finalreport

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Quantifying tenderness polymorphisms and discovery of associated biological pathways

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Abstract

This research aimed to assess effects of calpain system polymorphisms for tenderness in Brahman cattle and their interactions with hormonal growth promotant, gender, method of hand and aging period. The improvement in *longissimus* shear force in normally (achilles) hung carcasses after 7 days aging was 1.20 kg for the NSW herd and 0.93 kg for the WA herd for the cattle with the favourable calpastatin/calpain 3 alleles compared to those with the unfavourable alleles (i.e. 0*0* *vs.* 2*2*). Significant improvements were also evident in the tender-stretched sides due to the favourable gene markers. Calpain-calpastatin system protein abundance and activity and gene expression results are consistent with the phenotypic findings in relation to shear force, and are helping to elucidate mechanisms responsible for the phenotypic effects. There were no significant adverse effects of the favourable calpastatin-calpain 3 alleles on growth during backgrounding and feedlotting, feed intake, feed efficiency, behaviour, stress responsiveness, or commercial carcass and MSA chiller assessment traits. An MLA Donor Company is being established to have samples we collected for MSA eating quality assessment eaten, to allow incorporation of the effects of the gene markers into the MSA model as an integral component of the path to adoption. The extent to which the tenderness gene markers are incorporated into breeding programs and for fine-tuning of processing to improve product quality and efficiencies will largely depend on successful outcomes of the MLA Donor Company. It is recommended that further research to elucidate the mechanisms by which the favourable alleles in the calpain system, and other factors, enhance tenderness be undertaken using data and samples obtained within this experiment.

Executive Summary

The beef industry is in the early stages of adopting the use DNA markers in breeding programs. Amongst the initial targets are DNA markers for gene variations which influence beef tenderness with the objective to increase the rate of genetic gain for this trait. The number of DNA markers used in breeding programs will increase as more are discovered, commercialized and adopted for use by industry. Knowledge of the biology underpinning the DNA marker associated gene variation is required to optimize industry outcomes.

To fully understand the outcome from breeding programs using marker assisted selection for tenderness, knowledge of the mode of action, i.e. the biological pathways, of the genes that the markers represent is required. This knowledge will enable calculation of the size of effect of individual markers and also enable an analysis of the probability of inadvertent selection for linked but undesirable traits that maybe associated with marker assisted selection breeding programs.

This research aimed to assess effects of calpain system polymorphisms for tenderness in Brahman cattle and their interactions with hormonal growth promotant, gender, method of hand and aging period.

The improvement in *longissimus* shear force in normally (achilles) hung carcasses after 7 days aging was 1.20 kg for the NSW herd and 0.93 kg for the WA herd for the cattle with the favourable calpastatin/calpain 3 alleles compared to those with the unfavourable alleles (i.e. 0*0* *vs.* 2*2*). This benefit was mostly due to favourable markers for calpastatin. Significant improvements in *longissimus* tenderness due to the favourable gene markers were also evident in the tender-stretched sides.

There were no significant adverse effects of the favourable calpastatin-calpain 3 alleles on growth during backgrounding and feedlotting, feed intake, feed efficiency, behaviour, stress responsiveness, or commercial carcass and MSA chiller assessment traits.

The Project objectives have been successfully met. Milestones required to meet the Beef CRCs and MLAs go/no go barrier to further elucidate the calpain system if striploin *longissimus* shear force results showed benfits from the gene markers were met.

Calpain-calpastatin system protein abundance and activity and gene expression results are consistent with the phenotypic findings in relation to shear force, and are helping to elucidate mechanisms responsible for the phenotypic effects. It is recommended that further research to elucidate the mechanisms by which the favourable alleles in the calpain system, and other factors, enhance tenderness be undertaken using data and samples obtained within this experiment.

An MLA Donor Company is being established to have samples we collected for MSA eating quality assessment eaten by taste panels, to allow incorporation of the effects of the gene markers into the MSA model as an integral component of the path to adoption.

The extent to which the tenderness gene markers are incorporated into breeding programs and for fine-tuning of processing to improve product quality and efficiencies will largely depend on successful outcomes of the MLA Donor Company.

Contents

1 Background

The beef industry is in the early stages of adopting the use DNA markers in breeding programs. Amongst the initial targets are DNA markers for gene variations which influence beef tenderness with the objective to increase the rate of genetic gain for this trait. The number of DNA markers used in breeding programs will increase as more are discovered, commercialized and adopted for use by industry. Knowledge of the biology underpinning the DNA marker associated gene variation is required to optimize industry outcomes.

To fully understand the outcome from breeding programs using marker assisted selection for tenderness, knowledge of the mode of action, i.e. the biological pathways, of the genes that the markers represent is required. This knowledge will enable calculation of the size of effect of individual markers and also enable an analysis of the probability of inadvertent selection for linked but undesirable traits that maybe associated with marker assisted selection breeding programs.

This project will:

- Quantify the magnitude of effects of gene markers for tenderness
- Identify and quantify interactions between gene markers for tenderness, sex, hormonal growth promoters, method of carcass hang, and major muscles.

This project will lead to the understanding the biology of gene markers for tenderness and the calpain-calpastatin system.

This project is part of Beef CRC project 1.1.3 and the objectives and milestones are a subset of 1.1.3 objectives and milestones.

2 Project Objectives

By June 2008, to quantify the magnitude of effects of gene markers for tenderness, and to identify and quantify interactions between gene markers for tenderness, sex, hormonal growth promoters, method of carcass hang, and major muscles.

3 Methodology

- *Bos indicus* (~2,000) and *Bos taurus* (~600) cattle were genotyped for markers for beef tenderness (calpastatin, calpain 1 and calpain 3). From the *Bos indicus* cattle, animals were selected into groups for levels (0*, 1* and/or 2*) of gene markers in calpastatin and calpain 3, with groups as balanced as possible for calpain 1. Small groups of Angus cattle with favourable gene markers (2*, 2*, 2*) were used as positive controls.

- In NSW, steer and heifer cattle with either 0* or 2* for calpastatin and with 0* or 2* for calpain3 (i.e. 0*/0*, 0*/2*, 2*/0* and 2*/2* groups) were selected, with groups as balanced as possible for calpain 1. Half were treated with an aggressive hormonal growth promotant during feedlotting. To maximise this effect animals were slaughtered within the payout period. A small group of Angus cattle with 2* for calpastatin and 2* for calpain3 were included in the design as positive controls.
- In WA, steer cattle with either 0*, 1* or 2* for calpastatin and with 0*, 1* or 2* for calpain3 (i.e. 0*/0*, 0*/1*, 0*/2*, 1*/0*, 1*/1*, 1*/2*, 2*/0*, 2*/1* and 2*/2* groupswere selected, with groups as balanced as possible for calpain 1. Half were treated with an aggressive hormonal growth promotant during feedlotting. To maximise this effect animals were slaughtered within the payout period. A small group of Angus cattle with 2* for calpastatin and 2* for calpain3 were included in the design as positive controls.
- At each slaughter, one half from each carcass was conventionally hung, and the other half tender-stretched, to allow us to determine the extent to which genetic variation can be reduced by processing methods.
- Samples for meat quality and other assessments were taken from the striploin, eye round, oyster blade and rump.

4 Results and Discussion

The improvement in *longissimus* shear force in normally (achilles) hung carcasses after 7 days aging was 1.20 kg for the NSW herd and 0.93 kg for the WA herd for the cattle with the favourable calpastatin/calpain 3 alleles compared to those with the unfavourable alleles (0*0* *vs.* 2*2*) (highlighted in Tables 1 to 3). Significant or tendencies towards significant differences between the 0*0* and 2*2* cattle were also evident for the *longissimus* of the tenderstretched sides (Tables 1 to 3). Significant effects of the markers on other *Longissimus* objective meat quality measurements or on *Semitendinosus* (eye round) objective meat quality measurements were not evident.

Table 1. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers at 1 and 7 days ageing for normally (AT) and tenderstretch (TS) hung Brahman cattle (steers and heifers) from the NSW tenderness herd on *Longissimus* shear force (N, newtons).

Cast_Capn3	*n*	AT1day	AT 7days	TS1day	TS7days
0_0	38	80.8	78.5	48.2	47.3
0_2	26	81.9	71.3	47.9	46.7
2_0	45	78.4	73.8	46.7	45.1
2_2	41	79.5	66.5	45.4	44.6
sed		4.49	4.13	1.42	1.33
00-22 (N)		1.4	12.0	2.8	2.7
00-22 (kg)		**0.14**	**1.20**	**0.28**	**0.27**

Table 2. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers at 1 and 7 days ageing for normally (AT) and tenderstretch (TS) hung Brahman cattle (steers) from the WA tenderness herd on *Longissimus* shear force (N, newtons).

Cast_Capn3	*n*	AT1day	AT7days	TS1day	TS7days
0_0	9	51.3	54.7	54.9	48.3
0_1	17	53.7	55.3	58.1	49.0
0_2	15	51.8	53.3	55.4	46.6
1_0	14	51.1	49.2	52.9	47.3
1_1	19	53.4	49.7	55.3	48.1
1_2	16	51.5	47.8	51.8	45.6
2_0	12	50.3	46.8	49.5	44.2
2_1	22	52.6	47.3	53.4	44.9
2_2	16	50.7	45.4	48.8	42.5
sed		2.82	2.34	3.09	2.34
00-22 (N)		0.58	9.34	6.13	5.79
00-22 (kg)		**0.06**	**0.95**	**0.63**	**0.59**

Table 3. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers at 1 and 7 days ageing for normally (AT) and tenderstretch (TS) hung Brahman cattle, combined for steers from the NSW and WA tenderness herds, on *Longissimus* shear force (N, newtons).

Cast_Capn3	*n*	AT1day	AT7days	TS1day	TS7days
0_0	34	60.2	61.3	46.9	45.9
0_2	25	59.7	59.0	47.1	44.7
2_0	33	58.6	54.5	43.0	42.8
2_2	34	58.1	52.1	42.3	41.6
sed		2.53	2.5	2.2	1.71
00-22 (N)		2.2	9.2	4.7	4.2
00-22 (kg)		**0.26**	**0.94**	**0.48**	**0.43**

Tenderness gene marker effects on carcass characteristics and chiller assessment

Significant effects of the calpastatin and calpain 3 markers on carcass characteristics and MSA chiller assessments were not evident (Tables 4 and 5).

