final report

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Mapping Net Feed Efficiency Genes

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Abstract

In beef cattle production systems, the feed requirement of the breeding herd is a large proportion of the total production costs. Previous studies have demonstrated that selecting animals that eat less can lower this cost. However, feed intake measurements are expensive and time consuming. This study has identified five genomic regions that should be pursued with the aim of developing a commercial test. Furthermore, four of these regions were supported by results in a model species (mice!). One DNA region contains genes that could lower feed intake by 14% and improve net feed efficiency by 10%. Economic analysis demonstrated that a DNA test for this effect would significantly increase profitability even if the DNA diagnostic were expensive.

Executive Summary

In beef cattle production systems, the feed requirement of the breeding herd is a large proportion (around 50%) of the total production costs. Competing meat production systems (e.g. pigs and poultry) have much lower maintenance feed requirements because of larger reproductive rates and faster growth rates of progeny. In addition to the costs of cow maintenance, steers fed for the Japanese market have very high feed costs.

Adelaide University established a cattle gene-mapping program ten years ago. The basis of this program was a cross between two very different breeds of cattle (Limousin and Jersey). Specifically, three crossbred (F_1=X) bulls with genes from both parent breeds were mated to purebred cows to produce large numbers (120-130 per sire) of Jersey (XJ) or Limousin (XL) backcross calves. The aim of this cross was to locate genes that affect many production and meat quality traits. These locations are known as Quantitative Trait Loci (QTL) and form the basis for developing genetic tests in cattle selection programs.

The first objective was to identify gene markers for feed efficiency. Many QTL were identified for a range of traits. One QTL for feed intake was particularly large and if cattle were selected based on this QTL, the result would be a large reduction in feed intake (14%) with a small effect on cattle size (5%). This would improve net feed efficiency by 10%.

This project has gone beyond the original objectives by conducting additional analyses (e.g. principal components) and by mapping QTL in the mouse as well as cattle

The second objective was to report synergies between production traits for selection to improve net feed efficiency. This is being conducted in collaboration with AgResearch (New Zealand) and can not be reported until a multi-party intellectual property agreement (currently under development) is signed.

A cost benefit analysis (third objective) was conducted for a system producing steers for the Japanese market with long periods of grain feeding. The analysis clearly showed that utilising a DNA test would be more profitable than current (expensive) systems of measuring feed intake in young bulls.

The next stage in developing a gene marker test is termed "fine mapping" (final objective) and involves collecting additional data on many animals but in specific regions of interest. In total, it is recommended that five of the QTL located should be pursued. Four of these were also significant in the model species (mouse) studied in a sister project. Candidate genes for these regions are being identified based on results from both species. In addition, over the last three years parallel work has been conducted with the aim of developing a single nucleotide polymorphism (SNP) platform which will aid both fine mapping and commercialisation.

It is recommended that the Trangie animals that were part of the MLA funded project (DAN.075) be utilised for fine mapping. These animals have good pedigree and performance records, DNA available, and staff that are good collaborators. As anticipated at the beginning of this project, the Beef CRC is the obvious network for development of the commercial test.

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Acknowledgements

Contract feeding was conducted by the Cattle and Beef Quality Cooperative Research Centre at Tullimba Feedlot, Armidale NSW 2351 ('96-drop) and the South Australian Research and Development Institute at Struan Agricultural Centre, Naracoorte SA 5271 ('97 and '98-drop). Feedlot staff at Tullimba were Ried Geddes and Chris Smith, at Struan John Cooper, Ross Kuchel, and Ben Hebart.

This project would not be possible without the dedication of Tony Weatherly, Stockman Extradinaire! Mating, calving, tagging, regular weighing, blood sampling, animal health, and various tasks imposed by academics. In addition, data processing was conducted by Dr Judith Pitchford and Adam Kister. DNA processing and laboratory work was conducted by Jan Cook and Helena Kojevnikov.

The enormous help from Stockyard and T&R Pastoral Companies during the slaughter programs made collection of measurements on many traits possible. Paul Reynolds, Andrew Blakely and Matt Woolcott (Beef CRC, Armidale) provided outstanding assistance for slaughters conducted at Stockyard. In addition to those already listed, the Adelaide slaughter team included Elke Stephens (now with PIRSA), Andrew Ewers and Mick Deland (SARDI), Brian Siebert, Enoch Aduli, Raphael Afolayan, Chris Smyl, Hamidreza Mirzaei, Madan Naik, and Veronica Ingham.

Lastly, the economic analyses would not have been possible without the generous input of Dr Jason Archer (NSW Agriculture, Trangie).

Objectives

By December 2001 to:

1. Have identified the gene markers associated with the trait in cattle for feed efficiency;

2. Report any potential synergies between selecting for various production traits and the trait for feed intake;

3. Provide a cost benefit analysis of using gene markers to select for feed efficiency to influence cattle turnoff, both in terms of quality and quantity of the end product. This is to be done in collaboration with the feed conversion efficiency work being done at Trangie in project DAN.075 and CRCII Project 2.3.

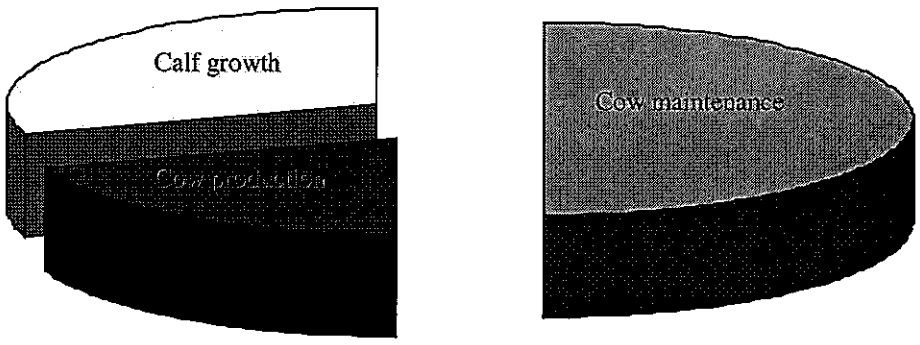
4. Provide a 2-3 page summary report of findings as well as a detailed final report incorporating the above objectives and the implications of findings for industry.

5. Begin commercialising the gene marker test in conjunction with MLA and the Beef CRC.

Background

In typical beef cattle production systems, the breeding herd accounts for 65-85% of the total feed requirements (Ferrell and Jenkins 1984, Montaldo-Bermudez et al. 1990) and 65-75% of this is used for maintenance (Figure 1). Primarily, this very large maintenance requirement is because cattle are a large, slowly maturing species with a low annual reproductive rate. Furthermore, only a single product is harvested (meat). Essentially, the 'machinery' of production represented by the breeding cow requires a proportionately higher level of raw 'inputs' to maintain itself than is required to produce the actual 'product', represented by the cow's offspring. The large maintenance requirement is in contrast to other production systems such as pigs or poultry, where the breeding animal has a small intake relative to the total intake of all progeny. Any improvement in the efficiency with which breeding cows maintain body weight will result in an increase in total meat production for a given amount of feed. In addition to the costs of cow maintenance, long-fed cattle for the Japanese market have a large maintenance feed cost because they are close to their mature weight and are fed on a very expensive diet.

