8a	9.0	878
High ^{Het}	58	222	2.4a	3.0a	43.6b	11.0	852
sed		3.9	0.14	0.21	0.81	0.26	17.5
signif.		ns	***	†	**	***	ns
Calf sex							
Heifer	86	218a	2.9b	3.6b	40.5	7.8	844
Steer	80	230b	2.5a	2.9a	42.1	8.8	892
sed		3.3	0.12	0.65	0.67	0.22	14.5
signif.		**	***	***	*	***	***

abc Letters denote means which are significantly different. †*P*<0.1, **P*<0.05, ***P*<0.01, ****P*<0.001, ns *P*>0.1

The effect of prior nutrition on calf growth to weaning and weaning weight was not significant (Table A3.3.). There was a small residual effect of prior nutrition on fatness of the calves at weaning, with calves from NMH being slightly fatter (0.3 mm rib fat and 0.5 mm rump fat) than those from NL. There was no difference in calf

weaning EMA or MS due to prior nutrition. The MNH cows utilised more subcutaneous fat during lactation than the NL cows, and it is possible that a difference in milk quality provided to their calves resulted in the fatness differences in the calves at weaning (Chilliard et al. 2000). It may also be a result of the higher in-utero growth of calves from MNH, as birth weight has previously been shown to be associated with body composition at weaning (Cafe et al. 2006).

There were no differences between calves from the three muscle lines in growth to weaning or weaning weight (Table A3.3). Both EMA and MS of the calves at weaning followed the pattern seen in the cows, whereby the Low muscle calves had the smallest EMA and MS, the High^{Het} calves had the greatest, and the High calves were intermediate. Rib fat at weaning was lowest for the High^{Het} calves, and not different between the Low and High calves, whilst High^{Het} calves had the lowest rib fat, High calves had the greatest, and Low calves were intermediate. The level of fat at weaning was quite low, so whilst it is difficult to draw too much from the results, they appeared to follow the usual pattern for seen across the muscle lines.

There were no interactions between prior nutrition and calf muscle line for weaning weight or body composition traits at weaning (all $P \geq 0.1$). Similar to the results for cow liveweight and body composition, there was no indication of any effect of selection for muscling on the residual impacts of longer-term low nutrition on subsequent calf liveweight or body composition.

Ruminants have evolved to cope with variation in pasture availability, and to utilise body reserves of both fat and muscle tissue in periods of feed deficit, and are obviously very successful at this. Additional demand is placed on nutritionally restricted cows during gestation and lactation, as they provide for the calf as well as their own needs. This is important in regards to the effect that early-life growth and variations in growth path have on the ability to optimise carcase endpoints. Results from the initial weight loss phase of this experiment indicated that cows with different levels of muscling utilised tissues relative to their body composition during weight loss (Cafe et al. 2012), and it appears that those tissues were returned in the same proportions when weight was re-gained.

A3.2.3 Conclusions

Cows which had experienced Low nutrition for a period of three years showed compensatory growth for 4 months prior to parturition, but had not fully compensated and remained lighter than cows from Medium/High nutrition at this stage. Under drought conditions, cows from both prior nutritional treatments utilised body reserves to fuel lactation, such that there was no difference in liveweight at weaning 6 months later, though body composition traits revealed that cows from prior restricted nutrition remained slightly poorer at this stage. The cows from prior Low nutrition gave birth to lighter calves. The difference in calf weight was not significant at weaning, though the calves form prior Low nutrition were less fat at weaning. There was no indication of any effect of selection for muscling on the residual impacts of longer-term low nutrition on cow liveweight, body composition or maternal performance.

A 3.3 Biological indicators of body condition

As proposed in the concluding remarks of the NMR section of the Final Report (p 31), an analysis of the glucose concentration of some representative plasma samples was undertaken using a Dade Behring Dimension RxL clinical chemistry analyser. Glucose levels in these samples were found to be 3.8 ± 0.5 mM.

From the relative integration of the α-glucose H-2 resonance and the betaine methyl resonance in the ^{1}H NMR spectra of these samples, the betaine concentration in these plasma samples was able to be estimated. Betaine concentration in plasma samples from fat cows was in the range 70 – 250 µM, while that in thin cows was in the range 150 - 410 µM. As can be seen in the scores plot from principal component analysis in the Final Report (Fig 4.9a, p. 29), where there is considerable overlap of scores of fat and thin cattle in the intermediate region of the plot, it is not possible to state a betaine concentration that would denote a clear cut-off point in determining a cow's condition. However, it is apparent that an animal with a plasma betaine concentration over 250 µM could be considered at risk of being undernourished.

A paper discussing this work, entitled "Markers of poor nutrition in cows using ^{1}H NMR spectroscopy of plasma" is currently in preparation for submission to the journal Metabolomics. Authors are David Tucker, Linda Cafe, Helen Smith, Ian Brereton, Horst Joachim Schirra, and Greg Pierens. A draft manuscript should be ready for circulation to the co-authors for comment within 1-2 weeks.