Table 4. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers in Brahman cattle (steers and heifers) from the NSW tenderness herd on carcass characteristics

Cast_ Capn3	*n*	HSCW	EMA	uOSS	HotP8	RibFat	Uslean (colour)	Temp at pH6	pHu
0_0	38	246	59.1	151	12.7	6.0	171	22.1	5.49
0_2	26	244	59.0	153	12.4	6.1	171	22.3	5.49
2_0	45	246	60.1	153	12.5	5.9	153	19.6	5.49
2_2	41	243	59.9	151	12.3	5.9	152	19.8	5.48
sed		5.6	1.55	3.7	0.6	0.44	14.3	1.55	0.011

Table 5. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers in Brahman cattle (steers) from the WA tenderness herd on carcass characteristics

Cast_ Capn3	*n*	HSCW	EMA	uOSS	HotP8	RibFat	Uslean (colour)	Temp at pH6	pHu
0_0	11	241	64.5	141	8.1	4.6	216	35.2	5.58
0_1	22	236	61.9	137	7.9	4.3	213	35.8	5.59
0_2	18	238	65.9	141	7.5	4.5	216	35.9	5.58
1_0	18	249	62.4	140	8.5	5.3	200	35.8	5.57
1_1	23	247	64.4	136	8.3	5.0	198	36.4	5.59
1_2	19	246	64.3	140	7.9	5.2	200	36.4	5.57
2_0	14	247	63.0	142	8.7	5.9	202	35.6	5.58
2_1	28	247	63.9	138	8.4	5.6	199	36.2	5.60
2_2	19	242	65.7	142	8.0	5.8	202	36.2	5.58
sed		5.5	1.88	3.6	0.59	0.60	18.3	0.78	0.021

Tenderness gene marker effects on growth, intake and efficiency

Significant effects of the calpastatin and calpain 3 markers on growth, feed intake and efficiency measurements were not evident (Tables 6 to 8).

Table 6. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers in Brahman cattle (steers and heifers) from the NSW tenderness herd on growth characteristics

Cast_Capn3	*n*	Background ADG (g)	Feedlot entry wt	Feedlot ADG (kg)	Feedlot exit wt
0_0	38	737	322	1.22	441
0_2	26	752	318	1.20	437
2_0	45	719	321	1.14	439
2_2	41	734	317	1.13	435
sed		21.2	7.2	0.054	10.2

Table 7. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers in Brahman cattle (steers) from the WA tenderness herd on growth characteristics

Cast_Capn3	*n*	Background ADG (g)	Feedlot entry wt	Feedlot ADG (kg)	Feedlot exit wt
0_0	11	611	343	1.31	449
0_1	22	588	341	1.12	442
0_2	18	575	335	1.32	442
1_0	18	590	357	1.27	464
1_1	23	568	354	1.31	462
1_2	19	555	349	1.26	459
2_0	14	627	350	1.29	459
2_1	28	604	348	1.33	458
2_2	19	591	342	1.29	452
sed		25.2	6.5	0.079	10.5

Table 8. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers in Brahman cattle (steers and heifers) from the NSW tenderness herd on feed intake and efficiency

Cast_Capn3	*n*	DMI	FCR	RFI
0_0	35	8.4	7.0	0.148
0_2	26	8.3	7.2	0.235
2_0	45	8.0	7.6	-0.147
2_2	40	8.0	7.4	-0.060
sed		0.27	0.48	0.176

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5 Success in Achieving Objectives

The Project objectives have been successfully met.

Milestones required to meet the Beef CRCs and MLAs go/no go barrier based on striploin *longissimus* shear force results were met, and further research on the calpain-calpastatin system is now included in the Project 1.1.3 Beef CRC Operational Plan for 2008/9.

6 Impact on Meat and Livestock Industry – now & in five years time

An MLA Donor Company is being established to have samples we collected for MSA eating quality assessment eaten, to allow incorporation of the effects of the gene markers into the MSA model as an integral component of the path to adoption. The extent to which the tenderness gene markers are incorporated into breeding programs and for fine-tuning of processing to improve product quality and efficiencies will largely depend on successful outcomes of the MLA Donor Company.

7 Conclusions and Recommendations

Favourable calpain system tenderness markers have beneficial effects on eating quality of beef from Brahman cattle.

in combination, 2 favourable markers for calpastatin plus 2 favourable markers for calpain 3 improve *longissimus* shear force by approximately 1 kg, compared to 0 copies of each of the favourable markers, in meat from normally hung carcasses aged for 7 days. Significant, although lesser, effects due to the gene markers were also evident for tender-stretched carcasses. Improved tendereness was mostly due to favourable markers for calpastatin.

There were no significant adverse effects of the favourable calpastatin-calpain 3 alleles evident for growth during backgrounding and feedlotting, feed intake, feed efficiency, behaviour, stress responsiveness, or commercial carcass and MSA chiller assessment traits.

The extent to which the tenderness gene markers are incorporated into breeding programs and for fine-tuning of processing to improve product quality and efficiencies will largely depend on successful outcomes of the MLA Donor Company.

It is recommended that further research to elucidate the mechanisms by which the favourable alleles in the calpain system, and other factors, enhance tenderness be undertaken using data and samples obtained within this experiment.

8 Appendix

Project 1.1.3 2007/8 Progress Report to the Beef CRC Scientific and Industry Review, May 2008

Project 1.1.3 - Proof of Concept and Biology Underpinning Gene Markers

Project Number: 1.1.3
Project Name: 1.1.3 Proof of concept and biology underpinning gene markers
Project Leader: Paul Greenwood
Program: 1 High Quality Beef for Global Consumers
Sub-Program: 1.1 Full utilisation of genetic markers for improved beef quality
Start Date: 01/07/2005
End Date: 30/06/2012
% of Project Complete: 63.16%
Description:

Objective/Outcome: Proof of concept to demonstrate the production systems necessary to optimise expression of genes for carcase and beef quality and to quantify the profit delivered by the DNA test suite in specific cattle populations and production systems

Delivery Targets:
Proof of concept and profit delivered to industry by June 2012

Progress

Milestone 1: Proof of concept – Tenderness

Summary

- The milestones required to meet the Beef CRCs and MLAs go/no go barrier based on striploin *longissimus* shear force results have been met, and research to complete the existing MLA Strategic Science contract (BSC.050) on the calpain-calpastatin system is now included in the 2008/9 Operational Plan.
- The improvement in *longissimus* shear force in normally (achilles) hung carcasses after 7 days aging was 1.20 kg for the NSW herd and 0.93 kg for the WA herd for the cattle with the favourable calpastatin/calpain 3 alleles compared to those with the unfavourable alleles (i.e. 0*0* *vs.* 2*2*).
- Calpain-calpastatin system protein abundance and activity and gene expression results are consistent with the phenotypic findings in relation to shear force, and are helping to elucidate mechanisms responsible for the phenotypic effects.
- There were no significant adverse effects of the favourable calpastatin-calpain 3 alleles on growth during backgrounding and feedlotting, feed intake, feed efficiency, behaviour, stress responsiveness, or commercial carcass and MSA chiller assessment traits.
- Negotiations are in progress to have the samples we collected for MSA eating quality assessment eaten, to allow incorporation of the effects of the gene markers into the MSA model as an integral component of the path to adoption.

Tenderness gene marker effects on objective beef quality

The improvement in *longissimus* shear force in normally (achilles) hung carcasses after 7 days aging was 1.20 kg for the NSW herd and 0.93 kg for the WA herd for the cattle with the favourable calpastatin/calpain 3 alleles compared to those with the unfavourable alleles (0*0* *vs.* 2*2*) (highlighted in Tables 1 to 3). Significant or tendencies towards significant differences between the 0*0* and 2*2* cattle were also evident for the *longissimus* of the tenderstretched sides (Tables 1 to 3). Significant effects of the markers on other *Longissimus* objective meat quality measurements or on *Semitendinosus* (eye round) objective meat quality measurements were not evident.

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Cast_Capn3	*n*	AT1day	AT 7days	TS1day	TS7days
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2_0	45	78.4	73.8	46.7	45.1
2_2	41	79.5	66.5	45.4	44.6
sed		4.49	4.13	1.42	1.33
00-22 (N)		1.4	12.0	2.8	2.7
00-22 (kg)		**0.14**	**1.20**	**0.28**	**0.27**

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Cast_Capn3	*n*	AT1day	AT7days	TS1day	TS7days
0_0	9	51.3	54.7	54.9	48.3
0_1	17	53.7	55.3	58.1	49.0
0_2	15	51.8	53.3	55.4	46.6
1_0	14	51.1	49.2	52.9	47.3
1_1	19	53.4	49.7	55.3	48.1
1_2	16	51.5	47.8	51.8	45.6
2_0	12	50.3	46.8	49.5	44.2
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00-22 (N)		0.58	9.34	6.13	5.79
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2_0	33	58.6	54.5	43.0	42.8
2_2	34	58.1	52.1	42.3	41.6
sed		2.53	2.5	2.2	1.71
00-22 (N)		2.2	9.2	4.7	4.2
00-22 (kg)		**0.26**	**0.94**	**0.48**	**0.43**

Tenderness gene marker effects on carcass characteristics and chiller assessment

Significant effects of the calpastatin and calpain 3 markers on carcass characteristics and MSA chiller assessments were not evident (Tables 4 and 5).

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2_0	45	246	60.1	153	12.5	5.9	153	19.6	5.49
2_2	41	243	59.9	151	12.3	5.9	152	19.8	5.48
sed		5.6	1.55	3.7	0.6	0.44	14.3	1.55	0.011

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Tenderness gene marker effects on growth, intake and efficiency

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Table 6. Effect of calpastatin (Cast) and calpain 3 (Capn3) markers in Brahman cattle (steers and heifers) from the NSW tenderness herd on growth characteristics

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2_0	45	8.0	7.6	-0.147
2_2	40	8.0	7.4	-0.060
sed		0.27	0.48	0.176

Influences of gene markers on behaviour and temperament, and of behaviour and temperament on performance

This work is being undertaken as part of Linda Cafe's PhD program.

Brahman weaners (n = 165) with 0 or 2 copies of the favourable alleles of two calpain system tenderness gene markers were sourced from central Qld. They were backgrounded at Glen Innes Research Station, N.S.W. and finished on grain at *Tullimba* feedlot, N.S.W. where feed intake was measured. Slaughter and carcass data were collected at John Dee Abattoir, Warwick, Qld where sides were hung using the achilles and tenderstretch methods, and muscles collected and aged for 1 and 7 days for meat quality analysis. The herd was composed of similar numbers of heifers and steers, and half received an HGP (Revalor-H®) at feedlot entry.

During backgrounding and feedlot growth, flight speed (exit speed from crush, m/s) was measured and crush score (1=very calm to 5=highly agitated) assessed regularly (5 times during backgrounding and 10 times at the feedlot). Cattle were stratified into 3 temperament classes based on the mean and SD of their average background flight speed: Quiet, slower than 1 SD below mean; Flighty, faster than 1 SD above mean; Average, all other cattle. During grain feeding, signs of laminitis were observed in some individuals, and were associated with reduced flight speeds, feed intake and growth. Occurrence and scores of severity of laminitis were combined into a severity index, and 13 cattle removed from the following analyses on the basis of their index.

