

IMPROVING THE ACCURACY OF GENOMIC SELECTION FOR LACTATION ANOESTROUS INTERVAL IN BRAHMAN CATTLE

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SUMMARY

Lactation anoestrous interval (time from the beginning of the annual mating period to the identification of the cows' first subsequent *corpus luteum* by ultrasound scanning) was measured in 898 Brahman females. Previous analyses, including genomic prediction, were performed using a trait defined in cows which successfully weaned a calf (as 3 year olds) from their first mating (LAI: $n = 629$). The current study expanded this dataset by including an additional 269 records for cows from the same experiment, whose first calf was weaned from their second annual mating, increasing the number of records for the new trait (LAI12) to 898. Heritability for LAI12 (0.43 ± 0.13) was consistent with the previous estimate for LAI (0.51 ± 0.18). A genome wide association study identified more significant SNPs at the $P < 0.01$ level for LAI12 ($n = 16,886$) than were previously identified for LAI ($n = 597$). Importantly, a five-fold cross-validation analysis showed that the accuracy of genomic EBVs was increased from 0.14 to 0.24. Expanding the definition of lactation anoestrous interval to that described for LAI12 identified more significant SNPs associated with the trait and increased the accuracy of the associated genomic EBVs for lactation anoestrous interval.

INTRODUCTION

Female reproduction is a key driver of profitability for beef producers in northern Australia. Research examining the genetics of reproductive traits in northern Australia's tropically adapted beef cattle has shown that extended post-partum anoestrous intervals contributed significantly to low weaning rates (Baker 1969; Entwistle 1983). This was more recently confirmed by Johnston *et al.* (2014) who showed that lactation anoestrous (LAI: a measure in lactating first calf females of the time in days between the start of the second annual mating period and the estimated date of first ovulation based on regular ultrasound scanning to detect ovarian function) was more heritable ($h^2 = 0.51$) than measures of net female reproduction traits. The study also showed that in Brahman females, lower (more favourable) LAI was significantly genetically associated with higher lifetime calving and weaning rates ($r_g = -0.71 \pm 0.21$ and -0.62 ± 0.24 respectively) in Brahman cows. However, in the cows examined for that study, reproductive rates from their first mating meant that only 63% of females ($n = 629$) were eligible to receive a LAI record.

Hawken *et al.* (2012) conducted a genome wide association study to identify single nucleotide polymorphisms (SNPs) which explained genetic variation for a range of female reproduction traits in the same females described by Johnston *et al.* (2014), and Zhang *et al.* (2014) estimated genomic breeding values, and their accuracy, from the same data. This research showed that some opportunities exist to exploit genomic selection for female reproduction, but that LAI had the lowest accuracy of genomic prediction (0.14) due, in part at least, to low numbers of records for the trait. This study, therefore, aimed to maximise the data which could be analysed for lactation anoestrous interval in Brahman females by re-defining a trait to include records from cows which failed to wean a calf from their first annual mating, to estimate variance components for this trait and to determine whether it can improve the accuracy of genomic prediction in Brahman cattle.

* AGBU is a joint venture of NSW Department of Primary Industries and the University of New England

MATERIALS AND METHODS

Animals and measurements. Animals evaluated for this study were a subset of the Brahman females (N = 1,026) from the Co-operative Research Centre for Beef Genetic Technologies Northern Project. Breeding and management of heifers up to their first annual mating was described by Barwick *et al.* (2009), and Johnston *et al.* (2009; 2014) described management through their first (as 2 year olds), and subsequent matings. Cows which failed to wean a calf in consecutive years were removed from the experiment, which meant that no culling on the basis of reproductive performance was undertaken in females prior to their second annual mating. At the start of the second and subsequent annual mating period, regular ultrasound scanning of the ovaries to identify the presence of a *corpus luteum* was undertaken in lactating cows. This identified the onset of cycling and this interval defined LAI (Johnston *et al.* 2014). For the current analysis, females whose first calving and lactation was the result of their mating as 3 year olds (i.e. for cows which failed to wean a calf from their first mating) also had a record included in the analysis of lactation anoestrous interval. All records for this trait (LAI12) were for the animal's first lactation, and no cow had more than one record included in the analysis.

Statistical analysis. Fixed effect modelling for LAI12 followed the protocols described by Johnston *et al.* (2014) for LAI, and initially included descriptors of the cow (property of origin (4 levels), month of birth (5 levels), dam age (7 levels), mating group (26 levels)), and the calf (calf month of birth (5 levels) calf sex (2 levels)), and all first order interactions. For females whose LAI12 record was from their mating as 3 year olds, mating group described both current and previous mating groups, ensuring that cows which had a record from their first mating were never analysed in the same contemporary group as those whose record came from their second mating. Terms were sequentially dropped from the model based in order of non-significance, with final models containing effects which were significant descriptors of systematic variation in LAI12 at $P < 0.05$. Following the methods described by Johnston *et al.* (2014), variance components for LAI and LAI12 were calculated in ASReML (Gilmour *et al.* 2009), with animal fitted as random and relationships between animals described using a three generation pedigree.

Genotyping and genome wide association study (GWAS). The Beef CRC database includes 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 SNP chips using the BEAGLE software package, with an accuracy of 90% (as described by Bolormaa *et al.* 2013). Of the 898 BRAH females with a record for LAI12, 875 had genotypes which could be analysed for this study. Thirteen percent of these were from the HD SNP chip, with the remainder imputed from 50K. SNPs with low minor allele frequencies (< 0.05) were excluded from the analyses. The magnitude and significance of SNP effects were estimated as the solutions for each SNP when fitted as a fixed effect in a model with animal fitted as random and including all significant descriptors of environmental variation. The expected false discovery rate (FDR) was calculated as $FDR = p(I-s/t)/[(s/t)(1-p)]$, where p is the threshold significance level tested (e.g. 0.001), s is the number of significant markers, and t is the total number of markers evaluated.

