Final report

Southern Multibreed Immune Competence Project – Improving the resilience of Australian beef cattle

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Abstract

Immune competence (IC) reflects the ability to cope with disease by mounting an effective immune response.

The measurement of IC in this study, from 3,000 cattle, extends the availability of the trait across Angus, Brahman, Charolais, Hereford, Simmental, Wagyu and associated Crossbreeds. Results reveal the contemporary distribution of the IC trait, the relationship to productivity traits and has delivered genomic tools for breeding strategies.

Distribution of the IC values was assessed by breed and found to be overlapping, with all breeds having individuals that span low to high IC. A slight negative relationship was observed between IC and growth-related traits confirming that IC should be considered in breeding objectives to ensure no inadvertent deterioration of herd IC status. A genomic prediction equation has been produced that can be used to estimate IC values across beef cattle breeds.

Ensuring cattle are immune competent is vital for minimising productive loss caused by disease, maintaining high standards of animal welfare, maximising the response to vaccines and reducing reliance on therapeutics such as antibiotics to prevent and treat disease. Demonstration that IC levels are maintained within the Australian herd may provide an important and convenient metric to demonstrate the beef industry commitment to sustainability, and more generally help maintain consumer confidence in products of the beef industry.

Executive summary

Background

Immune competence is an important component of animal resilience, reflecting the ability of the animal to cope with disease challenges through the mounting of an effective immune response. Ensuring cattle are immune competent is vital for minimising production loss caused by disease, maintaining high standards of animal welfare, maximising the response to vaccines and reducing reliance on therapeutics such as antibiotics to prevent and treat disease. A low-level negative relationship has been observed between immune competence and growth/productivity traits, meaning that selective breeding for growth, with no consideration of immune competence, has the potential to inadvertently reduce immune system effectiveness.

The measurement of immune competence in this study extends the availability of the trait across major Australian beef breeds (Angus, Brahman, Charolais, Hereford, Simmental, Wagyu, and Crossbreeds) and as such will allow the beef industry to better understand the contemporary distribution of the trait, the relationship of the trait to other key productivity traits and ultimately deliver tools allowing optimal breeding strategies to be developed which simultaneously target improved disease resistance and productivity.

Objectives

This study aimed to characterise the distribution of immune competence within the main beef cattle breeds in Australia, to determine relationships between immune competence and other productivity traits and to deliver a genomic prediction equation for immune competence for these cattle breeds.

- Refined resilience testing protocols, incorporating measures of immune competence, stress coping ability (through walk over weighing (WOW) and temperament, which are practical to conduct on-farm, and are targeted at specific breeds and specific environments.
- Information on the distribution of performance for immune competence traits within and across breeds and breed specific genetic parameter estimates associated with immune competence and resilience-related traits.
- Formulas to calculate breed-specific immune competence index values for individual animals. Immune competence index values are weighted based on the heritability of component traits (which may vary between breeds), to encourage genetic gains for each trait to occur at the same rate.
- Information describing associations between the resilience-related traits; immune competence, stress coping ability and temperament, within and across breeds and associations between these traits and health and welfare outcomes in commercial production systems.
- Define specific attributes associated with an improved ability of cattle of particular breeds to cope with specific environmental challenges which could be utilised to improve the resilience of cattle of other breeds.
- Identify specific genetic markers associated with an improved ability of cattle of particular breeds to cope with specific environmental challenges which could be targeted in genetic selection programs aimed at improving resilience in other breeds.
- Development of GEBVs for resilience-related traits within and across breeds. Monitoring genetic improvement in resilience traits in beef cattle over time has the potential to be used by industry as a sustainability metric to demonstrate their on-going commitment to improving animal health and welfare.

- On-going communication of the project and its outcomes through Southern Multibreed extension activities in consultation with MLA and NSW DPI.

Methodology

Both heifer and steer progeny from the 2022 (S) and 2023 (T) cohorts of the Southern Multibreed (SMB) project were enrolled in the study. A total of 1408 S and 1775 T progeny were tested, and these represent multiple breeds and sites. Total number of progeny = 3,183.

- Progeny were immune competence tested at weaning using the phenotyping methodology developed in MLA project B.STU.0244. Additional immune response related measures were collected on a small subset of animals from each breed in Yr2 of the project to help identify attributes of particular breeds which allow them to better cope with specific disease challenges.
- Additional resilience-related phenotypes were collected on all progeny at weaning including measures of temperament/docility (already being collected as part of broader SMB project) and stress coping ability. The potential of using 'weight change over weaning' as an indirect measure of stress-coping ability was investigated in Yr2 of the project. A pilot trial was conducted using walk over weighing (WOW) technology (initially a single unit incorporated into a weaning yard at two separate locations). Data collected using the WOW unit was correlated with periodic weight data collected using traditional methods to assess accuracy and reliability of weight measures.
- Detailed 'whole of life' health and performance data to be recorded on all resilience tested progeny. Performance traits were recorded in line with already established SMB protocols. Health data recorded will include disease incidence during backgrounding and feedlot finishing for steers and disease incidence on pasture of retained females. Longitudinal worm egg count (WEC) measures will also be recorded (as part of broader SMB project). Where possible new and novel traits will be recorded on SMB cohorts such as lung lesions.
- Associations between resilience traits both within and between breeds will be investigated.
- Breed specific attributes associated with their improved ability to cope with specific environmental challenges will be investigated.
- Breed specific genetic markers associated with improved resilience will be identified and gEBVs for resilience-related traits within breeds generated (this may require testing of additional cohorts of SMB progeny).
- Collection of second blood sample stored for further analyses of project outcomes if required.