Data were analysed using linear mixed models. The final model comprised fixed effects of sex, HGP treatment, temperament category and tenderness genotypes; and random effects of origin property, background replicate, feedlot replicate, and slaughter date where appropriate.
The gene markers for tenderness did not significantly affect any of the behavioural, temperament or performance and carcass measurements apart from *Longissimus* shear force (see above)

Table 9. Predicted means for flight speed (m/s) and crush score (1-5) across two growth phases (B = backgrounding; F = feedlot) for Brahman cattle categorised on their average background flight speed, and predicted category means for feedlot ADG (kg/day), feed intake (kg DM/day), carcass weight (kg), carcass rib fat (mm) and achilles hung 1 day aged *M. longissimus* shear force (LDSF, N).

Category	N	B Flight Speed	B Crush Score	F Flight Speed	F Crush Score	Feedlot ADG	Feed intake	Carcass weight	Rib fat	LD SF
Quiet	21	1.02^{a}	1.72^{a}	1.54^{a}	1.32^{a}	1.19^{b}	9.72^{b}	254^{b}	6.56^{b}	74.1
Average	105	1.74^{b}	2.09^{b}	2.01^{b}	1.47^{b}	1.17^{b}	9.34^{ab}	247^{b}	6.37^{b}	75.3
Flighty	25	2.96^{c}	2.68^{c}	3.04^{c}	1.94^{c}	1.01^{a}	8.66^{a}	229^{a}	5.24^{a}	79.3
s.e.d.		0.091	0.120	0.133	0.101	0.062	0.370	6.2	0.500	4.24
P-values		<0.001	<0.001	<0.001	<0.001	0.013	0.029	0.001	0.035	ns

Within columns, means with different superscripts differ significantly

Cattle classed as Flighty on background flight speed also had significantly higher crush scores during backgrounding and in the feedlot, as well as significantly higher flight speeds in the feedlot. Temperament was not related to the tenderness genotypes. Flighty cattle grew more slowly and produced the smallest carcasses with the least fat cover (Table 9).
Flighty cattle ate less than Quiet cattle but did not differ in feed conversion ratio or net feed efficiency. There was no significant difference in *M. longissimus* shear force between categories when sides were achilles hung

and aged for 1 day, though values trended higher with flightier temperament. Both tenderstretch hanging and ageing for 7 days reduced the effect of temperament on *longissimus* shear force. HGP implant did not affect temperament, significantly increased feedlot ADG and carcass weight, improved feed efficiency and increased *longissimus* shear force.
It is planned to use data and samples from this herd, with its divergent and persistent temperament phenotypes, as a basis for detailed analyses of the effects of temperament on meat quality and stress responsiveness, and to study biological associations between temperament, productivity and meat quality.

Tenderness gene marker effects on calpain system protein abundance and activity

Measurements of activity for CAPN1, CAPN2 and CSTN have been completed and reported to Beef CRC and MLA. Results for ELISA quantitation of these proteins is complete for NSW and WA samples for CAPN1 and CSTN, quantitation of CAPN2 using ELISA is complete for NSW but not for WA samples. Gene marker effects on activity of CAPN1, CAPN2 and CSTN has been determined and reported, with gene marker effects on expression of CAPN1 and CSTN also reported.

With respect to the CAST gene marker, we have shown that animals carrying 2 copies of the marker (2*) show less calpastatin protein levels and 15% less calpain inhibitory activity ($p<0.001$; Figure 1). This suggests that the CAST gene marker is associated with reduced calpastatin levels and subsequently, reduced calpain inhibition during the post-mortem period. Significant effects of HGP ($P<0.05$) and sex ($P<0.001$) were observed for calpastatin activity, with HGP increasing activity by 7% and heifers having approximately 20% higher activity compared to steers.

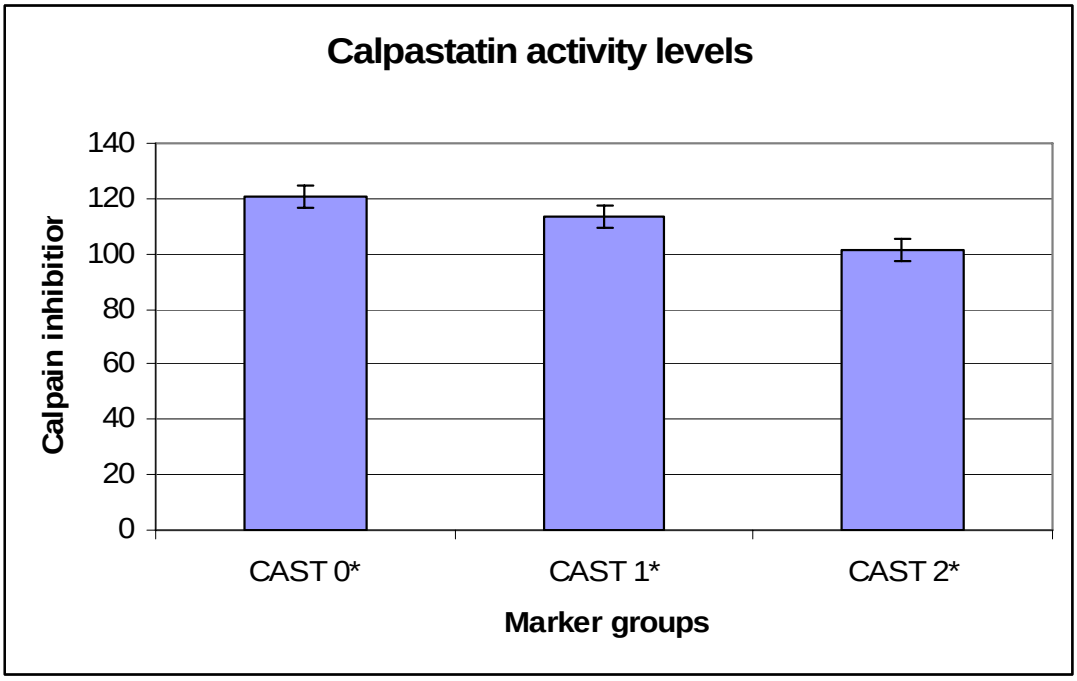

Figure 1. Calpain inhibition by calpastatin within 0*, 1* and 2* groups of WA and NSW tenderness herds.

Gene marker effects for CAPN1 were not significant at the protein amount level or at the activity level for calpastatin, calpain 1 or calpain 2 (P>0.05 for all). Gene marker effects for CAST did not influence protein amount level or activity of calpain 1 or calpain 2 (P>0.05 for all). Peptide sequences for synthesis of epitopes for production of monoclonal antibodies for CAPN3 have been investigated and opportunities for producing antibodies raised against these peptides have been identified. We anticipate that results for CAPN3 will be delivered in August 2008 following development of ELISA or other suitable assay.

Tenderness gene marker effects on calpain system gene expression

This study investigated the relationship between the calpain system tenderness genotypes (0, 1 and 2 copies) and the mRNA levels of the respective calpain system genes (calpain 1, calpain 3 and calpastatin) in bovine skeletal muscle.
Quantitative reverse transcriptase PCR (qRT-PCR) was employed to measure the mRNA levels of the calpain system genes (calpain 1, calpain 3 and calpastatin) and a variety of N-terminal calpastatin splice variants (Table 10). The mRNA levels were measured in LD samples collected at slaughter from the NSW and WA tenderness herds (n = 409). The gene expression data were normalised to the reference gene RPLP0 and statistically analysed with a General Linear Model (GLM, SAS 9.1).

Table 10. qRTPCR assays used in this study to measure mRNA transcript levels of the calpain system genes and calpastatin splice variants.

Assay number	Gene
1	Calpain 1
2	Calpain 3
3	Total calpastatin (C-terminus)
4	Calpastatin variant 1
5	Calpastatin variant 2
6	Calpastatin variant 3
7	Calpastatin variant 4
8	Calpastatin variant 5
9	Calpastatin variant 6
10	Calpastatin variant 7
11	RPLP0 (normalisation reference gene)

Calpastatin

Total calpastatin mRNA was measured with a qRTPCR assay that targeted a region in the C-terminus, which is common to all calpastatin mRNA transcripts. Splice variant specific assays were used to measure calpastatin N-terminal variants. The calpastatin N-terminal variant assays were subsequently expanded to include qRTPCR assays that detected these variants with and without a specific exon.

Total calpastatin mRNA levels showed no relationship with the Cast3_84 DNA marker. However, the mRNA levels of calpastatin variant 2 were significantly associated with Cast3_84 (Figure 2). The association between calpastatin variant 2 mRNA levels and Cast3_84 bears striking similarity to the calpastatin protein levels measured in these LD muscle samples. This finding suggests that the Cast3_84 polymorphism may be associated with the expression level of calpastatin variant 2.

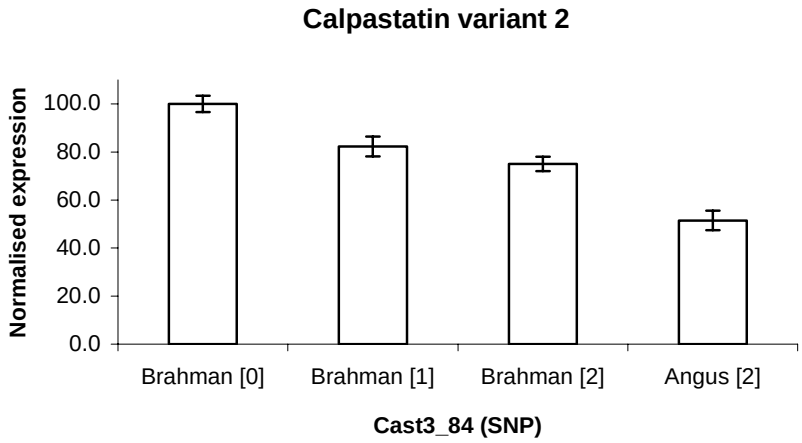

Figure 2: Calpastatin variant 2 mRNA transcript levels in the LD muscle of cattle with 0, 1 and 2 copies of the GeneStar Tenderness DNA marker, Cast3_84 ($p < 0.0001$)**.**

Another calpastatin variant (variant 7), exhibited a similar trend with the Cast3_84 DNA marker as observed for calpastatin variant 2. However, the difference in mRNA levels of variant 7 between 0 and 2 copies of Cast3_84 was significantly less than observed for the variant 2 (Figure 3). Intriguingly, variant 6 exhibited an expression profile that in comparison to the other calpastatin transcripts was unique. Calpastatin variant 6 was expressed at higher levels in Angus compared to Brahman cattle (Figure 4), and heifers expressed higher levels of this particular variant than steers (Figure 5). The latter finding may well account for muscle shear force differences in the NSW herd that were associated with sex.

