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THE AGRESEARCH/UNIVERSITY OF ADELAIDE QTL STUDY OF CARCASS COMPOSITION AND MEAT QUALITY TRAITS IN BEEF CATTLE

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SUMMARY

Over the last twelve years, a collaborative study with beef cattle has been carried out by AgResearch and the University of Adelaide to search for quantitative trait loci (QTL), primarily for carcass and meat quality traits. This paper reviews the QTL and related data collected so far. The main trial design in both countries involved crosses between the Limousin and Jersey breeds, with six first-cross (F1) sires mated to Limousin and Jersey cows. The resulting heifer and steer calves were grown out to slaughter. In New Zealand, all were fed on pasture; in Australia, the animals were finished in a feedlot. Up to 25.7 ± 5.5% of the differences in meat tenderness in cooked *M. longissimus dorsi* steaks were explained by the genotypes produced by two unlinked loci (the calpain-1 and calpastatin genes). The Limousin breed carries high frequencies of the 'F94L' mutant at the myostatin locus, and all six F1 sires were heterozygous. The effect of one copy of the 'F94L' mutant was to increase saleable meat weight by between 5.9 and 7.3%. QTL effects on fatty acid composition in adipose tissue, and effects of a candidate gene, fatty acid synthase, were also found.

INTRODUCTION

A large experiment to search for quantitative trait loci (QTL) for beef carcass and composition traits has been undertaken since 1995, by AgResearch (New Zealand) and the University of Adelaide (Australia). The objective was to find QTL, DNA markers linked to measured traits, and ultimately to use this mapping information to find causal gene variants. This paper describes the design and results obtained to date. An earlier update was presented to the AAABG six years ago (Morris *et al*. 2001).

MATERIALS AND METHODS

Jersey-Limousin backcross cattle. The trial design involved a double-backcross experiment with beef cattle. Six first-cross Jersey (J) x Limousin (L) sires were generated, and were used (three in each country) to breed backcrosses from both breeds of straight-bred dams, the so-called 'JL' trial. The offspring were phenotyped primarily for carcass composition and meat and fat quality traits, but many additional traits were also recorded, including resistance to facial eczema disease (New Zealand), food conversion efficiency (Australia), growth and behaviour traits, and age at puberty in heifers. Calves were born in 1996 and 1997 in New Zealand, and in 1996-98 in Australia. In each country, about 400 cattle (steers and heifers) were reared. In New Zealand, they were finished on pasture, whilst in Australia a feedlot finishing period of at least 180 days was used (*c*.65% grain). Cattle were grown to slaughter at ages of 22 to 28 months in New Zealand, and at ages of 34 to 40 months in Australia. Weights and levels of fatness at slaughter were greater in the Australian cattle.

Other cattle recorded. After some of the QTL effects on performance and carcass traits in the JL trial were initially identified, other animal resources were combined with the JL resource to follow up these findings. For tenderness studies, genotypes and phenotypes were added from research Angus cattle (Morris *et al*. 2006a), and from industry Herefords and Hereford-crosses with no known pedigree data. For fatty acid (FA) composition (adipose fat), milk fat phenotypes and associated genotypes were added from milk samples in two dairy trials: the first, in 2000-01, consisted of samples from 21 industry herds with daughters of 11 widely-used Friesian sires, average = 373 daughters per sire (Morris *et al*. 2002); the second, in March 2003, used 248 Friesian cows, Jerseys and their crosses from an AgResearch herd (a wide sample of sires, but few daughters per sire).

Measurements taken. For meat tenderness, shear-force measures were obtained from cooked *M. longissimus dorsi* steaks over a series of ageing stages *post rigor mortis* (Morris *et al*. 2006b). For retail beef yield% (RBY%), one carcass side from each backcross JL animal was butcher-dissected for RBY% and for its meat, fat and bone components (as described by Morris *et al*. 2006a). For FA composition, subcutaneous fat over the *M. longissimus dorsi* in JL cattle was analysed by standard methods (Morris *et al*. 2007).

