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PROGRESS IN DNA MARKER STUDIES OF BEEF CARCASS COMPOSITION AND MEAT QUALITY IN NEW ZEALAND AND AUSTRALIA

C.A. Morris[1], N.G. Cullen[1], S.M. Hickey[1], A.M. Crawford[2], D.L. Hyndman[2], C.D.K. Bottema[3] and W.S. Pitchford[3]

[1]AgResearch, Ruakura Agricultural Centre, PB 3123, Hamilton, New Zealand
[2]AgResearch, Molecular Biology Unit, PO Box 56, Dunedin, New Zealand
[3]Dept. of Animal Science, Roseworthy Campus, Adelaide University, SA 5371

SUMMARY

Two beef cattle DNA-marker trials in New Zealand and Australia are reviewed, where the trial objectives were to identify DNA markers for carcass composition and meat quality traits. The collaborative beef cattle project between AgResearch and Adelaide University is described in detail, and reference is also made to published data from the Australian 'Cooperative Research Centre (CRC) for Meat Quality' DNA-marker study. In the Beef CRC, F1 Charolais x Brahman bulls were mated to composite dams to breed experimental offspring. The AgResearch/ Adelaide study used two extreme breeds, Jersey and Limousin, with F1 Jersey x Limousin bulls mated to produce both back-crosses in both New Zealand and Australia. Detailed information is presented here from New Zealand only (416 animals), comprising firstly live-animal records including ultrasound fat- and muscle-depth data, metabolites from fat and muscle biopsies, and plasma cortisol at slaughter, secondly carcass dissection data and organ weights, and thirdly shear-force tenderness, pH measures and some detailed biochemistry from the right striploin. A whole-genome scan was carried out with an average of 185 informative microsatellite markers per sire (range 170-196), spread widely across the autosomes. Quantitative trait loci (QTL) were identified for 14 phenotypes (with a genome-wide threshold level of significance), from the New Zealand half of the data analysed, including composition traits (ultrasound, carcass and eye-muscle data), dressing %, bone weight, fat weight, meat tenderness, millicalpain, polled/horned status, and plasma cortisol. Corresponding data from the Beef CRC, which (from their interim publication) had detected 18 QTL for 54 carcass and meat quality traits (some markers above a significant lod-score threshold of 2.5), included birth weight, carcass weight and dressing %, saleable meat yield (kg), eye muscle area, marbling score, tenderness, rumpfat depth and fat colour. The sizes of significant effects for each New Zealand phenotype ranged from 2.0 to 31% of the respective trait means, and the Beef CRC effects ranged from 3.0 to 33%, although in both studies most traits (except for fat traits) were in the 3 to 10% range. Further opportunities for exploiting the results in the beef industry are discussed.

Keywords: Cattle, beef, carcass composition, meat quality, DNA marker.

INTRODUCTION

One of the recent innovations being developed to assist seed-stock beef producers with genetic improvement is DNA-marker technology. Making genetic changes in within-breed beef carcass composition and meat quality is difficult and expensive. Improving carcass composition currently involves ultrasound or progeny testing for carcass traits, whilst improving meat quality involves progeny testing for detailed meat measurements. It is difficult and time-consuming to keep track of

specified carcasses for this through a commercial meat works, and the measurement of meat quality traits in particular is a specialist field. The use of DNA markers may avoid these difficulties for carcass composition and beef quality traits, provided the markers are sufficiently accurate indicators of the biological traits predicted, and the price of the marker service is right.

Georges (2001) describes three phases in the development of Marker Assisted Selection programmes. Phase I is where flanking markers are identified near a gene significantly affecting a production trait of interest, i.e. a quantitative trait locus (QTL). The association between markers and QTL however needs to be re-established within each specific family. Phase II is where a QTL is fine-mapped with respect to closely linked markers, and where marker haplotypes and their associations with QTL hold across the population and therefore do not need to be re-established for each family. Phase III is where the specific genes and mutations for a given phenotype have been identified, and a "marker" is physically identified on the gene. The present review summarises up-to-date "Phase I" results from a DNA marker project in New Zealand (NZ) and Australia, and also published interim data from the 'Cooperative Research Centre (CRC) for Meat Quality' study, administered from Armidale, NSW (Hetzel *et al*. 1997, Davis *et al*. 1998). Results are summarised and compared from the two projects.