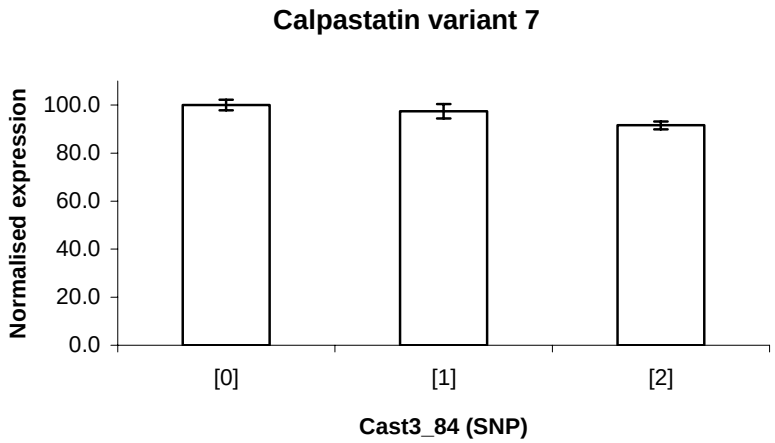

Figure 3: Calpastatin variant 7 mRNA transcript levels in the LD muscle of cattle with 0, 1 and 2 copies of the GeneStar Tenderness DNA marker, Cast3_84 ($p = 0.008$)**.**

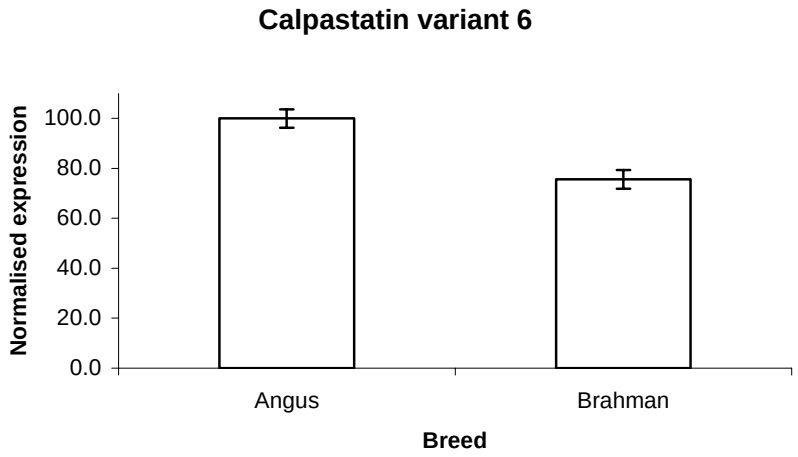

Figure 4: Calpastatin variant 6 mRNA transcript levels in the LD muscle of Angus and Brahman cattle ($p < 0.0001$)**.**

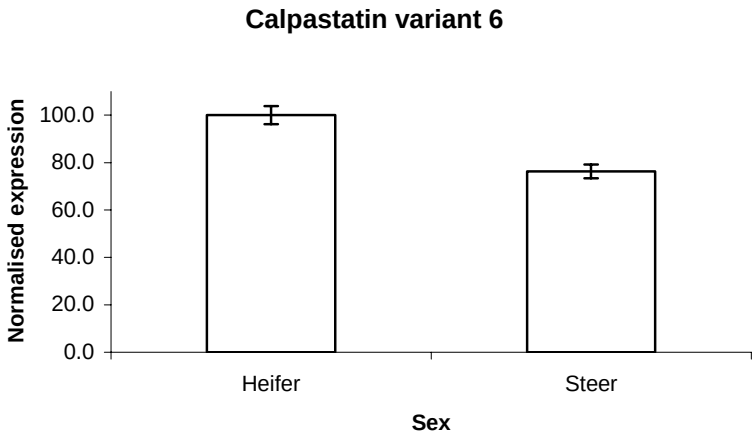

Figure 5: Calpastatin variant 6 mRNA transcript levels in the LD muscle of heifers and steers ($p < 0.0001$)**.**

Calpain 3

The qRTPCR data from this study indicates that calpain 3 is abundantly expressed in bovine skeletal muscle. Calpain3 mRNA levels were approximately 8 times higher than calpain 1 and calpastatin. LD muscle from cattle with 0 copies of the C3JK marker had significantly higher calpain 3 mRNA transcript levels than cattle with 2 copies of the same marker (Figure 6).

In relation to meat science, calpain 3 activity is not inhibited by calpastatin, and calpain 3 knockout mice exhibit normal post-mortem degradation of muscle structural proteins. These findings suggest that the C3JK polymorphism is probably associated with cellular processes in skeletal muscle ante-mortem. Lower calpain 3 mRNA levels in cattle with 2 copies of C3JK compared to 0 copies of the same marker are somehow associated with the tenderness differences attributed to this gene.

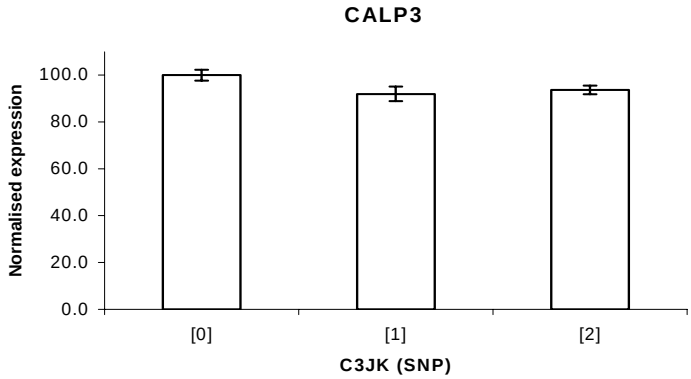

Figure 6: Calpain 3 mRNA transcript levels in the LD muscle of cattle with 0, 1 and 2 copies of the GeneStar tenderness DNA marker, C3JK ($p = 0.06$).

Calpain 1

The mRNA levels of calpain 1 were not assessed against the capn1_316 and capn1_4751 GeneStar tenderness DNA markers. The WA and NSW herds did not contain sufficient cattle with 2 copies of either calpain 1 marker to accurately assess the relationship between these SNPs and calpain 1 mRNA levels.

Candidate gene expression associated with meat tenderness

This work was undertaken as part of Yin San Leong's Honour's project.

A total of 16 candidate genes were selected for gene expression study (qRT-PCR) in LD tissue collected from WA Braham tenderness herd. The gene expression profiles were analysed against the genotypes (tenderness markers for calpain1, calpain3 and calpastatin) and HGP treatment. While the mRNA expression profiles of some of candidate genes were not differentially expressed by the genotypes or due to HGP treatment, calpain1, calpain3 and calpastatin gene expression were related to their genotypes, consistent with the above findings. The gene expression levels were also positively correlated with the tenderness objective measurements and calpain system protein measurements from these animals. In addition, gene expression of muscle fibre type 2A and insulin-like growth factor were significantly differentially expressed between HGP treated and untreated animals.

Alternative splicing and meat quality

This work is being undertaken as part of Nadia De Jager's PhD program.

The alternative splicing and its relationship with meat quality are under the investigation. Several candidate genes were selected. One of the genes, Slow Troponin T (*TNNT1)* which encodes a myofibrillar structural protein which undergoes degradation during post-mortem tenderization, is measured in LM muscle samples of Braham in tenderness herd. The aim of this study is to investigate the effect of sex and the use of a hormone growth promotant (HGP) on the mRNA levels of the two splice variants of *TNNT1.*

Brahman steers (M) and heifers (F), of similar live weight (311+/-31kg), were either administered HGP (M+HGP, n=20; F+HGP, n=21) or not (M-HGP, n=17; F-HGP, n=22) and finished on a similar concentrate diet in a feedlot for 120 days. Muscle samples were collected from the *longissimus dorsi* (LM) at slaughter, and total RNA was used for gene expression by qRT-PCR.

The abundance of the *TNNT1* splice variant was 27 and 23-fold greater than the abundance of the full transcript, in the LM muscle of +HGP and –HGP animals, respectively ($P<0.01$; Figure 7). The ratio of the splice variant to full transcript is greater in M+HGP than F+HGP ($P<0.01$; Figure 1); there was no difference in this ratio when animals were not treated with the HGP. The *TNNT1* splice variant appears to be up-regulated in males compared to females receiving HGP, relative to the full transcript, it may due to the differential response of males and females to growth promotants. The relationship of the *TNNT1* splice variant with objective measurements for tenderness in these cattle will be conducted in the future.

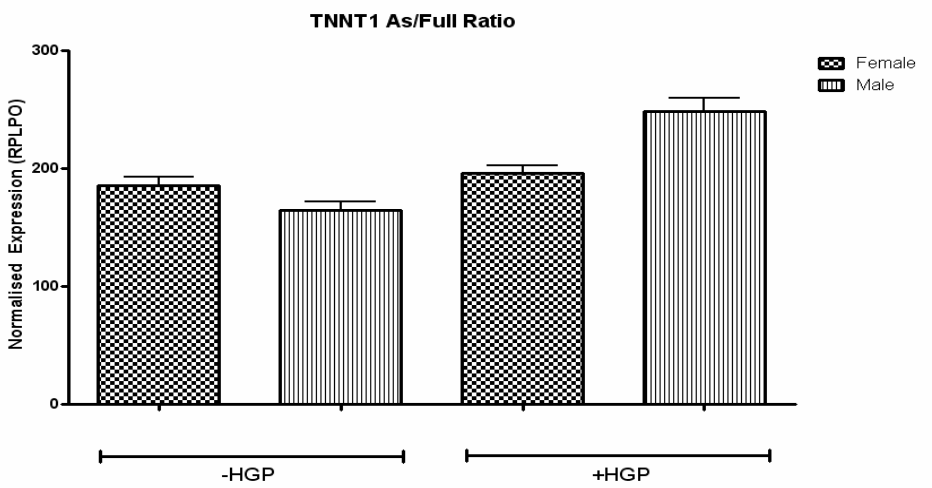

Figure 7. The expression profile of alternative splicing vs full length transcript (AS/Full length) in male and female cattle with and without HGP implant.

Milestone 2: Proof of concept – Marbling

Cattle studies on marbling and fat distribution

Dave Pethick and co-authors published a review of marbling for the ISEP meeting in France on 9-13 September 2007. This review on current understanding of regulation of marbling has highlighted potential for nutritional modification of marbling. However, the review concluded this is only worth pursuing in cattle with the genetic potential to shift fat distribution to the marbling site i.e. high IMF in relation to other depots. As a result, we conducted workshops at which we designed an experiment to study the development of marbling and fat distribution (and, by inference, retail beef yield) in elite and poor marbling genotypes that differ in their fat distributional characteristics. The experiment plans to also study effects of and interactions with industry production systems, namely pasture backgrounding or early-weaning coupled with concentrate feeding to feedlot entry, followed by feedlotting. The experiment has been designed to study in detail the biology of development of marbling and to assess commercial phenotypic outcomes, with a view to identifying potential early predictors or markers of marbling and fat distributional characteristics, to refining beef production systems to enhance marbling and, subsequently the Program aims to validate these outcomes within beef supply chains.

In vitro investigation of intramuscular and subcutaneous fat deposition in Wagyu cattle

This work is being undertaken as part of Bronwyn Bevan's PhD program.