Figure 1. Feed requirements in average production system



Cow maintenance requirements 50%, Lactation and pregnancy 20%, Calf growth 30% of total feed intake.

Attempts have been made previously to select animals for gross efficiency (weight gain/feed intake). However, due to a strong genetic correlation between gross efficiency and growth rate, this resulted in faster growing animals that grew to larger mature sizes with correspondingly higher feed intakes at maturity. What is needed is a means of selecting animals at a young age to reduce feed intake without affecting mature size. With this in mind, studies have been undertaken to examine genetic variation in feed intake that is independent of body size and growth rate, termed 'net feed intake' or 'net feed efficiency'.

During the last 9 years, Dr Wayne Pitchford's group at Adelaide University has been examining the genetics of feed intake including some evaluation of physiological differences in animals that differ in feed intake and efficiency. This has been in collaboration with the cattle research group at both Trangie and Armidale (NSW Agriculture). Recent work in both groups has highlighted possible carcass composition changes associated with selection for net feed intake.

Selection for net feed intake provides an exciting opportunity to significantly reduce feed costs in livestock breeding programs. With this in mind, recent beef industry workshops have been held to develop strategies for measuring net feed intake in large numbers of beef cattle in stud herds. During these workshops, it became clear that measurement of net feed intake will cost around $500 ($300 test plus $200 feed) per animal which is prohibitively expensive. A cheap selection alternative with the potential to significantly reduce generation interval would be to use a DNA test for markers of genes affecting intake. Currently, the GeneSTAR Marbling test is being marketed for $80 (+GST). Additional tests could be cheaper by testing for a number of genes at one time. New DNA technologies, in which Dr. Bottema (Adelaide University) is part of a development team, are also likely to lower this cost even further.

The aim of this project is to locate regions in the cattle genome that contain genes affecting intake with the Davies Gene Mapping Herd. Subsequently, there can be more precise mapping in performance recorded families of different breeds to identify the specific alleles that segregate in specific breeds and cause variation in feed intake and efficiency. These markers can then be tested in selection programs of Angus (utilising Trangie data) and other breeds.

Davies Cattle Gene Mapping Project

The Adelaide Animal Genetics Program was established within the Department of Animal Science at the University of Adelaide in 1992. The Program grew quickly into a substantial research group with expertise in molecular genetics, quantitative genetics, biochemistry and cytogenetics. The emphasis of the group is on the genetics related to livestock production.

The Davies Cattle Gene Mapping Project has been the foundation of the Program and has been made possible through a grant provided by the J. S. Davies Bequest to The University of Adelaide. In addition, a series of related research projects have been developed. These other projects are independent in terms of funding and outcomes. However, they strengthen the Gene Mapping Project by providing results and techniques that extend the gene mapping work and maximise the use of resources. The largest of these projects was the Southern Crossbreeding Project in collaboration with Mr Mick Deland at Struan Research Centre, Naracoorte SA.

The Davies Cattle Gene Mapping Project has two primary goals: i) to study the mode of inheritance of important meat quality traits, and ii) to map major genes controlling these traits (known as Quantitative Trait Loci, QTL). Once it is understood how traits are inherited, then improved selection strategies will be available for producer breeding programs. Mapping the major genes will lead to their identification for study and manipulation.

The ideal design for mapping genes for a large number of traits is to develop a population with large amounts of genetic variation. A common design is to cross two very different breeds to produce F_1 bulls. The next aim is to have many progeny from these bulls.

The Davies Cattle Gene Mapping Project is part of a worldwide effort to map the cattle genome, and complements the other beef cattle gene mapping programs. However, the Gene Mapping Project has several important features that distinguish the Project from all other current beef cattle mapping work. These include the:

- Diverse *Bos taurus* breeds (Jersey and Limousin),
- Large number of phenotypic traits that have been measured (around 300 traits),
- Unique herd design (double backcross) which allows testing of QTL by breed interactions,
- Large herd size (one of largest in the world with almost 800 gene mapping progeny),
- Additional measurements (fat and protein metabolic traits),
- Only herd in two environments (Australia and New Zealand) representing both grain and grass finishing systems, and
- Measurement of feedlot efficiency – net feed efficiency of grain finished cattle.

The Adelaide Gene Mapping progeny comprise 366 backcross calves: 77 born April 1996, 153 born April 1997, and 136 born April 1998. Phenotypic measurements and molecular analyses are being completed on these 366 calves.

Collaboration with AgResearch

As an extension of the Gene Mapping Project, in New Zealand, there are progeny from another 3 Jersey-Limousin crossbred bulls. Each crossbred bull is half-sibling brother of one the crossbred bulls used in Australia. These bulls were mated to Jersey and Limousin cows as in Australia. However, all progeny in New Zealand were finished on grass (not grain) to enable studies of genotype by environment interactions as well as greatly increasing the power of the current project. For the gene mapping, there are almost 800 progeny (from 6 sires) born over 3 years (1996-1998).

Drs Alan Crawford and Chris Morris lead the AgResearch Project. The genotyping for the whole project was completed in Dr Crawford's laboratory. For most traits, AgResearch and Adelaide are equal partners. Exceptions to this are for disease resistance (internal parasites and facial eczema) where AgResearch owns the IP and feed intake / efficiency where Adelaide owns the IP in partnership with MLA.

Traits measured

To maximise return from the investment in genotyping, it is crucial that the animals have as many traits measured as possible. Routinely cattle were kept on grass for 800-900 days then grain fed for around 200 days. It was intended to slaughter calves at a much younger age but there were significant delays in measuring feed intake for every cohort.

To target a specific trait, it is important that initial markers are confirmed by both comparative and fine mapping. Therefore, comparative mapping has been conducted in the Adelaide University mouse efficiency selection lines. Fine mapping will provide appropriate markers for testing in breeds such as Angus.

Results from Adelaide, Trangie and elsewhere all point to possible correlated response in carcass traits when selecting for net feed intake. Thus, it is important to be able to quantify meat yield and distribution as well as net feed efficiency. Consequently, the animals were trucked to Brisbane (Valley Beef Abattoir, Grantham) for slaughter and bone out similar to the Beef CRC cattle ('96 and '97-drop). Similar information was collected for the '98-drop, slaughtered and processed by T&R Pastoral, Murray Bridge SA. The carcass composition information will enable mapping of genes affecting meat yield and distribution of muscle mass, in addition to, feed efficiency.

