

## GENOMICS CAN CONTRIBUTE TO SELECTION TO IMPROVE BOTTLE TEATS IN TROPICAL BEEF GENOTYPES

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### SUMMARY

Beef CRC research showed that a subjective score of teat size (small (1) to large (5)) was heritable in tropically adapted Brahman (BRAH) and Tropical Composite (TCOMP) cows, and that higher teat scores (bottle teats) were genetically associated with higher calf losses from birth to weaning. Teat traits are only expressed in females, and the research showed that they tended to display more variation in later life; making them ideal candidates for genomic selection. Front and rear teat scores (TSF and TSB respectively) were recorded in cows at calving through up to 6 matings. From these, a trait was created which described a cows maximum lifetime teat score (TSM), as well as a binary trait which distinguished cows that received a teat score of 4 or 5 at any time through their lives (1) from those which did not (0) (MSB). Results confirmed the heritability of TSF and TSB ( $h^2 = 0.30$  to  $0.40$ ), and variation in both TSM and MSB was also shown to have a genetic basis ( $h^2 = 0.49$  and  $0.46$  respectively for BRAH, and  $0.29$  and  $0.22$  for TCOMP). Genome wide association analyses identified large numbers of significant SNPs but did not suggest a likelihood of identifying a small number of SNPs of large effect. It is unlikely therefore, that a simple diagnostic test (based a small number of SNPs) could be developed for the traits. Conventional genomic selection, however, is likely to present opportunities to improving teat traits by selection in tropically adapted beef genotypes, with accuracies of genomic prediction of  $0.23$  to  $0.35$  for TSM and MSB across both genotypes.

### INTRODUCTION

Results from the Co-operative Research Centre for Beef Genetic Technologies' northern breeding project (Beef CRC) showed that a subjective score of teat size (1 (smaller) to 5 (larger)) was heritable ( $h^2 = 0.30$  to  $0.38$ ) in Brahman and Tropical Composite cows (Bunter *et al.* 2014). That study also showed that higher teat scores (bottle teats) were genetically associated with increased calf losses from birth to weaning ( $r_g = 0.54 \pm 0.18$ ), and that teat score tended to increase with cow age in both genotypes. This, combined with the sexually dimorphic nature of the trait makes it a prime candidate for genomic selection. This study aimed to determine whether SNPs of large effect for variation in teat score could be identified in the Brahman and Tropical Composite females, and to estimate the accuracy of genomic prediction for teat traits.

### MATERIALS AND METHODS

**Cow management and trait definitions.** A comprehensive account of cow herd management is provided by Johnston *et al.* (2009) and Johnston *et al.* (2014), and Bunter *et al.* (2014) presented a description of teat score measurement in the Beef CRC Brahman (BRAH:  $n = 969$ ) and Tropical Composite (TCOMP:  $n = 1085$ ) females. In brief, females were transported from their properties of origin (5 BRAH and 4 for TCOMP) to on one of four research properties. Defining features of each location were described in detail by Barwick *et al.* (2009), and were selected to be representative of the major production environments of northern Australia. Cows were first mated, in multiple sire groups, to calve as 3-year-olds, and were re-mated annually thereafter, unless culled for failing to wean a calf from consecutive matings.

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At calving, calves and their dams were located within 24 hours of birth, and calves were individually identified, and their gender, date of birth and dam's teat scores recorded. Teat scores were assigned for front (TSF) and back (TSB) teats on a 1 – 5 scale, with higher scores representing greater teat size. For this study, a trait describing a cow's maximum teat score (across all years for which cows had a record) was created for each animal (TSM), and a binary trait (MSB) was also generated which identified whether cows had a TSM of less than or equal to 3 (coded 0) or greater than 3 (coded 1). As a cow could have teat scores recorded at multiple calvings, there were a total of 3661 TSF and TSB, and 939 TSM and MSB for Brahman cows; with 5006 TSF and TSB, and 1053 TSM and MSB for Tropical Composite females (Table 1).