The initial experiment was to establish the culture condition for preadipocyte differentiation. Primary preadipocytes were isolated from SCF tissue obtained from Wagyu cattle at slaughter and cultured in media comprised of DMEM/Ham's F12 media with 10% fetal bovine serum (FBS) + 2% antibiotics. The SCF preadipocytes were then treated 111µg/mL 3-Isobutyl-1-methylxanthine (IBMX, a c-AMP agonist) and one of a combination of treatments (Table 11). RNA was isolated from the treated cells and mRNA expression of essential lipogenic genes, including peroxisome proliferator activated receptor γ (PPARγ), CCAAT/enhancer binding protein α (C/EBPα), and glyceraldehyde-3-phosphate dehydrogenase (G-3-PDH), measured by quantitative RT-PCR.

Table 11. Conditions optimised for the differentiation of bovine SCF preadipocytes *in vitro*

Treatment	Concentration (µg/mL)		
Insulin	100	10	1
Dexamethasone	1	0.1	0.01
Biotin	10	1	0.1
Ciglitizone	10	1	0

The optimal concentration of insulin, biotin and dexamethasone was 1µg/ mL of each. The optimal concentration of ciglitizone was found to be 10µg/ mL. When combined with insulin and/ or biotin, dexamethasone did not appear to have an effect on adipogenesis. When combined with only insulin, dexamethasone, biotin and ciglitizone, the lipid mix combination of lecithin, cholesterol and sphingomyelin resulted in the accumulation of extracellular lipid, with a small increase in differentiation of preadipocytes. It was therefore concluded that while the optimal concentration of each additive has been determined, further study is required to determine the optimal combination of additives.

Milestone 3: Proof of concept – Retail beef yield

Summary

- Eighteen polymorphisms in the myostatin gene and nine myostatin haplotypes have been identified and association of haplotypes with indices of muscling and fatness determined in over 1,000 animals in various cattle populations.
- Significant phenotypic differences between haplotypes in muscling and fatness have been identified.
- Low muscle, high muscle, and high muscle plus myostatin steers from the NSW DPI selection line herd have been examined in detail to understand their physiological response to anabolic and catabolic hormones, and aspects of gene expression relating to muscle growth and development.
- We have also examined the commercial and financial feasibility of managing a loss of function myostatin mutation on farm to increase lean meat yield and so profit – at least in supply chains where this can be measured and rewarded.

Feasibility of using cattle heterozygous for major functional mutations in myostatin

The objective of this work is take the findings of the experimental results for effects of the heterozygote condition for non-functional myostatin and test these in a commercial setting as potential commercial application. This has become feasible given the ability to commercially genotype individual animals for the myostatin trait and to use this in managing a commercial beef operation

The Beef-N-Omics program has been selected for this purpose and is the model of choice for enterprise level modeling and economic evaluation of beef technologies relevant to the Southern Australian beef production systems within the Beef CRC program.

Four scenarios were tested in this analysis. Firstly the use of a self-replacing management system two herds would be run containing either normal cows and heterozygous bulls or heterozygous cows and normal bulls. In this case half the steers and heifers progeny sold would carry the myostatin deletion mutation in heterozygous form. Further half the cows and half the bulls would also be heterozygous for the myostatin mutation deletion and consequently attract the muscle score premium. In this management system all female calves would need to be genotyped, incurring a $50/hd cost. The second scenario incorporates the higher premium of 30 c/kg for heterozygous stock within the previously described self-replacing herd. The third scenario models a terminal system where a homozygous bull for the myostatin deletion mutation is purchased and bred to normal cows (homozygous normal). All progeny would be sold and replacement females purchased as 1 year olds. A 15 c/kg premium for all progeny from this terminal system is included. In the case of scenario 4, a 30 c/kg lw premium is attached to all progeny from the terminal system is applied.

The results show that each scenario has financial advantages above those of the Base herd with which comparisons were made (Table 12). These estimates are deemed conservative as the cost of testing is likely to decline with improved technologies and reduced testing costs would improve the financial attractiveness of the self-replacing scenarios. For example, a 20 per cent reduction in genetic testing cost for screening of the myostatin deletion mutation results in an additional improvement of approximately 1 per cent in the beef enterprise gross margin for the self-replacing production system.

Table 12. Comparison of management systems designed to produce offspring heterozygous for non-functional myostatin (see scenarios described above)

Scenario	Cow herd (hd)		$GM/cow	$GM/ha	Change in GM over Base (%)	Premium assumed (c/kg lw.)
Base herd	200	107,523	538	269	-	0
Self replacing – rotation 1	200	109,606	548	274	2	15
Self replacing – rotation 2	200	114,224	571	286	6	30
Terminal 1	200	114,364	572	286	6	15
Terminal 2	200	126,990	634	317	18	30

Phenotype of high and low muscling cattle and of cattle heterozygous for major functional mutation in myostatin

Three studies have now been undertaken in which the carcass and yield characteristics of the the cattle heterozygous for a non-functional myostatin mutation have been compared with herd mates in a commercial herd, and with high and low muscling selection line cattle from NSW DPI's Glen Innes herd.

These have consistently shown yield advantages in the heterozygotes or the high line cattle, without adverse effects on objective measures of beef quality apart from IMF% and marbling (Tables 13 to 16).

Table 13. Percent bone, fat and muscle from carcasses of Angus steers heterozygous for a non-functional myostatin compared with herd mates including high muscling and low muscling selection line Angus steers.

	Low Muscling	High Muscling	Myostatin hetero.
	Study 1		
Retail yield (%)	61.8a	63.0a	66.5b
Fat trim (%)	18.3b	17.1b	14.5a
Bone (%)	18.7b	19.0b	17.9a
	Study 2		
Retail yield (%)	62.4a	65.1b	67.6c
Fat trim (%)	18.2b	15.7a	14.4a
Bone (%)	18.5b	18.2b	17.0a
	Normal		Myostatin hetero.
	Study 3		
Retail yield (%)	68.5		71.8b
Fat trim (%)	10.3b		8.4a
Bone (%)	21.1b		19.8a

Values within rows followed different letters differ (P<0.05)

Table 14. Carcass characteristics of Angus steers heterozygous for a non-functional myostatin compared with herd mates including high muscling and low muscling selection line Angus steers.

	Low Muscling	High Muscling	Myostatin hetero.
	Study 1		
Liveweight (kg)	666	659	638
Carcass weight (kg)	359	360	357
Dressing %	53.9a	54.9b	56.0c
Ossification score	200	189	189
P8 fat	24.3	21.2	24.1
Rib fat (mm)	18.7	16.2	16.4
Eye muscle area (cm^2)	70.4a	76.9b	85.0c
AUS marble score (0-6)	1.32	1.13	0.92
MSA marble score (100-1100)	329	324	288
	Normal		Myostatin hetero.
	Study 3		
Liveweight (kg)	490		488
Carcass weight (kg)	281		291
Dressing %	57.2a		59.6b
Ossification score	127.5		124.2
P8 fat	11.4		11.0
Rib fat (mm)	6.5b		4.9a
Eye muscle area (cm^2)	59.3		61.6
AUS marble score (0-6)	0.67b		0.08a
MSA marble score (100-1100)	291b		223a

Values within rows followed different letters differ (P<0.05)

Table 15. Objective beef quality characteristics of *Longissimus* in Angus steers heterozygous for a non-functional myostatin compared with herd mates including high muscling and low muscling selection line Angus steers.

	Low Muscling	High Muscling	Myostatin hetero.
	Study 1		
Shear force (kg)	4.70b	5.11b	3.98a
Compression (kg)	1.64	1.75	1.61
Cooking loss (%)	20.5	20.3	20.0
Colour L	40.3	41.4	41.8
Colour a	25.0	23.9	25.0
Colour b	12.6	12.1	12.3
pHu	5.54	5.52	5.54
Intramuscular fat (%)	3.97b	4.11b	2.93a
	Study 3		
	Normal		Myostatin hetero.
Shear force (kg)	4.13		4.10
Compression (kg)	1.90		1.92
Cooking loss (%)	23.5		23.3
Colour L	38.7		39.0
Colour a	23.6		23.7
Colour b	11.8		12.1
pHu	5.51		5.51
Intramuscular fat (%)	2.69		1.81

Values within rows followed different letters differ (P<0.05)

Table 16. Objective beef quality characteristics of *Semitendinosus* in Angus steers heterozygous for a non-functional myostatin compared with herd mates including high muscling and low muscling selection line Angus steers.

	Low Muscling	High Muscling	Myostatin hetero.
	Study 1		
Shear force (kg)	4.70b	5.11b	3.98a
Compression (kg)	1.64	1.75	1.61
Cooking loss (%)	20.5	20.3	20.0
	Normal		Myostatin hetero.
	Study 3		
Shear force (kg)	5.06b		4.42a
Compression (kg)	2.51		2.34
Cooking loss (%)	23.5		23.3

Values within rows followed different letters differ (P<0.05)

Myostatin haplotypes and their association with muscling and fatness

The research is based on Brendon O'Rourke's PhD program.

The research has identified 18 DNA variants in the *myostatin* gene of Angus cattle. A sub-population of Angus and Belgian Blue cattle have since been genotyped at these 18 sites. The information has enabled the paternal and maternal contributions at these sites (haplotypes) to be deduced, and 9 different combinations identified. Further analyses of the 9 haplotypes have revealed a series of variants that are linked together, and are therefore predictive of each other.
This means that a minimum of 6 variants can be used to extract all the genotypic information from the 9 haplotypes, providing a very efficient strategy for studying larger populations.

More than 1000 cattle have now been genotyped at the 6 selected sites. Haplotypes for each animal have been inferred, and statistical analyses performed to determine if any haplotypes are associated with variation in muscularity. The variants were assessed as haplotypes to maximise the likelihood of identifying a statistical association. Multiple linear regression has revealed many haplotypes were significantly different from each other with respect to eye muscle area, a key indicator of muscularity in cattle (Table 17) and fatness measures. Further analyses on each of the individual variants found one site to be significantly associated with a substantial decrease in eye muscle area and the loss-of-function mutation was associated with a large increase in eye muscle area (Table 18).