MATERIALS AND METHODS

Trial design. The main project described here is an international collaboration established in 1995 between AgResearch and Adelaide University, to identify DNA markers for QTL affecting beef carcass composition and meat quality. It uses two extreme breeds, the Jersey (J) and Limousin (L), in a double back-cross design (Morris *et al*. 2000). The two breeds differ, for example, in carcass composition, fat colour, marbling, milk yield, body size, age at puberty and meat tenderness (Cundiff *et al*. 1986). Three pairs of first-cross half-brothers were generated in this project, and one of each pair was used for mating in each country with both J and L cows. Over 400 experimental ¾J or ¾L calves were born in each country. The marker-research involves identifying those sire-derived alleles of the calves whose presence is associated with one or more performance traits ("phenotypes"). The primary traits of interest were carcass composition and beef meat quality traits, but other simple traits during the animals' growing phase were also recorded, such as live weights and ultrasound measurements. The second beef QTL project reviewed is from the Beef CRC, whose design consisted of three large half-sib families sired by Charolais x Brahman F1 bulls and out of composite *Bos taurus* x *Bos indicus* cows.

Experimental details. In NZ 261 experimental back-cross calves (162 ¾J and 99 ¾L) were born in spring 1996, with a further 155 born in spring 1997 (102 ¾J and 53 ¾L).The ¾J calves were born on dairy farms and were bucket-reared. The ¾L calves were born by embryo transplant as singles or twins in 1996 to Hereford x Friesian recipients, whilst in 1997 they were born following artificial insemination of F1 bulls over L cows. In both years the ¾L calves were reared on their dams. Calves were grown out on pasture only and slaughtered at approximately 2 years of age, with ¾J and ¾L animals both represented in every slaughter group. DNA marker results from only the NZ part of the AgResearch/ Adelaide study are summarised here.

In the Beef CRC, calves were weaned in 1992-94, with 237, 167 and 170 progeny per sire. These calves were born and grown out to 18 months of age at "Belmont", just north of Rockhampton,

Central Queensland. From there they were finished at one of two different sites in Central or South-Central Queensland up to target carcass weights of about 270 kg (steers) and 250 kg (heifers), with between 7 and 22 mm subcutaneous fat at the P8 site, and then slaughtered at abattoirs in Biloela or Rockhampton (Hetzel *et al*. 1997).

Traits recorded. On live animals in NZ, the recorded traits included: birth weights, live weights at regular intervals, polled/horned status, linear body measurements, ultrasound fat- and muscle-depth data, and pre-slaughter live weight. Fat biopsies and muscle biopsies were taken in order to provide, respectively, beta-carotene and muscle glycogen levels on live animals. Plasma cortisol was analysed from blood samples taken on the slaughter ramp immediately before stunning, and urinary cortisol was analysed from urine in the bladder immediately *post mortem*. On the carcass, hot carcass weight and right side weight were taken, along with several organ weights and depot-fat weight (the weights of pericardial, perirenal and omental fats). Dressing percentage was calculated from pre-slaughter weight and hot carcass weight. On the right side of each carcass, weights and then percentages of meat (M%), fat (F%) and bone (B%) were recorded, and a meat-to-bone ratio was calculated. On the right striploin muscle, pH was recorded at intervals from slaughter until *rigor mortis*, at which time the striploin was cut into steaks and shear-force measurements were taken on five cooked steaks (sampled at intervals from *rigor* until near ultimate tenderness). Components of the calpain system were measured on fresh striploin muscle sampled immediately *post mortem*. Percent intramuscular fat ("marbling") was measured from a video image of the cross-section of a steak from each animal. Glycogen and glycogen breakdown products were assayed from fresh striploin muscle taken immediately *post mortem*, to calculate a glycolytic potential at slaughter, and three enzymes were assayed, reflecting their activities in glycolysis (LDH), the tricarboxylic acid cycle (ICDH) and fatty acid oxidation (HAD). Full details of the protocols are given in Morris *et al*. (2001).

