INTEGRATION OF DNA MARKERS INTO BREEDPLAN EBVS

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SUMMARY

Genotypes for the four GeneSTAR® tenderness markers and phenotypes for shear force of meat and flight time were used to develop marker assisted estimated breeding values for shear force (SF EBV^M). The partial regression coefficients for the effects of the four GeneSTAR tenderness markers on shear force were estimated and these were used in a prediction equation to calculate a single value for use in a multiple trait animal model to predict SF EBV^M on all animals.

INTRODUCTION

Livestock selection has used estimated breeding values (EBV) based on phenotypic data and pedigree records for more than 40 years. More recently, advances in molecular genetic techniques, in particular DNA sequencing, have led to the discovery of regions of the genome that influence traits in livestock. However utilising both sources of data in genetic evaluation schemes, such as BREEDPLAN, has been a challenge due to the heterogeneity of data sources, the multi-trait nature of the evaluations, and unknown effects of the marker information on all traits in the evaluation. The SmartGene for Beef project identified significant effects of the Catapult Genetics GeneSTAR® tenderness markers on meat tenderness as recorded by the objective measure of shear force (Johnston and Graser 2008; Johnston and Graser 2009). These results have been used to further develop methods for combining EBVs (i.e. phenotypic and pedigree data) and gene marker information into a single marker-assisted EBV called an EBV^M. Flight time is an objective measure of an animal's temperament which has been shown to be heritable and moderately genetically correlated with SF (Kadel *et al.* 2006), thus representing a potential genetic indicator trait for meat tenderness. Therefore the aim of this study was to use phenotypic records and GeneSTAR tenderness markers for the Brahman breed to estimate their combined effects on SF and incorporate them, using a multi-trait framework, into a marker assisted SF EBV^M.

MATERIALS AND METHODS

Data. The computation of the new tenderness EBV^M used three different sources of data. Shear force (SF) records from meat samples from the *M. longissimus thoracis et lumborum* of carcasses were available from Brahman animals measured through the Beef CRC1 (Johnston *et al.* 2003) and CRC2 (Wolcott *et al.* 2009) pedigreed breeding programs (N=1,995). GeneSTAR (commercialized by Catapult Genetics, now Pfizer Animal Genetics) gene marker results for one or more of the four tenderness markers (viz: T1, T2, T3, T4) were available on 7,040 Brahman animals from the Beef CRC1 and CRC2 projects (genotyped through the SmartGene Project) and industry tested animals that had GeneSTAR results submitted to the Brahman breed database. Finally, flight time (FT) records (N=4,737) on Brahmans recorded in the two CRC projects were used, along with a small number of industry recorded animals.

Computation of Cumulative Marker Phenotype (CMP). The first step to enable the inclusion of marker information into an EBV^M, was to compute the effects of each of the markers (i.e. T1, T2, T3, T4) on shear force, specifically for Brahmans using models and datasets developed as part of the SmartGene Project. The model included fixed effects previously defined for the two CRC

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experiments (Johnston *et al.* 2003 and Wolcott *et al.* 2009) and the genotypes for the four markers were included as linear covariables in the one model. The number of animals per genotype class and the estimated effects of the four tenderness markers are shown in Table 1.

The estimated partial regression coefficients for the effects of the four markers were used to construct a prediction equation that was then applied to each animal's marker genotypes to give a single prediction of the cumulative effects of the markers (CMP) for all genotyped Brahmans. However, given the significance of the T1 and T3 markers, both had to be genotyped for an animal to have a CMP computed. In total there were 4,729 animals with a computed CMP with a mean of 0.001 and a range of -0.68 to 0.30 kg SF.

Table 1. Number of animals per genotype (0, 1, 2), gene frequency of favourable allele (gf_q) and estimated partial regression coefficients (b) and standard errors of the four GeneSTAR tenderness markers (T1, T2, T3, T4) on SF in Brahman from Beef CRC1 and CRC2

Genotype	T1	T2	T3	T4
0	386	2066	1908	794
1	1322	141	753	1359
2	1081	14	76	627
gf_q	0.62	0.04	0.17	0.47
b (kg SF/allele)*	**-0.139** (0.041)	-0.087 (0.105)	**-0.234** (0.054)	-0.032 (0.040)

* bold significant P<0.05

Multi-trait analysis. A three-trait BLUP model was constructed using SF and FT phenotypes as well as the CMP record for the genotyped animals. Fixed effects of contemporary group (as previously defined) were fitted for FT and SF as well as linear covariates for age and carcass weight, respectively. Variance components were derived using a trivariate model where the residual variance of the CMP was set at 0.001 to assist convergence (i.e. heritability close to 1). The heritabilities of SF and FT were 0.30 and 0.20 respectively and the genetic correlation between SF and FT was 0.25 and between SF and the CMP was 0.28. The estimated genetic correlation between CMP and FT was small (-0.05), as were the residual correlations with SF and FT. Therefore model configuration allowed the information from the markers to contribute to the SF EBV^M through the genetic correlation structure.