Table 17. Haplotype association with eye muscle area at weaning and yearling age in the Research herd. Haplotypes are ranked by regression coefficient (B)

Eye muscle area							
Weaning				**Yearling**			
Haplotype	***N***	**B**	**Pr > F**	**Haplotype**	***N***	**B**	**Pr > F**
10	35	-5.57 ± 1.23	**<.0001**	10	25	-5.94 ± 1.36	**<.0001**
2	57	-5.17 ± 1.42	**0.0003**	6	16	-4.72 ± 1.47	**0.0014**
1	162	-4.11 ± 0.93	**<.0001**	4	94	-4.15 ± 1.01	**<.0001**
4	136	-3.32 ± 0.95	**0.0005**	1	107	-4.03 ± 1.00	**<.0001**
7	555	-3.22 ± 0.82	**0.0001**	2	50	-3.80 ± 1.47	**0.0099**
3	75	-2.06 ± 1.03	**0.0449**	7	350	-3.79 ± 0.89	**<.0001**
11	2	-3.97 ± 3.64	0.2766	11	2	-3.85 ± 3.51	0.2735
9	1	-2.48 ± 5.09	0.6261	5	30	-2.24 ± 1.26	0.0777
6	32	-2.25 ± 1.20	0.0605	3	36	-2.05 ± 1.23	0.0963
5	68	-1.43 ± 1.08	0.1865	9	1	-1.38 ± 4.88	0.7779
12	65	0	-	12	47	0	-
8	0	-	-	8	0	-	-
Total	1188	-	-	Total	758	-	-

Table 18. Tag polymorphism association with eye muscle area at weaning and yearling age

	Eye muscle area			
	weaning		yearling	
Polymorphism	B	Pr > F	B	Pr > F
1	0.12 ± 0.48	0.8024	-0.54 ± 0.56	0.3347
2	-3.11 ± 1.06	**0.0035**	-2.56 ± 1.19	**0.0323**
3	-0.62 ± 1.14	0.5863	-2.27 ± 1.45	0.1222
4	6.05 ± 1.52	**<.0001**	4.43 ± 1.81	**0.0146**
5	-	-	-	-
6	-0.09 ± 1.10	0.9319	-1.65 ± 1.42	0.2448
7	0.66 ± 0.95	0.4899	-1.50 ± 1.27	0.3661

The focus is now to validate these findings in other cattle populations (CRC1 database) and to explore the underlying biological effect of these variants through gene expression studies to support the statistical findings. This study promises to unlock some secrets behind the biology of muscling that can be utilised by industry, allowing producers to exert informed selection pressure on *myostatin* variants to improve meat yield.

Evaluation of the impact of selection for muscling on the intermediary metabolism of Angus steers

The aim of this experiment within Peter McGilchrist's PhD program was to evaluate the impact of selecting cattle for muscling on intermediary metabolism.

Specifically, the metabolic responses of lactate (an indicator for muscle), non-esterified fatty acids (NEFA - an indicator for adipose tissue) and glucose (an indicator for the liver) to adrenalin challenges, and whole body responsiveness of these cattle to insulin was evaluated. Twenty four Angus steers were included in this experiment, 10 low muscled genotype, 11 high muscled genotype and 3 that were heterozygote for a myostatin polymorphism. At 18 months of age the steers were fitted with dual indwelling jugular catheters and subjected to 7 adrenaline challenges ranging from 0.2 to 3 ug/kg live weight, and hyperinsuliaemic/euglycaemic (HIEG) clamps, with insulin infusion rates of 0.6 and 6.0 mU/kg/min. For adrenalin challenges, blood sampling was carried out for 30 min prior to challenge, and then for 2 hours post challenge, such that changes in plasma concentrations of lactate, NEFA, and glucose could be determined. In the case of the HIEG clamps, glucose infusion rate required to maintain euglycaemia at both the 0.6 and 6.0 mU/kg/min insulin infusion rates was measured.

The results showed that the high muscled genotype had decreased ($P<0.05$) peak plasma lactate concentrations at high levels of adrenalin challenge, indicating reduced muscle responsiveness. The high muscled genotype also showed increased ($P<0.05$) peak plasma NEFA concentrations, signifying increased adipose tissue sensitivity across the entire range of adrenalin challenges. The response in plasma glucose concentrations of the high muscled genotype were higher ($P<0.05$) at the moderate ranges of adrenaline challenges (ie 1.0-2.2 ug/kg live weight). The high muscled genotype also exhibited a greater ($P<0.05$) sensitivity to insulin at both the high and low insulin infusion rates signified by higher rates of glucose infusion during the HIEG clamp.

The myostatin genotype generally showed hormone responses that were similar to the high muscled genotype, but never more extreme, thus suggesting that the hormonal differences associated with selection for muscling are unlikely to be specifically associated with this particular variant of the myostatin mutation. At slaughter, the high muscled and myostatin genotypes yielded more lean meat per kilogram of carcase weight ($P<0.05$), and less fat ($P<0.05$) (see *Study 2* in Table 13 above).

Thus, selection for muscling causes increased adipose tissue sensitivity and reduced muscle sensitivity to stress, as well as increased whole body insulin sensitivity. This is likely to result in greater muscle accretion and glycogen storage due to increased cellular uptake of glucose, and less fat deposition due to greater rates of fat turnover associated with increased adipose tissue sensitivity to adrenalin.

Gene expression in muscles of low and high muscling and heterozygote myostatin mutant Angus

This study was undertaken by Grant Parnell for his Honour's thesis. The study was an investigation of the allele specific expression of myostatin in a population of animals selected for high or low muscling, including animals heterozygous for the *nt821(del11)* loss of function myostatin polymorphism. In addition, the expression of other genes affecting muscle regulation, namely follistatin, myogenin, and *MYOD*, was studied.

Animals heterozygous for the *nt(821)del11* loss of function polymorphism expressed higher amounts of total myostatin but lower amounts of the functional (wild-type) allele compared to homozygous wild-type animals. This indicated an up-regulation of total myostatin (normal pus non-functional) expression in the heterozygotes. Myogenin expression was higher in *M. longissimus dorsi* than *M. semitendinosus*, but there was no difference in its expression between the 3 muscling lines. The level of *MYOD* expression was greater

in the wild-type high muscling line compared to the wild-type low muscling line. This indicates that selection for divergence in muscling score has influenced expression levels of genes such as *MYOD* in addition to myostatin. Follistatin expression was lower in *M. semitendinosus* of high muscling *nt821(del11)* heterozygote line compared to the high muscling wild-type line.

This study has contributed to the understanding of the genetic basis of muscle regulation. Investigation of expression levels of a broader range of candidate genes known to influence body composition, particularly on homozygous wild-type animals, may reveal additional polymorphisms of large affect for future use in marker assisted selection to improve retail beef yield.

Other

Simon Quigley was awarded an Australian Academy of Sciences scholarship and visited the laboratory of Jean-Francois Hocquette to study cell localisation methods in muscle.

Milestone 4: Proof of concept – Fat distribution

- Factors influencing fat distribution were reviewed in the by Dave Pethick and co-authors for the ISEP meeting in France on 9-13 September 2007.
- Milestone 4 was deleted from the Operational Plan and integrated into proposed studies on fat distribution within Milestone 2 (Proof of concept – marbling) and Milestone 3 (Proof of concept – Retail beef yield)

Milestone 5: Diagnostic mini-array for traits of interest

- A Strategy Committee has continued working on determining appropriate array platforms and elements to be represented on arrays. They work with the Underpinning Science Committee to ensure the appropriate platforms and experimental designs are used in expression studies

Milestone 6: General

Planning and progress workshops were held in December 2007 and and February 2008. Revised experimental and commercialisation strategies have been developed for future tenderness, marbling and fat distribution and retail yield studies, which are included in the 2008/9 Operational Plan.

Milestone 7: BSC 050 Milestone 1

- This Milestone was achieved on time in 2005/6.

Milestone 8: BSC 050 Milestone 2

- This Milestone was achieved on time in 2006/7.

Milestone 9: BSC 050 Milestone 3

- This Milestone go/no go barrier was successfully achieved and MLA are being invoiced for the outstanding $140,000 balance of this contract during 2007/8, and the work related to this funding to complete the contractual commitments will be undertaken in 2008/9.

Other Beef CRC commitments

Grafton early-life nutrition x sire-genotype studies

An economic analysis of the Grafton early-life nutrition experiment from Beef CRC2 has been completed was published as a NSW DPI Economic Research Report with Dr Andrew Alford as senior author. This work is being prepared as a scientific paper for an upcoming Beef CRC Special Edition of the *Australian Journal of Experimental Agriculture*.

A paper on effects of early-life growth and sire-genotype on growth, intake and efficiency in the feedlot has also been submitted for the upcoming Beef CRC Special Edition of the *Australian Journal of Experimental Agriculture*, and a paper on early-life growth and sire-genotype effects on primal cut yields has also been drafted for this volume.

Inputs continue to provided for Beef CRC workshops, Feeder Steer School, SABRC and other extension publications and articles on this research.

A number of other scientific and industry papers have also been published, submitted and drafted from this research project (see Publications and Presentations).

SABRC

Paul Greenwood has continued to act as the Beef CRCs representative on SABRC and has provided updates on Beef CRC RD&E at two meetings in 2007/8 (see Presentations).

Project Milestones and Tasks

Green = Not due yet, Purple < 14 days, Orange < 7 days, Red = Overdue, White = Complete, RedWhite = Revised Due Date

Type	WBS	Name	Responsible	Date Due-Revised Date	% of Project	% Complete	Status
Milestone	1	Proof of Concept - Tenderness	Greenwood, Paul	30/06/2012	20	83	Green
Task	1.02	Phenotypic data available for validation of effects of polymorphisms on tenderness	Greenwood, Paul	31/12/2007	4	100	White
Comments/Results: Please note: This task was included in the Year 3 Operational Plan and was revised and updated to reflect the Operational Plan which includes that B. McIntyre was also responsible for this task.							
Task	1.03	Gene expression data available that maps the genes, proteins and metabolites directly relating to the calpain system and its control of tenderness	Greenwood, Paul	30/06/2008	4	0	Redwhite
Task	1.23	Completion of laboratory analyses	Greenwood, Paul	30/06/2008	5	0	Redwhite
Comments/Results: Please note: This task was included in the Year 3 Operational Plan and was revised and updated on 03.07.2007 to reflect the operational plan. Persons responsible for this project also include: Y. Wang, G. Nattrass, M. McDonagh and D. Pethick.							
Milestone	2	Proof of Concept - Marbling	Greenwood, Paul	30/06/2012	20	23	Green
Task	2.02	Establishment of SNP chip to profile cattle for marbling polymorphisms, in conjunction with Project 1.1.1	Greenwood, Paul	30/06/2008	7	0	Redwhite
Comments/Results: Please note: This task was revised and updated to reflect its inclusion in the Year 3 Operational Plan. Persons responsible also included: B. Barendse Milestone to be covered within 2008/9 operational plan for marbling							
Task	2.03	Validation to assess value and transportability of SNP chip in Australia and Hanwoo cattle, in conjunction with Project 1.1.1	Greenwood, Paul	30/03/2008	7	0	Redwhite
Comments/Results: Please note: This task was revised and updated on 03.07.07 to reflect its inclusion in the Year 3 Operational Plan. Persons responsible also include: J. Thompson, B. McIntyre and B. Barendse							

Task	2.04	Pending the outcome of the above study: genotyping of ~2,000 cattle for SNP's associated with marbling, in conjunction with Projects 1.1.1 and 1.2.2	Greenwood, Paul	30/06/2008	7	0	Redwhite
Comments/Results: Please note: This task was revised and updated to reflect its inclusion in the Year 3 Operational Plan. Persons responsible also include: B. Barendse and B. McIntyre.							
Task	2.05	Establish a herd comprising cattle with most and least favourable SNP profiles	Greenwood, Paul	30/06/2008	7	0	Redwhite
Comments/Results: Please note: This task was not revised and updated to reflect its inclusion in the Year 3 Operational Plan. Persons responsible also include: B. Barendse.							
Task	2.06	Concurrent with the above Australian study, sampling and establishment of a herd of Hanwoo cattle with most and least favourable marbling and SNP profiles	Greenwood, Paul	30/06/2008	7	0	Redwhite
Comments/Results: Please note: This task was revised and updated to reflect its inclusion in the Year 3 Operational Plan. Persons responsible for this task include; B. Barendse.							
Task	2.11	Identification, in conjunction with Strategy 1.1.1, of polymorphisms influencing marbling to be investigated	Greenwood, Paul	30/06/2008	7	0	Redwhite
Comments/Results: Please note: This task was not included in the Year 3 Operational Plan Milestone to be completed within 2008/9 operational plan for marbling experiment							
Task	2.12	Development (incl. refereeing) of specific projects	Greenwood, Paul	30/06/2008	7	0	Redwhite
Comments/Results: Please note: This task was not included in the Year 3 Operational Plan Milestone to be completed with acceptance of 2008/9 operational plan for marbling							
Task	2.15	Idenification of polymorphisms influencing marbling, in conjunction with Project 1.1.1	Greenwood, Paul	30/09/2007	8	100	White
Comments/Results: Please note: This task was included in the Year 3 Operational Plan and was revised and updated on 03.07.07 to reflect the operational plan.							