For the Beef CRC study, similar primary traits for carcass and meat quality were recorded to those in the AgResearch/ Adelaide study, plus a number of "growth and morphological traits", although the experimental detail of the actual 54 traits was not given by Hetzel *et al.* (1997).

Marker analyses. Sire-derived alleles were determined in the AgResearch/ Adelaide study for a total of 253 informative microsatellite loci spread across the whole genome, excluding the X and Y chromosomes (an average of 185 loci (range 170 to 196) per sire group). In the Beef CRC study, the interim data reported by Hetzel *et al*. (1997) included results from 153 markers, providing an estimated 81% coverage of the genome, from 311 progeny by two of the F1 sires.

Data analyses. Phenotypes in the AgResearch/ Adelaide study were pre-adjusted to account for known fixed effects, such as contemporary group (back-cross x rearing group) and slaughter group (for carcass or meat traits), and residuals were stored. Linkage between each microsatellite and phenotype was tested within sire using genome-wide threshold levels of significance, and the multiple-marker regression approach of Knott *et al.* (1996). The programme produces a test at regular small intervals along the length of each chromosome (for all its markers together). A significantly linked marker ($P < 0.05$, genome-wide test) was required to have an F test statistic > 7.8 (all sires) or >16.8 (single sire group). The polled/ horned data were analysed using Knott *et al.* (1996)

Table 1. Summary of phenotypes which showed significant QTL (using genome-wide threshold levels), with the Jersey (J)-derived alleles and Limousin (L)-derived alleles characterised at the marker site [RSD = residual standard deviation]

Trait group	No. of records	Herd mean	RSD	Allele difference J-L		
				RSD units	Actual units	Effect, % of mean
Live Animal						
Horns[A]	416	-	-	-	0.67	-
Ultrasonic eye-muscle fat depth (mm)	412	4.36	1.30	-0.94	-1.22	-28
Plasma cortisol at slaughter (ng/ml)	415	48.39	17.80	-0.83	-14.77	-31
Carcass						
Depot-fat weight (kg)	412	11.73	3.01	0.78	2.35	20
Kidney fat weight (kg)[B]	416	7.21	2.13	0.90	1.92	27
				0.81	1.73	24
%Meat in side	404	69.36	1.81	-1.24	-2.24	-3.2
%Fat in side	404	8.70	1.72	1.06	1.82	21
Dressing %	412	52.82	1.46	-0.73	-1.07	-2.0
Meat: bone ratio	408	3.20	0.20	-0.89	-0.18	-5.6
Bone weight in side (kg)	404	23.96	1.86	-0.73	-1.36	-5.7
Fat weight in side (kg)	404	9.55	2.18	0.93	2.03	21
Eye muscle width (mm)	329	57.58	5.45	-0.81	-4.41	-7.7
Meat						
Average shear-force (kgF)	394	9.19	1.13	-0.84	-0.95	-10
Millicalpain (i.u./g wet tissue)	256	1.27	0.16	0.96	0.15	12

[A] 1=polled; 2=horns, by Knott *et al*. (1996) procedures here; confirmed by chi-square analysis.
[B] Two different chromosomes.

procedures and also by chi-square, assuming that 'horned' was recessive to 'polled'. Similar regression procedures were used in the Beef CRC study, except that the threshold was determined as a lod-score "of 2.5, corresponding to a Type I genome-wise error rate of 5%".