**Modelling and variance component estimation for teat score traits.** Fixed effect models for all teat traits initially included terms which described cohort (the location the cows were managed and their year of birth), the cow's property of origin, cow month of birth, previous mating outcomes and, for TCOMP, the genetic groups of their sire and dam. For both genotypes, all first order interactions were also tested in the initial models. Terms were dropped sequentially from the models in order of non-significance ( $P < 0.05$ ) to produce the final models for each trait-by-genotype combination. Following the methods described by Bunter *et al.* (2014), variance components for teat score traits were estimated in ASReml, with animal fitted as random and relationships between animals described using a three generation pedigree. For TSF and TSB, which included repeated records from animals, a permanent animal genetic effect was also fitted as random in the models.

**Genotyping and quality control for genotype data.** The genotype data used for study was a subset of Beef CRC genomic dataset. The database included high density Illumina genotypes (HD: 729,068 SNPs) for 1137 animals, with a further 14, 110 imputed to this level from the results of Illumina 50K or 80K SNP chips using the BEAGLE software package (Browning and Browning, 2011), with an accuracy of 90% (as described by Zhang *et al.* 2014). Of the 969 BRAH and 1085 TCOMP cows with records for teat score traits, 939 and 1053 respectively had SNP genotypes which could be analysed for this study. SNPs with low minor allele frequencies ( $< 0.05$ ) were excluded from the analyses, as were those which deviated significantly ( $P < 10^{-5}$ ) from Hardy-Weinberg Equilibrium, resulting in a total of 567,445 analysable SNPs.

**Genome wide association study.** The magnitude of individual SNP effects were estimated as a fixed effect in a mixed model that included animal fitted as random and all significant descriptors of environmental variation, as described by Hawken *et al.* (2012). The expected false discovery rate (FDR) was calculated as:  $FDR = p (1 - s/t) / [ (s/t) (1-p) ]$ , where  $p$  represents the threshold significance level tested (e.g. 0.01),  $s$  is the number of significant markers, and  $t$  is the total number of markers evaluated. To account for the multiple testing inherent in the GWAS methodology, further rigor was applied to the testing of significance of SNPs by applying a Bonferroni correction. This much more stringent evaluation of significance in multiple testing experiments divides the significance level applied by the number of tests carried out, and sets this as the threshold at which significance was evaluated.

**Genomic prediction and five-fold internal cross validation.** Genomic estimated breeding values (GEBV) for teat score traits were calculated using genomic best linear unbiased prediction (GBLUP). GEBVs were estimated with the genomic relationship matrix fitted as random (Zhang *et al.* 2014), and inverted using the Wombat software package (Meyer 2007). GEBV Accuracy (ACC) was calculated as:  $ACC = r/h$ , where  $r$  is the correlation between GEBVs and phenotypes (adjusted for fixed effects) and  $h$  is the square root of the heritability for the trait when estimated

in a model which contained all phenotypes and significant fixed effects, and with relationships described using a 3 generation pedigree. The accuracy reported for this study is the mean of five estimates from a five-fold internal cross validation of GEBV estimates (Zhang *et al.* 2014).

## RESULTS AND DISCUSSION

**Descriptive statistical and genetic parameters for teat traits.** Summary statistics, additive variances and heritabilities for teat score traits are presented in Table 1. Means show that on average, BRAH cows had higher TSF, TSB and TSM than TCOMP, and that it was more common for BRAH cows to record a teat score of 4 or 5 than it was for TCOMP (MSB = 0.30 and 0.21 for BRAH and TCOMP). For the subset of BRAH and TCOMP females with genotypes, heritabilities for TSF and TSB were consistent with those reported by Bunter *et al.* (2014), which was expected, as the estimates were based on very similar datasets ( $h^2$  TSF and TSB = 0.38 and 0.30 for BRAH and 0.37 and 0.31 for TCOMP from that study). Heritabilities for TSM and MSB were comparable with those for TSF and TSB. These results suggest that if breeders of tropically adapted beef cattle wished to apply selection to reduce teat size, or to select to reduce the incidence of high teat scores, this could be undertaken successfully.