Genomic estimated breeding values (GEBV) for LAI12 were calculated using genomic best linear unbiased prediction (GBLUP). The genomic relationship matrix was fitted as a random effect to estimate GEBVs (Zhang *et al.* 2014), using the method described by Yang *et al.* (2010), and inverted using the Wombat software package (Meyer 2007). GEBV Accuracy (ACC) was estimated as $ACC = r/h$, where r is the correlation between GEBVs and phenotypes and h is the square root of the heritability. The accuracy reported for this study is the mean of five estimates from a five-fold cross validation of GEBV estimates (Zhang *et al.* 2014).

RESULTS AND DISCUSSION

Table 1 presents the number of observations and descriptive statistics for LAI (from Johnston *et al.* (2014)) and LAI12. By including results for Brahman cows whose first lactation was from their second annual mating, the number of records available for analysis increased from 629 to 898 (by 43%), with LAI12 displaying a lower mean and slightly lower standard deviation than LAI. This shows that when Brahman cows' first lactation was from their second annual mating, lactation anoestrous interval was shorter than for cows when the trait was from their first annual mating. As more data becomes available for lactation anoestrous, it may be appropriate to test whether first lactation anoestrus records from the first and second annual mating were the same trait, and consider running a bivariate analyses if the relationship proved to be less than unity.

Table 1. Number of records (N), mean and standard deviation (s.d.), with additive and phenotypic variances (σ_a^2 and σ_p^2) and heritability (h^2) (standard error in parenthesis) for LAI and LAI12.

Trait	N	Mean	s.d.	σ_a^2	σ_p^2	h^2
LAI [†] (days)	629	134	109	5238	10271	0.51 (0.18)
LAI12 (days)	898	116	106	4115	9482	0.43 (0.13)

[†] From Johnston *et al.* (2014)

Table 1 also presents the additive and phenotypic variances for LAI and LAI12, and the resultant heritabilities for the traits. These show that expanding the definition of lactation anoestrous did not significantly change its heritability suggesting that selection to improve the trait would be similarly effective for LAI and LAI12.

Genome wide assessment of SNP effects for LAI12. The numbers of significant SNPs at levels from $P < 0.01$ to 0.00001, and the proportion in each of these categories expected to be identified by chance, are presented in Table 2. The magnitudes of SNP significance estimated in the current experiment were, higher than those reported for LAI in the study of Hawken *et al.* (2012), where only 530, 66 and 3 SNPs significant at the $p < 0.01$, 0.001 and 0.0001 levels were reported.

Table 2. Number of significant SNPs from genome wide association study (GWAS) for LAI12 in Brahman cows, and the proportion which could be expected as false positives (FDR) at significance levels from $P < 0.01$ to $P < 0.00001$.

GWAS outcomes	N (MAF > 0.05) [‡]	$P < 0.01$	$P < 0.001$	$P < 0.0001$	$P < 0.00001$
SNPs Observed	567,445	14,449	2,127	289	21
FDR		0.38	0.27	0.20	0.27

[‡] Total number of SNPs included in the analysis with minor allele frequency greater than 0.05

Similarly, the FDR for LAI12 from the current study represents a marked improvement for those reported by Hawken *et al.* (2012) for LAI at the 0.001 and 0.0001 levels of 0.67 and 1.00 respectively. These results suggest that expanding the definition of lactation anoestrous to include results for cows whose first calf was from their second annual mating, increased the capacity of genotypic data to describe genetic variation in lactation anoestrous in Brahman females.

Accuracy of genomic estimated breeding values for LAI12. The results of the five-fold cross-validation showed that the accuracies for GEBV for LAI12 in Brahman was 0.24. This result

was higher than the accuracy of 0.14 reported by Zhang *et al.* (2014) when the trait was defined exclusively in cows which had successfully weaned a calf from their first annual mating. Given the high inputs in management, operator expertise and therefore cost associated with accurate measurement of lactation anoestrous, the opportunity to evaluate the trait using genomic technologies is particularly attractive. The results of this study show that by expanding the definition of the trait to include data from cows whose first lactation was from their second annual mating, the accuracy of genomic breeding values was increased by a factor of almost 60%.

CONCLUSIONS

Lactation anoestrous is an important determinant of reproductive performance in northern Australia's Brahman cattle population. Results of this study have shown that expanding the definition of the trait to include results from cows whose first lactation was from their second annual mating, increased the number of records available for analysis while not significantly changing heritability for the trait. A genome wide association study showed that more significant SNPs were identified for LAI12 than were found for LAI. Importantly, the accuracy of genomic breeding values estimated for LAI12 were also significantly higher than those estimated for LAI. Lactation anoestrous is a difficult and time consuming trait to measure accurately, particularly under the extensive conditions which prevail in northern Australia. As more data becomes available for the trait from research, beef information nucleus and seedstock herds, genomics will provide opportunities to improve lactation anoestrous in Brahman cattle by selection.

ACKNOWLEDGEMENTS

The authors acknowledge Meat and Livestock Australia for their financial support through B.NBP.0761, and the CRC for Beef Genetic Technologies which generated the data analysed for this study.

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