Over the whole project, trait groups are defined as follows:

1. Pedigree and birth traits (e.g. birth weight, linear measurements at birth, gestation length).
2. Growth (live weights every 30-50 days).
3. Skeletal growth (regular live-animal linear measurements plus carcass length, pelvic dimensions, bone lengths, bone weights).
4. Puberty traits (age plus weight at puberty by interpolation; dentition and ossification as indicators of physiological maturity).
5. Other live-animal traits (horns, coat colour, blood enzymes, hormones, metabolites, and minerals from slaughter tissues).
6. Temperament scores (exit speed, flight distance, docility score) plus plasma and urinary cortisol.
7. Food intake / efficiency (plus eating rate). AUSTRALIA ONLY
8. Disease resistance (Faecal egg counts, facial eczema score). NEW ZEALAND ONLY
9. Carcass dissection (starting at HSCW, including meat distributions and meat yields).
10. Marbling, fat depths (ultrasound and carcass), fat distribution (including fat depots and IMF%).
11. Fatty acid composition, fat melting point, and fat colour.
12. Tenderness, pH, muscle glycogen, meat colour, calpains.
13. Taste panel scores including cooking loss.
14. Organ weights (except fat depots), hide traits, rumen and intestinal weights.

Results and Discussion

In all cohorts, as expected, just over 10% of animals were shy feeders and were removed from the intake testing pens and fed separately. Thus, there is feed intake data available for 323 animals (Table 1). Some animals had poor weight records so net feed intake was calculated for 319 animals. Additional data on number of feeding sessions and total time in feeders was obtained for 314 animals.

Feed intake data was processed by calculating least-squares means for each animal over the test period. Day was included in the model to allow for weather, personnel, time of feeding, and any other factors that would affect the intake of all cattle. Average daily gain was calculated as the regression coefficient for weight against day of test. Most traits were normally distributed with the exception being number of eating sessions and time eating (Table 1). These traits were transformed by taking the natural logarithm. When back transformed, the mean, minimum and maximum values were 7, 2, and 32 for number of meals (sessions) per day and 96, 46, and 215 for time eating (minutes) per day respectively. The maximum value for feeding sessions (32) was probably high because some animals had the tendency to come in and out of the feeders frequently while eating. Others just calmly ate their fill and then backed out to the pen when finished.

The relationship between feed intake and weight was stronger than for average daily gain (Figure 2 and Table 2). Net feed intake was calculated after modeling daily feed intake with a regression comprising metabolic body weight ($MMWt = MidWt^{0.73}$) and average daily gain (ADG) while on feed intake test (Table 3). Cohort was defined as the combination of year of birth ('96 - '98) and sex (heifer, steer). The main effect of cohort and interactions between MMWt or ADG with cohort were not significant (Table 3). Thus, a simple model comprising only MMWt and ADG was utilised.

The constant was basically zero and the slopes were 0.113 kg feed per kg metabolic weight and 0.8 kg feed required for 1 kg weight gain. The slope for metabolic body weight was equivalent to maintenance feed requirements being 2% of body weight. The 0.803 was less than expected but may have been a result of the low correlation between weight and weight gain (r=-0.20, Table 2). The correlation between DFI and NFI was 0.84 indicating that approximately 30% of the variance in intake was associated with variation in size (MMWt) and growth rate (ADG).

In addition to net feed intake, maintenance requirement (MR=DFI/MidWt) and gross efficiency (GE=ADG/DFI) were calculated to aid interpretation of results. Both MR and GE were expressed as percentages with a lower value being desirable for MR and a higher value being desirable for GE. GE was negative for those animals that lost weight during the test period. MR and NFI were highly correlated (0.92, Table 2) which not surprising since the cattle were relatively old when entering feed intake test. GE was far more highly correlated with ADG (0.94) than intake (-0.14) and was likely due to the large variation in ADG (CV=61%, Table 1) relative to DFI. Eating time and NFI were moderately correlated (0.54) indicating that more efficient animals (low NFI) spent less time eating rather than eating at a different rate (r=-0.08).

Since the traits studied were correlated, principal components were formed between the five primary traits (MidWt, ADG, DFI, LnMeals and LnTime, Table 2). Principal components convert five correlated traits into five uncorrelated but highly accurate measured components. The principal components (PC1 - PC5) accounted for 35%, 31%, 23%, 8%, and 3% of the variation in the component traits respectively. While principal components are sometimes helpful biological descriptors of data, in this case the only clearly interpretable component was PC2 which was highly correlated (0.91) with DFI and most highly correlated with NFI (0.73).

Table 1. Summary of data collected.

Trait	Abrev.	Number	Mean	CV (%)	Min.	Max.
Mid-weight (kg)	MidWt	324	625.6	13	382.6	824.3
Mid-w $t^{0.73}$ ($kg^{0.73}$)	MMWt	324	109.7	10	76.8	134.5
Weight gain (kg/day)	ADG	324	0.94	61	-1.43	2.35
Daily feed intake (kg/day)	DFI	323	12.94	17	6.37	18.95
Net feed intake (kg/day)	NFI	319	0.05	14*	-5.04	7.23
Maintenance requirement (% feed/MidWt)	MR	319	2.07	16	1.22	3.71
Gross efficiency (% ADG /kgfeed)	GE	319	7.36	65	-22.42	20.17
No. meals (log_n(No./day))	LnMeals	314	1.96	30	0.65	3.48
Time eating (log_n(min/day))	LnTime	314	4.56	7	3.83	5.37
Eating rate (g/sec)	Rate	314	2.44	30	1.05	4.71

*SD divided by mean DFI since mean NFI close to zero by definition.

Table 2. Raw correlations between traits and principal components.

Trait	MidWt	ADG	DFI	NFI	LnMeals	LnTime	Rate
MidWt	1	-0.20	0.50	0.00	-0.20	-0.33	0.66
ADG	-0.20	1	0.10	0.00	0.45	0.36	-0.32
DFI	0.50	0.10	1	0.84	-0.09	0.34	0.29
NFI	0.00	0.00	0.84	1	-0.11	0.54	-0.01
MR	-0.28	0.27	0.68	0.92	0.03	0.67	-0.25
GE	-0.31	0.94	-0.14	-0.21	0.46	0.26	-0.36
LnMeals	-0.20	0.45	-0.09	-0.11	1	-0.09	-0.03
LnTime	-0.33	0.36	0.34	0.54	-0.09	1	-0.78
Rate	0.66	-0.32	0.29	-0.01	-0.03	-0.78	1
PC1*	-0.73	0.75	-0.24	0.00	0.61	0.48	-0.67
PC2	0.40	0.37	0.91	0.73	-0.10	0.62	-0.05
PC3	0.47	0.29	0.12	-0.24	0.69	-0.57	0.60
PC4	-0.15	-0.46	0.22	0.48	0.36	0.12	-0.01
PC5	0.25	-0.06	-0.22	-0.41	0.10	0.24	-0.38

*Five principal components between five primary traits (MidWt, ADG, DFI, LnMeals and LnTime) related to intake. The principal components accounted for 35%, 31%, 23%, 8%, and 3% of the variation in the component traits respectively.