Genotyping. On average, 189 microsatellite loci per sire family (range, 178 to 206), which were spread as evenly as possible over the 29 bovine autosomes, were genotyped over the JL backcrosses. QTL of experiment-wide significance were identified on most chromosomes. Subsequent searches for candidate genes under the QTL in our resources, along with literature checks at about the same time, led to genotyping tests with single nucleotide polymorphisms (SNP). For tenderness, Smith *et al.* (2000) had hypothesised that the calpain-1 (*CAPN1*) gene on cattle chromosome (BTA)29 was a candidate gene with a major effect on meat tenderness. DNA samples from segregating sire families at both the U.S. Department of Agriculture, Clay Center, Nebraska and in the New Zealand JL resource were genotyped for two *CAPN1* SNP at amino acid positions 316 and 530 (Page *et al.* 2002). The Australian JL resource was also genotyped for the *CAPN1* SNP at position 316 and New Zealand animals were genotyped for a calpastatin (*CAST*) SNP (*CAST*:c.2959A>G) originally reported by Barendse (2002) to show an effect on tenderness (Morris *et al*. 2006b). For RBY%, a Limousin-derived mutant myostatin allele ('F94L': Dunner *et al*. 2003) was found to be segregating in all the first-cross JL sires, and a SNP test using RFLP-PCR was developed (*MSTN*: c.413C>A; p.Phe94Leu) by Sellick *et al*. (2007). Sire-derived effects on body composition were evaluated for the F94L allele compared with the wild-type allele. For FA composition, fatty acid synthase (*FASN*) was a positional candidate on BTA19, and the effects of five *FASN* SNP were tested on FA composition in adipose fat in the New Zealand JL resource and in milk fat in the two New Zealand dairy resources described above.

RESULTS AND DISCUSSION

Meat tenderness. The *CAPN1* gene effect was confirmed using American and New Zealand JL resources (Page *et al*. 2002). Subsequent work using the JL backcrosses and other independent New Zealand Angus and Hereford cattle, examined the combined effects of *CAPN1* and *CAST* on meat tenderness, with comparisons at six stages ('Cook groups') during the meat-ageing process (Morris *et al*. 2006b). These two genes had significant effects at all stages except at *rigor mortis* itself ('Cook group 1'). In Cook groups 2-4 combined, the difference between *CAPN1* GG and CC genotypes on tenderness was 1.17 ± 0.10 kg, or 20.1 ± 1.7% ($P < 0.001$). The difference between *CAST* AG and AA genotypes was 0.50 ± 0.12 kg (8.6 ± 2.0% of the mean) over Cook groups 2-4 combined ($P <$

0.001), about 85% of the *CAPN1* single-copy substitution effect. There were very few *CAST* GG genotypes for comparison. For the *CAPN1* and *CAST* effects combined, the maximum genotypic difference in average shear force was 25.7 ± 5.5% ($P < 0.001$) at intermediate stages of the ageing process. Although there are now commercial SNP tests available to detect tenderness genotypes, the causative SNP(s) in *CAPN1* or *CAST* have not yet been convincingly identified.

Carcass composition. The Limousin-derived 'F94L' myostatin mutant allele was shown to produce effects on body composition (Sellick *et al*. 2007), including possible pleiotropic effects on other traits such as meat tenderness (Esmaili-Koshkoih *et al*. 2007). Adding one mutant copy in Limousin backcross animals was associated with an increase in saleable meat weight (5.9% and 7.3%, New Zealand and Australian projects, respectively, $P<0.001$), reduced fat depths measured on live animals and in P8 fat on the carcass (-13.9% and -18.7%, respectively, $P<0.001$, in the Australian project), and total fat yields (-8.1% and -16.5%, New Zealand and Australian projects, respectively, $P<0.001$).

Fatty acid composition. A significant QTL for FA composition was identified on chromosome 19. Of five SNP tested for *FASN*, one codes for an amino acid change, two are silent exonic SNP and two are intronic SNP (Morris *et al*. 2007), but the causative SNP still has not been identified. Significant association with *FASN* was demonstrated for adipose tissue from Jersey x Limousin crosses, and for milk fat from Jersey and Friesian cows. However, the gene has an effect which is opposite in sign in adipose fat from that in milk fat (Morris *et al*. 2007), for reasons which are unclear.

Other traits

Facial eczema. Facial eczema is a metabolic disease which is common in New Zealand. It is caused by sporidesmin, a toxin produced by spores of the fungus, *Pithomyces chartarum.* In susceptible ruminants, the toxin produces liver injury, and resistance is heritable in cattle (Cullen *et al*. 2006). JL backcrosses were phenotyped for resistance, and four QTL were identified. These QTL are being fine-mapped, using DNA from 174 accurately ranked New Zealand dairy sires, and from selected bull calves and cows which were phenotyped and found to be elite for resistance or susceptibility.

Food intake. In the Australian JL resource only, data were recorded on all the backcross cattle for daily feed intake and growth, on high energy feedlot rations. Similar work in a parallel mouse genomics project has also been conducted. From five initial QTL identified for cattle feed intake (Pitchford *et al*. 2002), one was followed in further detail in one sire family, and "the QTL resulted in animals that ate 14% less, spent 4% less time eating, and had a 10% lower eating rate".

Miscellanea. Other QTL identified include: horns (Morris *et al*. 2001), birth weight (Morris *et al*. 2003), two coat colour QTL (Bottema *et al*. 2004), ultimate pH of steak samples (Navajas *et al*. 2002), cooking weight loss in steaks (Pitchford *et al*. 2003), and meat colour (Koshkoih *et al*. 2005).

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