**Table 1. Number of records analysed (N), mean and standard deviation (SD), with additive variance ( $\sigma_a^2$ ) and resultant heritability (and associated standard error (s.e.)) for teat score traits in Brahman and Tropical Composite cows.**

Traits	N	Mean	SD	$\sigma_a^2$	$h^2$	s.e.
<b>Brahman</b>						
TSF	3661	2.81	0.89	0.27	0.40	0.08
TSB	3661	2.70	0.82	0.18	0.32	0.07
TSM	939	3.39	0.88	0.39	0.59	0.12
MSB	939	0.30	0.46	0.10	0.46	0.12
<b>Tropical Composite</b>						
TSF	5006	2.65	0.84	0.17	0.30	0.08
TSB	5006	2.53	0.79	0.16	0.30	0.08
TSM	1053	3.22	0.77	0.15	0.29	0.10
MSB	1053	0.21	0.41	0.04	0.22	0.09

**Genome wide association study (GWAS) for teat score traits.** Tables 2 presents the number of significant SNPs identified at levels of  $P \leq 0.05$ , 0.01, 0.001 and 0.0001 for each of the teat score traits evaluated. Results for both genotypes suggest that there were high numbers of significant SNPs identified for the teat score traits evaluated. Across the four teat traits evaluated, the expected FDR averaged 0.41, 0.41, 0.42 and 0.52, for significance levels of 0.05, 0.01, 0.001 and 0.0001 respectively. FDR tended to be higher for TCOMP with results suggesting that for TSF and TSB, none of the SNPs identified as significant were beyond the expectations of chance. For the remaining teat traits, however, these results demonstrate a capacity to successfully identify significant SNPs, and suggest that genomic selection could be undertaken successfully in the genetic evaluation for tropically adapted beef genotypes to improve teat score. After accounting for the multiple testing associated with GWAS analyses through the application of a Bonferroni correction ( $P \leq 0.05 = 8.8 \text{ E-}8$ ), there were far fewer significant single SNPs associated with the teat score traits evaluated. For BRAH, none retained significance, while only a small proportion of SNPs identified for TCOMP TSM and MSB traits were still significant ( $n = 0$  to 24).

**Table 2. Number of significant SNPs identified from genome wide association studies and accuracy of genomic prediction based on 5-fold cross validation for teat score traits in Brahman and Tropical Composite cows.**

Breed	Trait	Number of significant SNPs at $P \leq$				Accuracy of genomic prediction
		0.05	0.01	0.001	0.0001	
Brahman	TSF	60352	11955	1063	13	0.12
	TSB	75518	17730	2015	13	0.08
	TSM	62820	13659	1458	12	0.30
	MSB	64107	14253	1656	8	0.27
Tropical Composite	TSF	67045	13971	1416	5	0.09
	TSB	49633	8433	579	3	0.10
	TSM	77891	20038	3181	136	0.23
	MSB	66195	15211	2068	88	0.35

**Genomic prediction for teat score traits.** Table 2 also presents the accuracies of GEBVs estimated from five-fold internal cross-validation for teat score traits. The results suggest that GEBVs for TSF and TSB would provide only limited opportunities to change the trait by genomic selection, due to the low accuracies of GEBVs (ACC = 0.08 to 0.12). TSM and MSB displayed higher accuracies of genomic prediction (0.23 to 0.35), suggesting that genomic information could make a useful contribution to genetic evaluation for these traits.

## CONCLUSIONS

This study confirmed the heritability of repeated measures of teat scores, and found that new traits, describing maximum lifetime teat score and whether cows ever recorded a high score (4 or 5), displayed similar heritabilities ( $h^2 = 0.22$  to  $0.59$ ). A genome wide association study showed that variation in teat traits was associated with a large numbers of genes, and that development of a genomic test, based on small numbers of genes of large effect, was unlikely to be successful. Results indicated however, that GEBVs for teat traits had accuracies between 8 and 30 percent. Beef CRC research showed that high teat scores (bottle teats) were significantly genetically associated with early calf survival in tropical beef genotypes. The results of this study suggest that genomics could contribute to the genetic evaluation for teat traits, and correlated female reproduction traits, if they were included in the evaluation for tropical beef breeds.

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