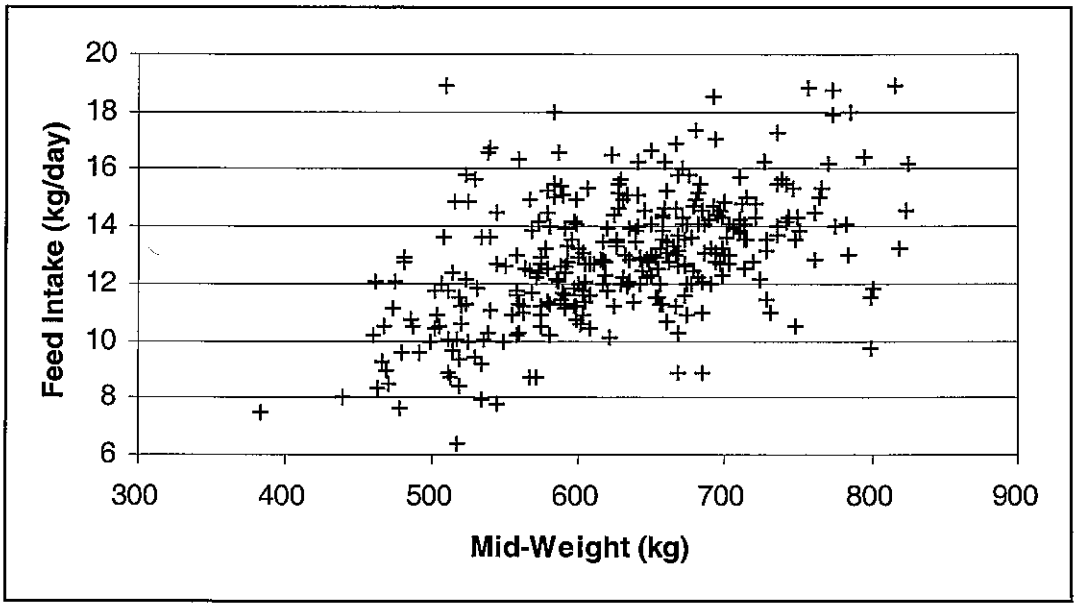
Correlations greater than ±0.10 were generally significant ($P<0.05$).

Table 3. Analysis of variance for calculating Net Feed Intake.

Source	DF	SS	F-value	Parameters
Initial model				
Cohort	5	6	0.5	
Metabolic mid-weight	1	294	126.2***	
Daily wt. Gain	1	20	8.5**	
Cohort x MMWT	5	11	0.9	
Cohort x ADG	5	22	1.9	
Residual	301	700		
Final model				-0.295±1.14
Metabolic mid-weight	1	454	131.7***	0.113±0.010***
Daily wt. gain	1	67	19.4***	0.803±0.182***
Residual	320	1103		
Total	322	1575		

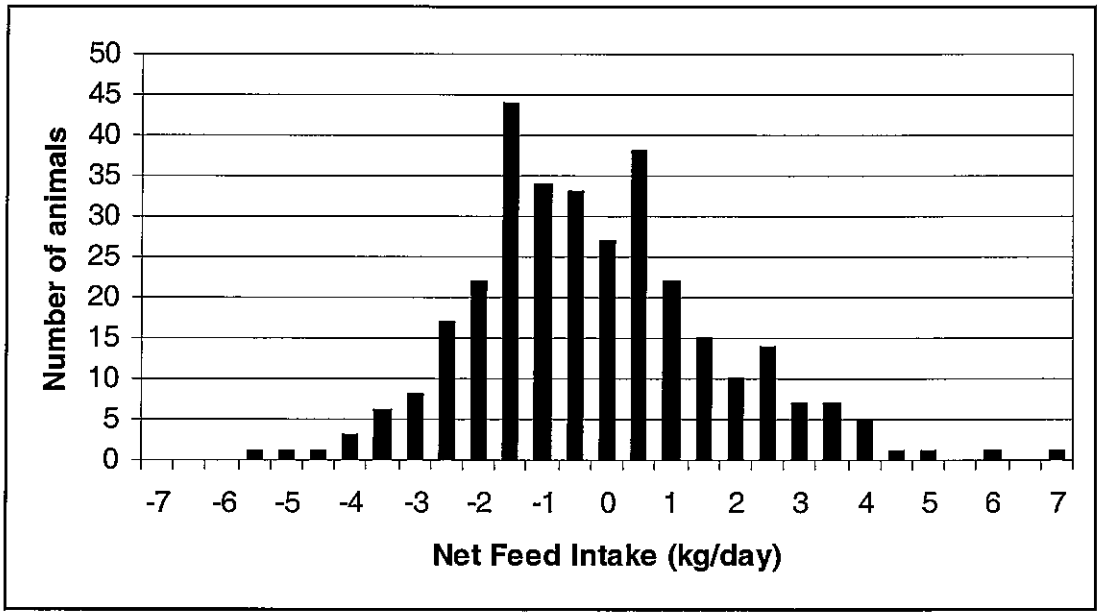
*P<0.05, **P<0.01, ***P<0.001.

Figure 2. Relationship between feed intake and body weight.



Indicates large variation in both weight and feed intake. Also demonstrates significant relationship between the two traits i.e. big animals eat more!

Figure 3. Distribution of Net Feed Intake.



Indicates that Net Feed Intake has a large amount of variation and is normally distributed.

Least squares analysis

Before proceeding with mapping, the data were investigated by fitting a model containing fixed effects of cohort ('96 heifers, '98 steers), breed of dam (Jersey or Limousin) and sire (Ryan, Lou or Tom). Cohort differences were highly significant for every trait (Table 4). The 1996 and 1997 drops were tested at similar weights but 1998 drop had lighter heifers and heavier steers. The '98-drop steers had very low gains (average 0.03kg/d) because they entered the feed test after spending significant time on feed. Standard errors were higher for the '96-drop than other cohorts because there were fewer animals.

The 1996-drop was the only group where steers and heifers were fed at the same time. For the 1997 and 1998 progeny at Struan, heifers were fed first and then slaughtered while the steers were on feed test. Hence, the differences between sexes was greater at Struan than Tullimba. From the '96-drop results, it can be concluded that compared to heifers, steers were 8% heavier, gained weight 17% faster, ate 4% more, had a 2% lower NFI, ate 13% less meals, spent 4% less time eating, ate 2% faster (Table 4), had 3% lower maintenance feed requirements, and a 12% higher gross efficiency.

Feed intake was generally higher at Struan ('97 and '98-drop) than Tullimba ('96 drop). Also, the number of meals at Struan was far less than Tullimba (1996 drop) indicating possibly less than *ad libitum* intake. However, the results for total feed eaten was reasonable and total time eating was generally higher at Struan than at Tullimba. Careful examination of the intake data revealed total box usage values were all less than 85% indicating ample opportunity for animals to eat *ad libitum*.

Significant breed and sire effects indicate the presence of genetic variation in a trait. When there is significant genetic variation, it is likely that QTL can be mapped. Breed of dam differences were significant for weight and feed intake (Table 4). Limousin cross (¾L¼J) cattle were 16% heavier, gained 11% more weight and ate 12% more than the Jersey cross (¼L¾J). It could be expected that the difference between purebred Limousin and Jersey would be approximately double these. While the differences appear large, there was no significant difference in net feed intake, number of meals or time spent eating (Table 4, Figure 4).