Table 2. Genetic correlations (r_g) and percent additive genetic variance ($\%V_A$) explained by the GeneSTAR tenderness MVP on SF in four CRC1 datasets

	MVP	SF records			SF, MVP	SF, MVP
CRC1 Dataset	N	N	V_A	h^2	r_g	$\%V_A$
Straightbred temperate	659	3322	0.433	0.08 (0.04)	0.170 (0.14)	2.9
Straightbred tropical	585	3254	0.612	0.30 (0.06)	0.283 (0.08)	8.0
Crossbred temperate	253	785	0.658	0.26 (0.10)	0.126 (0.14)	1.6
Crossbred tropical	225	762	0.871	0.31 (0.10)	0.547 (0.13)	29.9

Adapted from www.beefcrc.com.au/Assets/473/1/Pfizer2.pdf Table 3

Comparison with new Molecular Value Predictions (MVP™). Whole genome associations studies using tens of thousands of SNP are finding large numbers of SNP, generally with small effects, associated with a range of economically important traits. In March 2009, Pfizer Animal Genetics computed a molecular breeding value (MBV) for meat tenderness (marketed as GeneSTAR® MVP™) using an extended panel of 56 SNP, including the four GeneSTAR

tenderness markers. This development signalled the move away from single SNP markers to the computation of MVPs. The effects of the GeneSTAR tenderness MVP were calibrated in four Beef CRC1 datasets and estimates of the genetic correlation and the percent additive genetic variance explained are presented in Table 2.

RESULTS AND DISCUSSION

Size of effects and gene frequencies. The results showed that the size of effects of the four markers differed (Table 1). Marker T3 had an estimated effect almost as large as the other markers put together, and markers T1 and T2 had intermediate effects. The gene frequencies of the four markers also differed. The frequency of the desirable form of the T2 marker was extremely low whereas that of T1 exceeded 60%. The different estimated size of the marker effects means that animals with the same total number of alleles (i.e. total stars) are likely to have different EBV^M.

Comparison with MVPs. Combining the effects of the four markers into the CMP in this study is a similar technique used to construct MBVs or MVPs. Results from this study showed that the cumulative effects of the four tenderness markers explained 8% of the additive genetic variance of SF (i.e. 0.28^2). This is comparable with variance explained by the new Pfizer tenderness MVP (Table 2) for tropical breeds (8%) and with the estimate of 7.4% in Brahmans from CRC2 (Johnston *et al.* 2009). Therefore the contribution to the SF EBV^M from the new tenderness MVP for Brahmans will be comparable to that from the original four GeneSTAR tenderness markers.

Computed EBV^M. A total of 22,052 animals (those with records and their ancestors) had SF EBV^M computed with a mean of 0.02 and a range of -0.98 to +1.36 kg. The units of the tenderness EBV^M are kg of SF and therefore lower (i.e. more negative) EBV^M have lower SF and more tender meat. The spread in the SF EBV^M of sires with an accuracy greater than 80% was almost 2 kg SF. Sires used in the Beef CRC projects were a random sample of the Brahman breed and the large spread generated was mainly the result of the large number of progeny recorded (N=16 to 68) for actual shear force for each of these sires, and the contribution of the other sources of information (i.e. markers and flight time) was minimal. When marker genotypes were the only available data then the SF EBV^M had a reduced spread equal to the maximum difference in the CMP, with an accuracy of 28%. The accuracy of the SF EBV^M for animals with their own SF record was approximately 55% and 79% when 20 progeny were recorded for SF. Adding marker information when phenotypic records were already available on the trait had little effect on increasing the accuracy.

Contribution of gene markers to EBV^M. The effects of the tenderness markers were included in the EBV^M using the multiple trait model where the relative contribution of the markers to the EBV^M depended on the estimated effect of each marker, their gene frequency and the genetic variation accounted for by the marker. Therefore if a marker has a reasonable size effect but is at high gene frequency (i.e. most animals have 2 copies) in a particular breed then this marker will be explaining very little of the differences between animals and therefore will have little contribution to differences in EBV^M between animals. To generate EBV^M with high accuracies from marker data alone will require finding numerous markers that explain a large amount of the genetic variation of a trait.

Once an animal's genotype has been established there is no benefit for that animal's EBV^M in testing relatives with the same panel of markers. This is different to recording phenotypic information, like flight time, where the records on relatives (i.e. sire, dam, half sibs and progeny) can be of considerable benefit in increasing the accuracy of the EBV and the EBV^M.

Future research. This new trial tenderness EBV^M is the start to a new chapter in the genetic evaluation of beef cattle in Australia. Research is underway to determine if the tenderness markers are genetically correlated with other economically important traits. Early indications, using Brahman BREEDPLAN EBVs, are that the tenderness markers are having no substantial effects on any of the published EBVs. Beef CRC2 research has also shown few antagonisms between shear force and other traits, but research is continuing to assess female lifetime reproductive performance and cow survival. To include tenderness EBV^M into a breeding program will require their inclusion into a selection index, and this will require the determination of the economic value of tenderness and estimates of genetic correlations with all other traits in the breeding objective.

The other important development is the expanding capacity to genotype animals for large numbers of potential markers and therefore the increasing opportunity to explain greater amounts of genetic variation. However with the trend towards panels of SNPs, and then the prediction of MBVs, there will be an ongoing need for the estimation of variances and covariances with phenotypic traits as well as any existing MBVs. To fully utilize this capacity the Australian beef industry needs to record many more phenotypes on animals with a DNA sample, particularly for traits difficult to record in industry. Efforts to do this are currently underway. Although there are theoretical predictions of completely replacing phenotypic records with large panels of markers in the future, this appears to be some way off for the beef industry. The current experience from the dairy industry, where the phenotypes used are the highly accurate EBVs of proven sires, is that the accuracies of EBV^M derived from only DNA marker information are around 70% (e.g. VanRaden *et al.* 2008). However this is unlikely to be achieved in the short-term in beef where unlike the dairy industry, there are relatively few animals with phenotypes or large numbers of sires with highly accurate EBVs for all economically important traits.

CONCLUSIONS

These SF EBV^M represent a first for the Australian beef industry, and a significant advancement in genetic evaluation. The methodology developed can be extended to use molecular breeding values from DNA companies, provided there are accurate estimates from independent datasets of the marker variance and, most importantly, the genetic correlations between the MBV and other traits.

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