Persons responsible also include: B. Barendese and T. Reverter. SNPs have been identified by Bill Barendse's laboratory and a 48 SNP panel synthesised. Initial testing to validate the 48 SNP panel has begun using 1,000 CRC 1 animals.							
Milestone	3	Proof of Concept - Retail Yield	Greenwood, Paul	30/06/2012	19	54	Green
Task	3.09	Complete laboratory analyses of muscling selection line samples	Greenwood, Paul	31/12/2007	4	100	White
Comments/Results: Completion date extended to allow additional molecular analyses by NSW DPI (G. Parnell, Honours project) and SARDI. All other analyses have been completed as per MLA contract G. Parnell honours thesis completed and awarded high distiction.							
Task	3.11	Identification, in conjunction with Strategy 1.1.1, of polymorphisms influencing retail beef yield to be investigated	Greenwood, Paul	31/12/2008	5	0	Redwhite
Comments/Results: Please note: This task was not included in the Year 3 Operational Plan WFS for RBY not being undertaken until during 2008							
Task	3.12	Manuscript(s) for refereed scientific journal on muscling selection line study – NSW DPI/Strategy team	Greenwood, Paul	31/12/2007	5	100	White
Comments/Results: Please note: This task was not included in the Year 3 Operational Plan Draft manuscript prepared							
Task	3.13	Muscle hypertrophy-related methodology development including establishment of Simon Quigley Post-Doc (UQ) program - SARDI/UQ/NSW DPI	Greenwood, Paul	30/06/2008	5	0	Redwhite
Comments/Results: Please note: This task was not included in the Year 3 Operational Plan							
Task	3.14	Development (incl. refereeing) of specific projects	Greenwood, Paul	30/06/2008	5	0	Redwhite
Comments/Results: Please note: This task was not included in the Year 3 Operational Plan							
Task	3.15	Phenotypic and gene expression data for retail yield preliminary study	Greenwood, Paul	31/12/2007	5	100	White
Comments/Results: Deadline extended to allow for additional gene expression analyses within Grant Parnell's Honours project Please note: This task was not included in the Year 3 Operational Plan							

Task	3.18	Myostatin commercialisation plan	Greenwood, Paul	30/12/2007	5	100	White
Comments/Results: Deadline extended to allow implementation of recommendations of November 2006 myostatin workshop Draft finalised and being prepared for printing							
Task	3.19	Gene expression data for MLA study on muscling selection line/myostatin Angus herd available	Greenwood, Paul	31/12/2007	5	100	White
Comments/Results: Please note: this project has been created to reflects its inclusion in the Year 3 Operational Plan. Persons responsible also include: Y. Chen							
Task	3.20	Identification, in conjunction with Project 1.1.1, or further polymorphisms influencing RBY	Greenwood, Paul	30/03/2008	5	0	Green
Comments/Results: Please note: this project has been created to reflects its inclusion in the Year 3 Operational Plan. Persons responsible also include: B. Barendse							
Task	3.21	Phenotypic data on effects of myostatin mutant heterozygotes on production characteristics in Angus cattle obtained	Greenwood, Paul	30/06/2008	5	0	Green
Comments/Results: Please note: this project has been created to reflects its inclusion in the Year 3 Operational Plan. Persons responsible also include: W. McKiernan							
Task	3.22	Metabolic and molecular studies undertaken on muscling selection line and myostatin heterozygote cattle	Greenwood, Paul	30/06/2008	5	0	Green
Comments/Results: Please note: this project has been created to reflects its inclusion in the Year 3 Operational Plan. Persons responsible also include: P. Greenwood.							
Milestone	5	Diagnostic mini-array for traits of interest	Greenwood, Paul	30/06/2012	20	100	White
Task	5.01	Confirm the array platforms for the tenderness study	Greenwood, Paul	30/09/2007	100	100	White
Comments/Results: Please note: this task was revised and updated to reflect its inclusion in the Year 3 Operational Plan. Persons responsible also include: Y. Wang and G. Nattrass. This Milestone is now the domain of the Underpinning Science Committee, within which Project 1.1.3 provides technical							

and scientific input. CRC 3 underpinning science committee has made the decision to purchase the USDA bovine long oligo set and have the slides printed by a local service provider (AGRF Melbourne). Two test printing runs have been conducted so far. The hydridizations of the 1st testing printing slides showed poor DNA deposition and poor spot morphology. The hybridization from the 2nd testing printing (done by Yizhou Chen) showed much improved printing . In the meantime, a number of USDA bovine long oligo slides (printed at USA) were purchased and tested by hybridization. In our understanding that the statistical analysis of both hybridization results will be undertaken by Toni Reverter and Eva Chan. If the results are promising, the long oligo array (printing by AGRF Melbourne) will be the choice for gene expression studies.							
Milestone	6	General	Greenwood, Paul	30/06/2012	10	45	Green
Task	6.01	Plannning and progress workshops	Greenwood, Paul	30/12/2007	11	100	White
Comments/Results: Workshop held in Brisbane in Dec 2007.							
Task	6.02	Planning and progress workshops	Greenwood, Paul	30/03/2008	11	0	Redwhite
Comments/Results: Please note: this task was revised and updated on 03.07.07 to reflect its inclusion in the Year 3 Operational Plan. This included changing previous completion sated and due dates.							
Milestone	8	BSC.050 - Milestone 2	Greenwood, Paul	15/07/2007	4	100	White
Comments/Results: Please note: this task was updated on 03.07.07 to reflect its inclusion in the Year 3 Operational Plan.							
Milestone	9	BSC.050 - Milestone 3	Greenwood, Paul	01/06/2008	4	5	Green
Comments/Results: Please note: this task was updated to reflect its inclusion in the Year 3 Operational Plan							
Task	9.01	BSC.050 - Milestone 3, Laboratory data collection complete - eating quality, calpain-calpastatin assay, calpain-calpastatin gene expression, proteomic analysis, microarray analysis of gene expression	Greenwood, Paul	01/06/2008	95	0	Green
Task	9.02	BSC.050 - Issue invoice to MLA $250,000 - dependent on Milestone 3	Webeck, Susan	01/06/2008	5	0	Green

Team Members

Member	Organisation
Andrew Alford	NSW Department of Primary Industries
Angus Tester	Victoria Department of Primary Industries
Barbara Waldoch	Murdoch University
Barry Grob	NSW Department of Primary Industries
Bill Barendse	CSIRO Livestock Industries
Bill Johns	NSW Department of Primary Industries

Bill McKiernan	NSW Department of Primary Industries
Brendan O'Rourke	NSW Department of Primary Industries
Brian Dalrymple	CSIRO Livestock Industries
Brian McIntyre	WA Department of Agriculture
Bronwyn Bevan	CSIRO Livestock Industries
Carmen Elvins	NSW Department of Primary Industries
Catherine Stockman	WA Department of Agriculture
Dave Pethick	Murdoch University
David Miller	Murdoch University
Dete Hasse	Victoria Department of Primary Industries
Diana Perry	NSW Department of Primary Industries
Emma Giumelli	WA Department of Agriculture
Erin Rutty	Victoria Department of Primary Industries
Graham Gardner	Murdoch University
Greg Nattrass	South Austalian Research Development Institute
Helen McLennan	NSW Department of Primary Industries
Ian Higgins	NSW Department of Primary Industries
Inho Hwang	National Livestock Research Institute, Korea
Jean Francois Hocquette	INRA France
Jeisane Accioly	WA Department of Agriculture
Joe Brunner	NSW Department of Primary Industries
John Thompson	University of New England
Kevin Williams	South Austalian Research Development Institute
Lakshmi Krishnan	Victoria Department of Primary Industries
Linda Cafe	NSW Department of Primary Industries
Matthew McDonagh	Victoria Department of Primary Industries
Moira Menzies	CSIRO Livestock Industries
Nadia De Jager	CSIRO Livestock Industries
Neil Bower	CSIRO Livestock Industries
Paul Greenwood	NSW Department of Primary Industries
Peter Kamphorst	NSW Department of Primary Industries
Peter Newman	NSW Department of Primary Industries
Phil Dawes	NSW Department of Primary Industries
Philip Bowman	Victoria Department of Primary Industries
Ross Barnard	University of Queensland
Shannon Dellar	WA Department of Agriculture
Sheridan Moll	University of New England
Sigrid Lehnert	CSIRO Livestock Industries
Simon Bawden	South Austalian Research Development Institute
Simon Quigley	University of Queensland
Snezana Zivcovic	Victoria Department of Primary Industries
Stuart McClelland	NSW Department of Primary Industries
Susan Webeck	CRC for Beef Genetic Technologies
Toni Reverter-Gomez	CSIRO Livestock Industries
Xuemei Han	University of New England
Yin San Leong	CSIRO Livestock Industries
Yizhou Chen	NSW Department of Primary Industries
YongHong Wang	CSIRO Livestock Industries

Publications and Presentations

Refereed papers

Wang YH, Bower NI, Reverter A, Tan SH, De Jager N, Wang R, McWilliam SM, Cafe L, Greenwood PL and Lehnert SA (2008) Intramuscular fat development in cattle: the early expression of adipogenic and lipogenic-related genes. J Anim Sci (*submitted*)

O'Rourke, B.A., Healy, P.J., McKiernan, W.A., Greenwood, P.L., Cafe, L.M., Perry, D., Walker, K.H., Arthur, P.F. Marsh, I., Parnell, P.F. and Dennis, J.A. Selection for increased muscularity amplifies allelic frequency of a disruptive mutation in the bovine *myostatin* gene Aust J Exp Agric (*drafted*)

O'Rourke, B.A., Hayes, B.J., Greenwood, P.L., Arthur, P.F. and Goddard, M.E. Haplotype and linkage disequilibrium analysis of genetic variation at the myostatin locus in Angus and Belgian Blue cattle. Anim Genet (*drafted*)