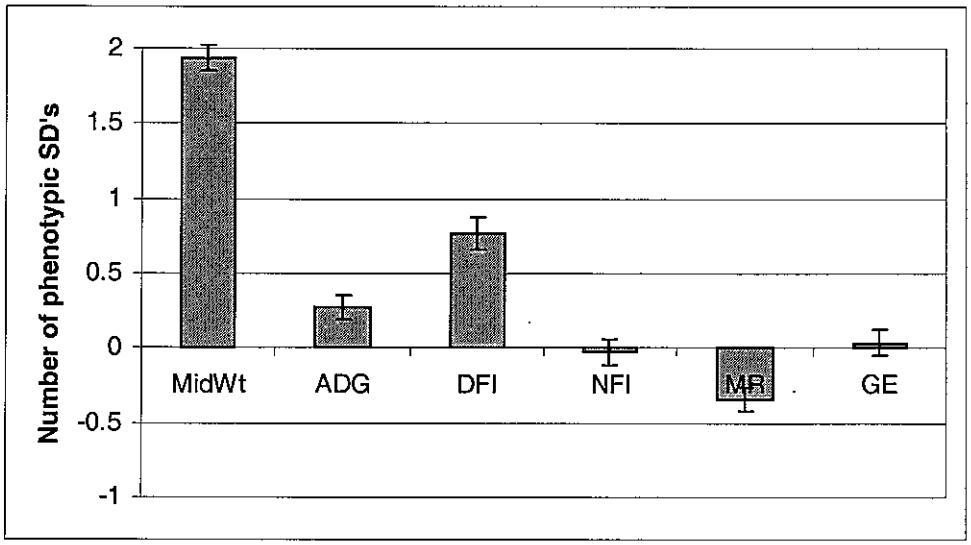
As for breed, sire differences were significant for weight (although not ADG) and feed intake. In contrast with breed, sire differences were also significant for net feed intake, number of meals and time spent eating. Generally "Tom" was different from the other two sires. Tom's progeny were 4% lighter but ate 7% less and so had a 4% lower net feed intake than the average of the other two sires' progeny. Tom's progeny also had 11% more meals but spent 9% less total time eating (Figure 5).

Table 4. Least squares means and tests of significance for main effects.

Trait	MidWt	ADG	DFI	NFI	LnMeals	LnTime	Rate
Cohort	***	***	***	***	***	***	***
'96 Heifers	594±7	1.00±0.05	11.4±0.3	-1.11±0.23	2.76±0.05	4.36±0.03	2.78±0.08
'96 Steers	644±9	1.17±0.07	11.9±0.3	-1.38±0.29	2.62±0.06	4.32±0.03	2.84±0.10
'97 Heifers	598±6	1.31±0.05	12.3±0.2	-0.49±0.19	1.98±0.04	4.66±0.02	2.06±0.07
'97 Steers	658±6	1.13±0.05	13.7±0.2	0.20±0.19	2.15±0.04	4.51±0.02	2.73±0.06
'98 Heifers	569±6	1.07±0.05	14.1±0.2	1.92±0.20	1.65±0.04	5.01±0.02	1.61±0.07
'98 Steers	701±6	0.03±0.05	13.4±0.2	0.14±0.20	1.26±0.04	4.31±0.02	3.17±0.07
Breed	***	*	***				***
Jersey	580±4	0.90±0.03	12.1±0.1	-0.10±0.12	2.08±0.03	4.54±0.01	2.33±0.04
Limousin	675±4	1.00±0.03	13.5±0.2	-0.14±0.13	2.06±0.03	4.53±0.01	2.73±0.04
Difference	16%	11%	12%	0.3%	-1%	-0.2%	17%
Sire	***		***	*	**	***	
Lou	631±5	0.98±0.04	12.9±0.2	-0.12±0.15	2.07±0.03	4.58±0.02	2.46±0.05
Ryan	641±5	0.95±0.04	13.3±0.2	0.20±0.15	2.00±0.03	4.55±0.02	2.61±0.05
Tom	610±5	0.92±0.04	12.2±0.2	-0.44±0.15	2.14±0.03	4.47±0.02	2.52±0.05

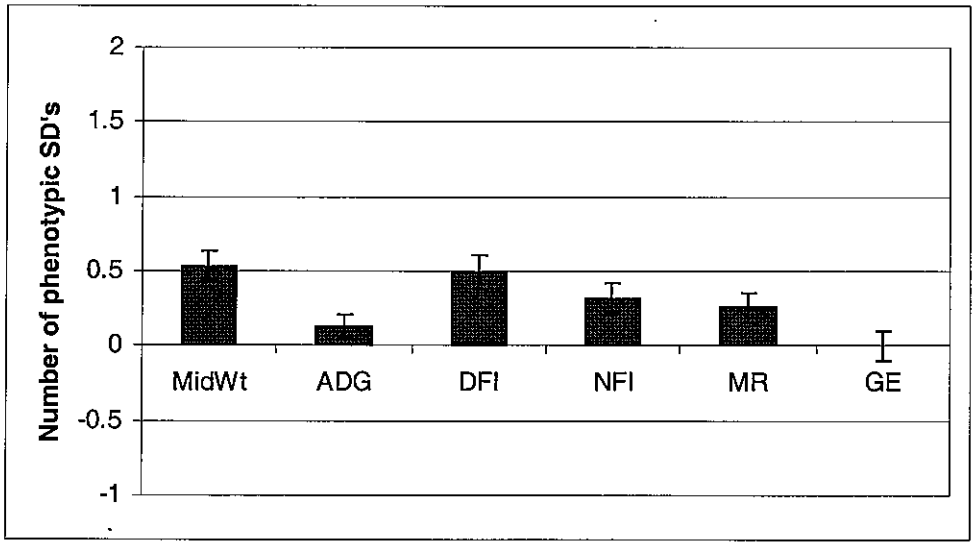
*P<0.05, **P<0.01, ***P<0.001.

Figure 4. Breed differences.



Indicates Limousin cross calves were much bigger, ate more but had lower maintenance feed requirements than Jersey cross calves.

Figure 5. Difference between "Tom's" and other progeny.



Indicates sire differences smaller than breed differences for weight but similar sized effects for feed intake and efficiency.

Mapping Quantitative Trait Loci

Microsatellite markers were genotyped for 366 calves that survived to weaning (77 '96-drop, 153 '97-drop, and 136 '98-drop). There were 3-9 markers typed per chromosome for all 29 autosomes. In the half-sib design utilised, sires were all classified as having the genotype "AB". Thus, progeny would either inherit an "A" or "B" from the sire and another allele from the dam. Therefore, the genotypes in the progeny were AA, AB, BB, AC, or BC where "C" = any other allele. If progeny were AB, then they were not informative for the analysis because it is not clear which allele they received from the sire (A or B). Genotyped probabilities were calculated using "QTL Express" (Seaton *et al.* 2001) so that at every point (1centi-Morgan) of every chromosome, calves were assigned a value of either 0 (=A) or 1 (=B) or somewhere in between depending upon the level of confidence. For example, close to a marker, values would be 0.05 or 0.95 whereas further from a marker they may be 0.3 or 0.7. When markers were uninformative, genotype probabilities were 0.5.