RESULTS

Table 1 shows 15 significant markers or QTL identified so far (at the genome-wide significance level) for 14 phenotypes in the NZ half of the AgResearch/ Adelaide study. One of them (kidney fat weight) had significant markers on two different chromosomes. It is important to note that the power to detect significant markers in the project was originally worked out using animal numbers from both countries combined, so only half of the data are available so far. Nevertheless significant markers for live animal traits, carcass traits and meat quality have already been identified. Some of the traits were phenotypically correlated (e.g. M% and F% at -0.82, depot fat weight and F% at 0.57), so that there were fewer than 14 independent phenotypes with significant linkage shown. The sizes of the significant marker effects, relative to the overall mean, are also shown in Table 1, ranging from 2.0 to 31%. In the NZ half of the data alone, we did not find any significant markers for birth weight,

live or carcass weight, B%, individual organ weights, pH or pH change, marbling (one year only analysed so far), muscle glycogen or beta-carotene by biopsy, nor for the traits recorded in one year only: glycogen potential, urinary cortisol or the three metabolic enzymes.

In the Beef CRC study, Hetzel *et al*. (1997) reported some details for nine of the QTL which showed significance, including QTL for carcass weight and dressing %, saleable meat yield (kg), eye muscle area, marbling score, tenderness, rumpfat depth and fat colour. Davis *et al.* (1998) also reported a series of markers on five chromosomes (numbers 5, 6, 14, 18 and 21) with significant effects on birth weight.

DISCUSSION

Significant markers. For the 15 significant markers already identified in the AgResearch/ Adelaide study, two were *highly* significant (F test statistic > 20; $P < 0.0001$; all sires combined), and the Adelaide data may not be required in addition. Other marker tests just exceeded the required threshold, and it is clearly of interest to see the new outcome for traits against the test threshold after addition of the Adelaide data.

Hetzel *et al.* (1997) found QTL for 18 of 54 carcass and meat quality traits. However it was not stated how many of these markers were significant on a genome-wide basis. The authors pointed out that "one, or in some cases two, QTL were detected for most of the traits". So far we have found one trait (kidney fat weight) where marker loci from two different chromosomes were significantly linked to it, although more may become apparent when the second half of the data set is analysed together with the NZ half. Hetzel's group reported the size of marker effects linked with carcass weight (3.4% of the mean), dressing percentage (3.0% of the mean), predicted kg of saleable meat yield (4.1%), eye muscle area (9.3%), tenderness peak force (6.9%), marbling score (33%), rump fat depth (24%) and subcutaneous fat colour (15%). We found similar values for markers linked to traits that were significant (Table 1) and in common to the two trials. However the significant alleles in the AgResearch/ Adelaide study may not necessarily be the same ones for any trait in common across the two trials. It was to be expected that the NZ markers identified would all be of reasonable size (at least 0.7 phenotypic standard deviations here), because of the trial design.

Breed effects. Breed effects on saleable meat yield and carcass composition in the AgResearch/ Adelaide project are already published (Pitchford *et al*. 1998; Morris *et al.* 2000). They are generally consistent with the published data on Limousin-crosses and Jersey-crosses alongside other breeds and crosses (Cundiff *et al*. 1986). The marker effect for ultrasound eye-muscle fat depth (Table 1) was of opposite sign to the published breed differences.

The next stage? Hetzel *et al*. (1997) described their next stage as re-evaluating the most useful linked markers, in cattle populations directly relevant to the industry. The same markers might not apply in different populations and, since the markers are only *linked* to the relevant gene, the linkage phase for the "improver allele" could be opposite in other families. This is also true in the AgResearch/ Adelaide project. Any new industry project in NZ, however, depends for viability on continuing interest and assistance from local breeders or Breed Societies (e.g. recording animals at home; assistance with following carcasses at slaughter). The source of genotyping funds would also

have to be negotiated. All this would complete what Georges (2001) referred to as Phase I of an industry marker-assisted-selection project. We are also continuing at AgResearch and Adelaide University with Phases II and III, fine-mapping and searching for specific genes of interest, such as some of those involved in meat/ muscle and fat metabolism.

Conclusions. Fifteen significant markers have been found so far for 14 phenotypes in the AgResearch/ Adelaide trial. Only half of the final data set has been analysed so far; some markers are expected to be confirmed whilst other new ones may be found when the study is complete. Opportunities exist for industry groups to follow up these results in collaboration with us, eventually leading to direct application of marker technology in industry herds as marker assisted selection. In the Beef CRC study, scientists are already working with industry groups to confirm the linkage and linkage-phase of markers for economically important traits.

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