Greenwood, P.L., Cafe, L.M., Hearnshaw, H., Hennessy, D.W., and Morris, S.G. (2008). Beef primal cut yields in 30 month-old Piedmontese- and Wagyu-sired cattle that differed in prenatal and pre-weaning growth. Aust J Exp Agric (*drafted*)

Alford, A., Cafe, L., Greenwood, P. and Griffith G. (2008). The economic consequences of early-life nutritional constraints in crossbred cattle bred on the NSW North Coast. Aust J Exp Agric (*drafted*)

Cafe, L., Hennessy, D.W., Hearnshaw, H., Morris, S.G. and Greenwood, P.L. (2008). Long-term consequences of birth weight and growth to weaning for feedlot growth and efficiency of Piedmontese- and Wagyu-sired cattle Aust J Exp Agric (*submitted*)

Greenwood, P.L. and Dunshea, F.R. (2008) Biology and regulation of carcass composition: an overview and future directions. In: Improving the Sensory and Nutritional Quality of Fresh Meat. Editors J. Kerry and D. Ledward. Woodhead Publishing. (*submitted*)

Greenwood, P.L., Bell, A.W. and Vercoe, P.E. editors (2008) Managing Prenatal Development to Enhance Livestock Productivity. Springer, The Netherlands, IAEA. (*submitted*)

Greenwood, P.L., Thompson, A.M. and Ford, S.P. (2008) Postnatal consequences of the maternal environment and of growth during prenatal life for productivity of ruminants. In: Managing Prenatal Development to Enhance Livestock Productivity. Editors P.L. Greenwood, A.W. Bell and P.E. Vercoe. Springer, The Netherlands, IAEA. (*submitted*)

Brameld, J.M., Greenwood, P.L., and Bell, A.W. (2008) Biological mechanisms of foetal development relating to postnatal growth efficiency and carcass characteristics in ruminants. In: Managing Prenatal Development to Enhance Livestock Productivity. Editors P.L. Greenwood, A.W. Bell and P.E. Vercoe. Springer, The Netherlands, IAEA. (*submitted*)

Greenwood, P.L., Finn, J.A., May, T.J. and Nicholls, P.J. (2008) Pre-slaughter management including continuous fasting and water deprivation influence carcass characteristics of young Australian goats. Aust J Exp Agric (*accepted*)

Greenwood, P.L. and Cafe, L.M. (2007) Prenatal and pre-weaning growth and nutrition of cattle: Long-term consequences for beef production. Animal 1:1283-1296.

Robinson, D.L., Cafe, L.M., Thompson, J.M. and Greenwood, P.L. (2007). Designing experiments that estimate genetic marker, major gene and treatment effects. Proc Assoc Advmt Anim Breed Genet 17:312-315.

O'Rourke, B.A., Hayes, B.J., Greenwood, P.L., Arthur, P.F. and Goddard, M.E. (2007). Genetic variation at the myostatin locus. Proc Assoc Advmt Anim Breed Genet 17:135-137.

Lehnert, S.A., Reverter, A., Byrne, K.A., Wang, Y., Nattrass, G., Hudson, N.J., and Greenwood P.L. (2007) Gene expression profiling of developing bovine muscle from two different beef cattle breeds. BMC Dev Biol 7:95

Greenwood, P.L., Harden, S. and Hopkins, D.L. (2007) Myofibre characteristics of ovine *longissimus* and *semitendinosus* muscles are influenced by sire breed, gender, rearing type, age, and carcass weight. Aust J Exp Agric 47:1137-1146

Gardner, G.E., Hopkins, D.L., Greenwood, P.L., Cake, M.A., Boyce, M.D. and Pethick, D.W. (2007). Sheep genotype, age, and muscle type affect the expression of metabolic enzyme markers. Aust J Exp Agric 47:1180-1189

Warner, R.D., Pethick, D.W., Greenwood, P.L., Ponnampalam, E.N., Banks, R.G. and Hopkins D.L. (2007). Unravelling the complex interactions between genetics, animal age and nutrition as they impact on tissue deposition, muscle characteristics and quality of Australian sheep meat. Aust J Exp Agric 47:1339-1238

Greenwood, P.L. and Thompson, A.N. (2007) Consequences of maternal nutrition during pregnancy and of fetal growth for productivity in sheep. Rec Adv Anim Nutr Aust 16:169-180

Harper, G.S. and Greenwood, P.L. (2007) Targets for nutrigenomics in production animals: a numbers game. Rec Adv Anim Nutr Aust 16:63-69

Pethick DW, Barendse W., Hocquette J.F., Thompson J.M. and Y.H. Wang (2007) Marbling biology - growth & development, gene markers and nutritional biochemistry. In Energy and protein metabolism and Nutrition. EAAP publication No. 124 pp 75-88. Ed. I. Ortigues-Marty, Wageningen Academic Publishers.

Bonnet, M. Faulconnier, Y., Leroux, C, Jurie, C., Cassar-Malek, I., Bauchart, D., Boulesteix, P., Pethick, D.W., Hocquette, J.F. and Y. Chilliard (2007) Glucose-6-phosphate dehydrogenase and leptin are related to marbling differences among Limousin and Angus or Japanese Black × Angus steers. Journal of Animal Science 85, 2882-2894.

Jurie, C., Cassar-Malek, I., Bonnet, M., Leroux, C, Bauchart, D., Boulesteix, P., Pethick, D.W. and J.F. Hocquette Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. Journal of Animal Science 85, 2660-2669.

Conference proceedings

Cafe, L.M., Ferguson, D.M., Robinson, D.L. and Greenwood, P.L. (2008). Temperament of young Brahman cattle assessed during backgrounding persists and is related to performance. Australian Society of Animal production Bieenial Conference Short Communication. June 2008. (*submitted*)

Coulter, C.L., Greenwood, P.L., Dunn, S.L. and Salkeld, M.D. (2007). Impact of genotype and fetal and pre-weaning growth on steroidogenic capacity of adult *bovine* adrenal. Early Human Dev 83: S100

Coles, C., White, J., Greenwood, P.L., and McDonagh, M. (2007). Variation in extracellular matrix structural

proteins and proteases related to differences in muscle fibre type in cattle. Matrix Biol Conf

Cafe, L.M., Ferguson, D.M. and Greenwood, P.L. (2007). Temperament of young Brahman cattle during backgrounding persists and is related to performance. Beef and Sheep CRC Annual Postgraduate Conference, 9-12 October 2007.

Coles, C., White, J., Greenwood, P.L., and McDonagh, M. (2007). Variation in extracellular matrix structural proteins and proteases related to differences in muscle fibre type in cattle. Beef and Sheep CRC Annual Postgraduate Conference, 9-12 October 2007.

O'Rourke, B.A., Hayes, B.J., Greenwood, P.L., Arthur, P.F., and Goddard, M.E. (2007). Genetic variation at the *myostatin* locus in Angus and Belgian Blue cattle. Beef and Sheep CRC Annual Postgraduate Conference, 9-12 October 2007.

McGilchrist, P., Pethick, D.W., Greenwood, P.L. and Gardner, G.E. (2007) Genetic selection for muscling in cattle increases insulin sensitivity and beef yield. Beef and Sheep CRC Annual Postgraduate Conference, 9-12 October 2007.

Parnell, G., Greenwood, P. and Chen, Y. (2007) Expression of Myostatin in Cattle Selected for High and Low Muscling and in High Muscling Cattle Heterozygous for a Myostatin loss of Function Mutation Genetics Soc of Australasia Ann Conf 54: 64

Reports

Alford, A., Cafe, L., Greenwood, P. and Griffith G. (2007). The economic consequences of early-life nutritional constraints in crossbred cattle bred on the NSW North Coast. Economic Research Report No. 33, NSW Department of Primary Industries, Armidale.

Pethick DW (2007) Investigating feed and water curfews for the transport of livestock within Australia – a literature review. Final report for Meat and Livestock Australia, Murdoch University, Perth, Australia.

Pearce K, North M, Micklander E, Jacob R, Devine C, Pethick D and P. Hanson (2007) Preliminary study of a Magritek low filed nuclear magnetic resonance device to measure meat attributes. Final report for Meat and Livestock Australia, Murdoch University, Perth, Australia.

International conferences/presentations

Invited presenter on Effects of Birth Weight and Nutrition on Postnatal Muscle Development and Energy Metabolism in Ruminants, Aspen Perinatal Biology Symposium on "Interaction of aternal, Placental and Fetal Systems in Perinatal Development", Perth, 25-28 August 2007

Convenor and Moderator, of Session on "Applications to Agriculture" at 5th International Congress on Developmental Origins of Health & Disease, Perth, Western Australia, 6-10 November 2007

Presenter on *Beef CRC Research Collaborations with Europe: ProSafeBeef*, seminar on Research Collaborations with Europe: Opportunities, Prospects and Challenges (FEAST), UNE Armidale, August 2007

Pethick DW., Barendse W., Hocquette J.F., Thompson J.M. and Y.H. Wang (2007) Marbling biology - growth & development, gene markers and nutritional biochemistry. Presented to the International Symposium on Energy and Protein metabolism in ruminants, September, Vichy, France

Pethick, DW "The MSA system" presented to the French beef industry task force, Paris, 14th September, 2007.

Industry talks and papers

Greenwood, P.L. and Oddy, V.H. (2008). How growth affects finishing growth and carcase and meat quality attributes. In: 2008 Armidale Feeder Steer School. Beef Quality CRC. Armidale, January 2008. (presented by Paul Greenwood)

Greenwood, P. and Cafe, L. (2007). Long-term consequences of growth and nutrition of cattle early in life for beef production. In: 2007 Agribusiness Livestock Updates, 24-25 July 2007. Edited by Anne Jones. Department of Agriculture and Food, Perth, Western Australia. (presented by Paul Greenwood)

Paul Greenwood. Invited presenter on *Nutrition and Offspring Performance*, seminars on Boosting Cow Herd Profitability in the New England, Australian Society of Animal Production, UNE Armidale, August 2007

Paul Greenwood. Presentation on Beef CRC growth path RD&E. Southern Australian Beef Research Council, meeting, South Australia, 30 October – 1 November 2007

Paul Greenwood. Presentation and paper on Beef CRC RD&E update. Southern Australian Beef Research Council, meeting, Victoria, 17-19 March 2008

Awards

Linda Cafe. First prize for 1st year post-graduate student. Beef and Sheep CRC Annual Postgraduate Conference, 9-12 October 2007.

Peter McGilchrist. Third prize for 1st year post-graduate student. Beef and Sheep CRC Annual Postgraduate Conference, 9-12 October 2007.

Brendon O'Rourke. Third prize for 2nd and 3rd year post-graduate students. Beef and Sheep CRC Annual Postgraduate Conference, 9-12 October 2007.

Grant Parnell. First prize for student presentation. Genetics Society of Australasia Annual Conference 2007.

Simon Quigley. Australian Academy of Sciences scholarship to visit the laboratory of Jean-Francois Hocquette (INRA) to study cell localisation methods in muscle.

Patents

None