Phenotypes (measured traits) were then regressed against the genotype probabilities for every chromosome. Cohort and breed of dam were included as factors in the model and the regression was nested within sire. Initially the tests were done at 4cM intervals. Once a significant test was obtained, they were conducted every 1cM to more accurately define the most probable location. Additional models that included two QTL and QTL by breed of dam interactions were tested for a number of traits. There was some evidence of a QTL by breed interaction for net feed efficiency (NFI). This could result from a maternal (milk supply) effect, dominance at the QTL, or genetic background (epistasis).

Significance was loosely defined as LOD scores greater than 2 as suggestive and greater than 3 significant. LOD scores close to 5 would be considered "confirmed linkage". LOD scores presented in this report are calculated (by QTL Express), not exact, and may be slightly biased. F-statistics are also reported by QTL Express and are exact for regression analyses. Lastly, bootstrapping and permutation tests were conducted to test the confidence of location of QTL and to determine appropriate significance levels (Figure 6).

All traits (Tables 1 and 2) were tested but this report has concentrated on net feed intake as the primary trait and reported accordingly (Table 5 and 6). [Note: Locations of QTL are not reported in this public report to enable future patenting options.]

Most QTL were segregating in one family only. The result is that the size of the effect could be reasonably large even at "borderline" significance levels. The difference between families also implies that the QTL or genes identified were not fixed in the parent "purebred" populations. Thus, the results are likely to be more widely useful than simply identifying THE Jersey or Limousin gene. The largest QTL for NFI in cattle (C1) had a calculated LOD score of 3.2 (Table 5). At the same location, the effect on DFI was large with a calculated LOD score of 4.5. The F-value for DFI was 7.5 (Table 6), a highly significant result (Figure 6).

QTL C1 was significant for a number of traits in addition to NFI. It was highly significant for daily feed intake (LOD 4.7, Table 5) but also for weight and average daily gain. Thus, initially it would appear that this QTL results in small animals that eat less! However, it was significant for NFI, but, as with most QTL identified in the present study, did not have the same effect in all families. The largest effect was clearly in Tom's progeny (Table 6, Figure 7). In contrast, the QTL was significant for MidWt in Ryan's and for ADG in Lou's progeny. Furthermore, the ADG QTL did not map to exactly the same location as NFI although was very close (only 7cM away).

Interestingly, principal component 2 (Table 2) also mapped to the same location as C1 and was highly significant (LOD 4.5). This was not surprising because PC2 was highly correlated with both daily (0.93) and net (0.73) feed intake. Also not surprising was the fact that PC2 was segregating with large effect (1.3 phenotypic standard deviations) in the same sire family (Tom). The first and third principal components also mapped to a similar position to PC2 and feed intake. However, they were far less significant and were segregating in different sires (Table 6).

In Tom's progeny (Table 6), the QTL C1 resulted in 5% smaller animals that ate 16% less, had an 11% lower net feed intake, 10% lower eating rate (i.e. same time spent eating but ate less), and 14% lower maintenance feed requirements. Consequently, they were slightly smaller, but they were also significantly more efficient. Results for NFI are presented as phenotypic standard deviations in Figure 7 and were large when compared to the magnitude of the breed (Figure 4) and sire differences (Figure 5).

Selection for the most significant QTL located would result in a large reduction (14%) in feed intake with a small sacrifice (5%) in growth (animal size).

In addition to the large QTL (C1), nine others are reported (Table 5). While of lower significance, C2 and C3 were mapped with principal components (although not PC2) to add confidence to the region. Both of these appear more independent of growth than C1. C4 was highly significant for ADG as well as being suggesting for DFI, PC2 and PC3. The high LOD score for ADG increases confidence of there being a QTL. However, the QTL is likely to affect ADG primarily rather than improving efficiency *per se*. C5 was supported by a high LOD score for PC3 and should be pursued since, based on correlations, it would appear PC3 reflected intake pattern (Table 2). MidWt and PC2, which was highly correlated with intake traits, supported C6. C7-10 were of much lower significance and are reported for completeness.

Table 5. QTL identified in cattle[a].

Cattle QTL	NFI LOD	NFI size[b]	DFI LOD	MidWt LOD	ADG LOD	PC1 LOD	PC2 LOD	PC3 LOD
1	3.2	98	4.7	3.0	3.9	2.3	4.5	1.9
2	2.2	65	1.6			1.7		1.9
3	2.0	57			1.7			1.5
4	1.9	50	2.2	1.8	3.4		2.4	1.6
5	1.7	46	2.2					3.2
6	1.6	50	2.1	2.2			1.7	
7	1.6	46	1.5		1.5		1.7	
8	1.5	51			2.5	1.5		1.5
9	1.5	46			2.9	1.9	1.5	
10	1.5	45	2.3	2.3			2.3	2.0

[a]LOD scores less than 1.5 not presented.

[b]Additive size of effect in percentage phenotypic standard deviations (1.55 kg/day in cattle) in family where QTL had greatest effect. Assuming no interaction between the QTL effect and sex or breed.

Table 6. Effects of cattle QTL1 on various traits.

Trait	F-value	LOD score	Sire: "Ryan"	Sire: "Lou"	Sire: "Tom"
MidWt	4.8	3.0		-22±11	-32±10
ADG*	5.8	3.7	-0.29±0.08		
DFI	7.5	4.7			-1.76±0.39
NFI	4.9	3.2			-1.25±0.32
MR	4.1	2.6			-0.27±0.07
GE*	3.6	2.3	-1.87±0.63		
Rate	1.6	1.0			-0.25±0.11
LnTime*	2.3	1.5			0.12±0.05
PC1	3.7	2.4	-0.33±0.13		-0.30±0.13
PC2	7.8	4.9			1.19±0.27
PC3	2.9	1.9	-0.30±0.13		
PC4*	2.4	1.6	0.33±0.12		
PC5	1.8	1.2			

*Average Daily Gain, Gross Efficiency, Time spent eating, and PC4 all mapped to slightly different positions from NFI but were similar to each other. LnMeals mapped to a totally different reqion and was not significant.

Mouse studies were conducted in a parallel project to the cattle studies. Mouse lines were selected for high or low net feed intake based on a post-weaning test (Hughes *et al.* 1997). After seven generations of selection, the lines were approximately 30% different in feed intake with little difference in growth and body composition. These lines were crossed to produce F_1 sires and dams that were *inter se* mated to produce F_2 progeny. Mapping was conducted in two sire families (Table 7). Fine mapping in two other mouse sire families has begun based on the results presented.

The most significant ten QTL in mice had higher LOD scores (Table 7) than in cattle (Table 5). This is likely because a more complex model was fitted to the data. Since an F_2 design was utilised, dominance was estimated as well as the additive effect of the QTL. Both the additive and dominance components were included in the LOD score. The more complex model also resulted in the large discrepancy between LOD scores and size of effect of the QTL (Table 7) in mice.

When QTL are mapped in more than one species to homologous regions, there is increased confidence that the results are real. The increased confidence aids decision making when pursuing specific genes involved. Once the genes are identified, a commercial test can be developed. The human genome has now been mapped and sequenced, and mouse will soon follow. As the genetic maps improve, it will become more certain where homologous (corresponding) genes are located in each species. We already know that the human and cattle genomes are very similar (i.e. there are large blocks of genes in the same order and inherited together in each species). Unfortunately, the mouse genome is quite different so that genes on one human chromosome are commonly located on three different mouse chromosomes. Therefore, having better genomic data available rfom mouse is imperative for comparative analyses.

While mice may not be expected to be a good model for cattle, FOUR QTL were in common between the two species (Table 7). C1 was homologous to M7, C3 to M8, C5 to M6, and C6 to M3. These results greatly add confidence to the QTL identified. Furthermore, they will help with targeting candidate genes which could eventually be commercialised as with GeneSTAR marbling. Initial examination of the human genetic map has revealed two exciting candidate genes worth pursuing in regions homologous to cattle C1.

Four QTL mapped to homologous regions in cattle and mice. These should be the target of future studies with the aim of producing a commercial test to improve efficiency.

In total, it is recommended that five of the QTL located (C1, C2, C3, C5, and C6) should be pursued. The next stage is termed "fine mapping" and involves collecting additional data on many animals but in specific regions of interest. This has already begun for the model species and will proceed as soon as intellectual property agreements are in place with the Cattle and Beef Quality Cooperative Research Centre.

Lastly, it is recommended that the Trangie animals that were part of the MLA funded project (DAN.075) be utilised for fine mapping. These animals have good pedigree and performance records, DNA available, and staff that are good collaborators. As anticipated at the beginning of this project, the Beef CRC is the obvious network for development of the commercial test.

Table 7. QTL for NFI in mice.

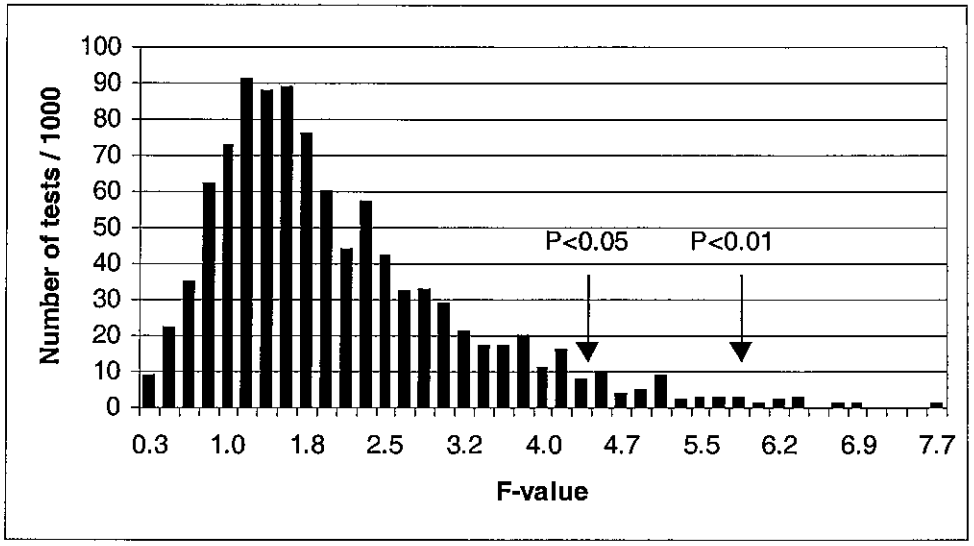
Mouse QTL	Mouse LOD	Mouse size[a]	Cattle QTL[b]
1	3.5	165	
2	3.2	50	
3	3.1	274	6
4	3.0	203	
5	3.0	28	
6	2.9	199	5
7	2.8	57	1
8	2.5	86	3

[a]Additive size of effect in percentage phenotypic standard deviations (0.32 g/day in mice) in family where QTL had greatest effect. Dominance values not reported.

[b]Homologous regions between cattle and mice.

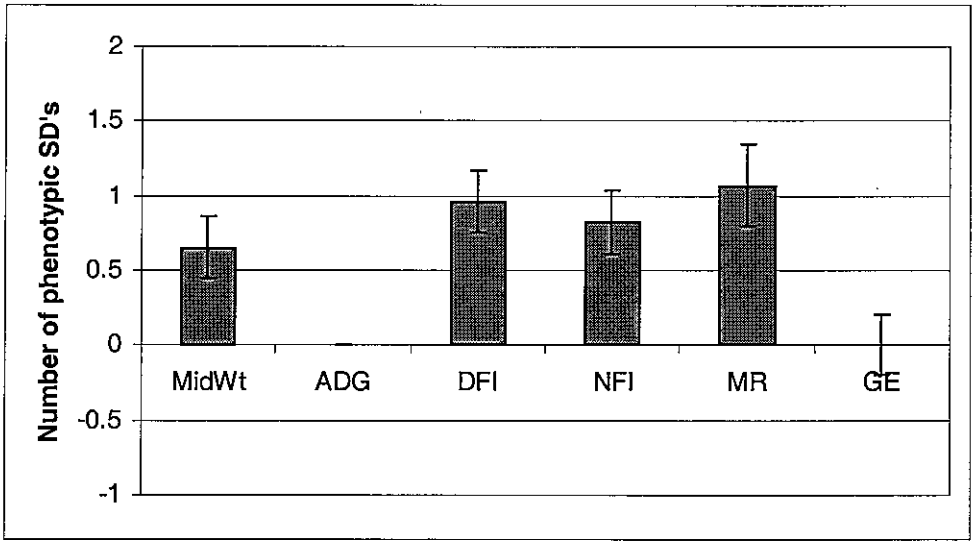
Discrepancy between mouse LOD scores and size of effect is because of either level of dominance effect and/or a significant interaction between sex and the QTL effect.

Figure 6. Permutation tests for NFI at C1 QTL.



Permutation tests randomly re-sort the data and then test the model used. The F-value for Daily Feed Intake (7.5, Table 7) was clearly significant and for Net Feed Intake (4.9) was suggestive compared to significance thresholds shown. Thus, conclude that the QTL located is real and not simply due to chance.

Figure 7. C1 QTL effects in "Tom's" progeny.



Indicates QTL effect is as big as the breed and sire effects for most traits and even bigger for Net Feed Intake (Efficiency). Direction of the effect was same as for sire difference.

Economic analysis

Net feed intake is a trait that is expensive to measure but has a high economic value, especially for animals bred for the Japanese market which requires long periods on expensive, high-energy feed. A DNA based test has the potential to make genetic gain with decreased cost of measuring NFI. Dr Jason Archer (NSW Agriculture, Trangie) has experience in modeling economics of genetic improvement in NFI and has published a number of papers in the area (e.g. Archer and Barwick 2001). With the assistance of Dr Archer, some of the economic aspects of a DNA test for NFI were briefly investigated with the program "Z-plan" (Graser *et al.* 1994, Nitter *et al.* 1994).

For the analysis, the breeding program consisted of a two-tier self-replacing population of 200,000 breeding cows, with 10,000 cows in the breeding unit and the remainder in the commercial unit following Archer and Barwick (2001). Genetic improvement was only generated in the breeding unit and transferred to the commercial unit through the use of bulls selected from the breeding unit. Twenty bulls per year were selected for use in the breeding unit as AI sires, and each sire was used for an average of 2.5 years.

The breeding objective was based on production of 650kg liveweight steers fed for the high quality Japanese market where marbling is valued. Selection criteria included weight at various ages (birth, 200d, 400d, 600d, and mature cows), fertility traits (days to calving, calving difficulty score and scrotal size), and scan traits (rib and P8 fat depth, eye muscle area, and intra-muscular fat percentage) in addition to NFI measurements and/or genotypes (see below). Information sources included records on individuals, paternal half-sibs, sire, dam, and half-sibs of the sire and dam. Criteria recording costs were similar to those used by Graser *et al.* (1994).

Assumptions:

$\sigma_p = 0.62$ kg/day	In the Trangie data (Archer pers. comm.) the phenotypic standard deviation was only 0.62kg/d whereas in this study it was 1.52kg/d.
$\sigma^2_A = 0.15$ kg^2/day^2	In the Trangie data (Archer pers. comm.) the genetic variance was 0.15kg^2/d^2 corresponding to a heritability of 39%.
$\alpha = 0.57$ kg/day	Size of gene effect would be 0.57kg/d. The gene effect in initial analyses was 1.4kg/d (actually 1.25 in Table 6) in the family with the largest effect. This was 0.92 phenotypic standard deviations. The Trangie test was post-weaning which is likely the reason for the difference. Thus, 0.92xSD's would be 0.57kg/d.
$\sigma^2_{QTL} = (\sigma^2_A)/3 = 0.05$	Assume the variance due to the QTL is a third of the genetic variance. This is a large, but conservative, effect based on the size of the QTL in this study (Table 6).
$p = 10\%$	If it is assumed that the average effect of the gene is 0.57kg/d and that the variance due to the QTL is a third of the total genetic variance (0.05kg^2/d^2), then the frequency of the QTL in the wider population would be approximately 10%.
$r_G = 0.57$	If the variance due to the QTL is a third of the genetic variance, then the genetic correlation between the trait and the QTL is the square root of a third ($0.33^{0.5} = 0.57$). It is only by chance that this is the same value as for the average effect of the gene (above). It was further assumed that the genetic correlation between the QTL and all net feed intake traits (during post-weaning test, as young animals, and as cows) was the same (0.57).
$r_G = 0.57 x r_G$	The genetic correlation with other traits in the breeding objective was 0.57 time the correlation previously used between post-weaning net feed intake and the trait of interest.

$h^2_{QTL} = 0.99$	By definition the heritability of a DNA marker is 1. However, it was possible that the program would not allow exactly one, 0.99 was the next best thing!
$\sigma^2_{P,QTL} = 0.051$	Follows from heritability of 0.99 that phenotypic variance of the QTL was slightly greater than the genetic variance.
Normally distributed	It is assumed that the QTL is normally distributed. This is clearly not the case but it is likely to be a reasonable assumption since large numbers of animals are involved. Perhaps a more concerning assumption is that the variance due to the QTL is constant as the frequency of the favourable gene increases.
L = 3.74 years	Assume generation interval of 3.74 years, the same as previous analyses (Archer pers. comm.)
Genotyping cost = $80	GeneSTAR marbling currently costs $80 (+GST) and it seemed sensible to assume the same cost. It should be cheaper if more than one DNA test was conducted at the same time as GeneSTAR marbling, tenderness etc. Thus, a value of $20 was also tested. The cost compares to $300 for the actual feed intake test.
Age = 400 days	The age of measurement was assumed to be 400 days which allows for early culling on other traits and is equivalent to the current post-weaning test.
AI utiltised	AI utilised and cost assumed $30/dose including mating costs.

Five scenarios were simulated (Figure 8):

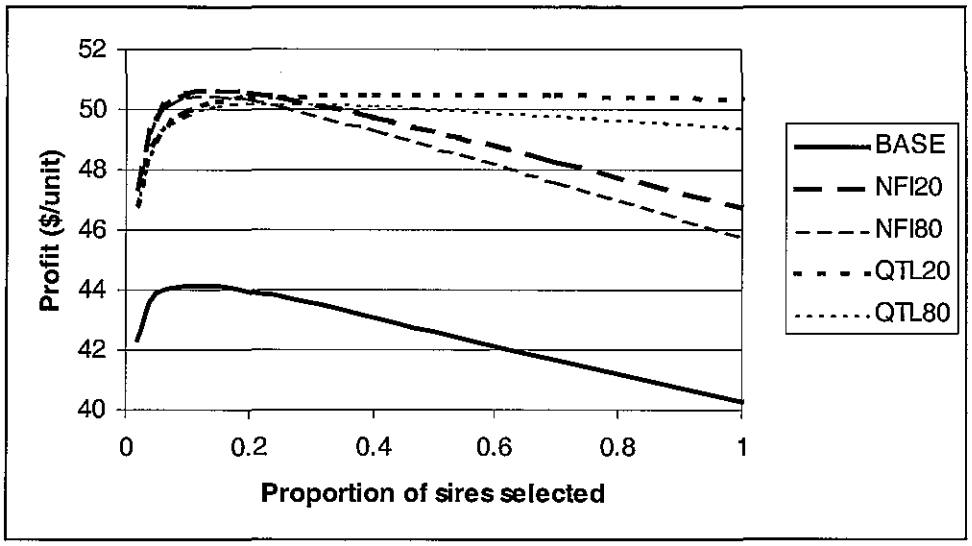
1. Measure actual NFI on all sires in the breeding unit (i.e. sires to breed stud sires, sires to breed commercial sires, and sires to breed stud cows). This is the "BASE" simulation.

2, 3. Measure actual NFI and genotype all sires in the breeding unit with genotype costs being $20 or $80. These are the "NFI20" and "NFI80" simulations respectively.

4, 5. Only genotype all sires in the breeding unit with genotype costs being $20 or $80. These are the "QTL20" and "QTL80" simulations respectively.

The results demonstrate that a QTL of the magnitude located in this study (C1) would have a high economic impact. Regardless of whether or not NFI was measured, QTL information would increase the accuracy of selection and, hence, profit per cow. This could be taken to extreme since genotyping without measuring the trait (QTL20) was more profitable that only measuring the trait (BASE). However, as stated above, this assumes that the variance due to the QTL is constant as the frequency of the favourable gene increases. If the favourable gene were of low frequency, then the variance would increase with selection. However, if the frequency were initially high, then the variance would decrease. Once the variance of the QTL decreases it is likely that it would become more profitable to measure the trait rather than rely solely on the DNA test. The cost of the DNA test ($20 versus $80) had an impact on profit but the effect was only small (around 10 cents/unit) compared to the effect of not genotyping at all.

Figure 8. Economic value of QTL for NFI



Measuring Net Feed Intake on stud bulls is profitable (BASE simulation) and hence recommended. In addition, all scenarios with information from genetic markers were more profitable than measuring NFI alone. Utilising only marker information (QTL versus NFI) was as profitable as markers plus measurement but is not recommended. The cost of the genetic test only had a small impact on profitability (NFI20 versus NFI80).

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