Results/key findings

Distribution of the immune competence trait values was assessed by breed and found to be overlapping, and as such all breeds assessed will have individuals that span low to high immune competence. A similar result was observed for crossbred animals. A slight negative genetic relationship was observed between immune competence and growth-related traits confirming that immune competence should be considered in breeding objectives to ensure no inadvertent deterioration of herd immune competence status when selecting for improved productivity. A genomic prediction equation has been produced that can be used to estimate Immune Competence values in the main Australian beef breeds and subsequent crossbred cattle.

Benefits to industry

The development of strategies aimed at improving the resilience of Australian beef cattle has the potential to:

- Increase the ability of Australian cattle to cope with environmental challenges posed by an ever-changing environment.
- Improve animal health & welfare.
- Reduce use of antibiotics in the food-chain and costs associated with treating disease.
- Reduce wastage resulting from animal mortality/morbidity.
- Provide an objective means of demonstrating industries commitment to achieving sustainability-based goals.
- Maintain consumer confidence in the Australian beef industry and the products they produce.

Future research and recommendations

- Collection of further IC phenotypes to improve accuracy of associated genomic predictions.
- Incorporation of detailed 'whole of life' health (disease incidence and health-related mortality data) phenotypes for association with the immune competence trait when available.
- Improve the genotype data using imputation to the 100K chip or HD level (700K).
- Adopt the CSIRO in-house genomic breed composition tool to confirm the breed composition of tested cattle (i.e. exploring the finding that the breed of some tested animals appeared to be misclassified as seen in the PCA plots).
- Explore alternative GBLUP models including effect of heterosis and inbreeding.
- Explore alternative GRM including breed specific allele frequencies to determine if a generic 'across breed' genomic prediction provides sufficient accuracy or if breed specific genomic predictions are required to provide improved GEBV accuracy.
- Cross-validation studies to assess accuracy of resulting GEBV (both within and across breeds).
- Develop genomic prediction equations for IC, with associated accuracy calculation, to identify elite animals for different breeds.
- Consider linking datasets to Angus and Brahman data (Immune competence and sensor behavioural data) collected during other projects (eg. Angus Sire Benchmarking Projects and Northern Repronomics Project).
- Consider opportunity to develop a resilience index including traits such as immune competence, temperament, weight change over weaning across and within breeds and across different production environments.

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1. Background

Cattle face a variety of challenges in production environments including exposure to infectious agents, climatic extremes, social stressors arising from mixing with unfamiliar animals and management induced stressors imposed by standard husbandry procedures and practices (Hine et al 2014). Animals respond to these challenges through a variety of host defence reactions involving immunological, behavioural and physiological responses. These responses are highly integrated and, in combination, determine an animal's resilience or capacity to cope in the production environment (Colditz & Hine, 2016).

Immune competence (IC) is an important component of an animal's resilience, reflecting its ability to cope with disease challenges. In a recently completed MLA funded project, B.STU.0244, associations between the resilience traits of immune competence, stress-responsiveness and temperament in 1149 performance recorded Angus calves during yard weaning, and production and disease traits during feedlot finishing were investigated. Immune competence was found to be moderately heritable and favourably correlated with stress-responsiveness and temperament (Hine et al 2021). In that study cattle were classified into one of three categories, high, average or low responders, based on an IC score that was calculated from a combination of antibody and cellular immune response parameters. Animals with high IC demonstrated a strong ability to deal effectively with disease, with no recorded mortalities and health costs of $4 per head. Conversely, the low IC group had a mortality rate of 6.1% and incurred substantially higher health associated costs (estimated at $103 per head). Animals classified into the average IC group displayed intermediate values with a mortality rate of 1.2% and health associated costs of $28 per head. Collectively these results suggest that selection for enhanced IC can improve an animal's ability to cope with disease challenges and, therefore, contributes to an animal's resilience. Removing animals with suboptimal, likely low level, IC from the herd is not only expected to improve the general disease resistance of the herd, but also improve the efficacy of vaccines commonly used in industry. Predictions of genetic merit for IC will also inform targeted management decisions aimed at matching animals with the most suitable production environment to optimise health and welfare outcomes. As an example, cattle with low immune competence scores would not be prioritised for feedlot entry as animals within this category have an increased likelihood of poor outcomes in a feedlot (Hine et al 2021).

A key to the future sustainability of the Australian beef industry is an ongoing ability to meet consumer demands for the highest standards of animal health and welfare, while at the same time, reducing the use of antibiotics in animals producing their food. Although there is limited evidence to support any contribution of antibiotic use in livestock to the global issue of antimicrobial resistance, the public perceive this as a risk (Etienne et al 2017; Doster et al 2022). The Australian beef industry is strongly committed to meeting these consumer expectations.

Contributing to this challenge, intensive genetic selection for productivity traits, with no emphasis on health and fitness traits, is known to reduce the ability of animals to cope with challenges posed by their production environment (Rauw et al 1998). A coordinated approach involving targeted management, improvements to environmental conditions and genetic strategies aimed at improving the resilience of livestock will be required to address these issues. A possible genetic solution is to combine production traits and health related traits into a weighted selection index with the aim of breeding highly productive animals with enhanced general disease resistance (Hine et al 2019).

Traditionally it has been difficult to compare animals from different breeds as an animal's 'life experience' can influence how they respond to subsequent environmental challenges. Therefore, the

Southern Multibreed project provides a unique opportunity to investigate resilience-related traits within and across breeds by assessing such traits in individuals from different breeds but with a similar 'life experience'. This project aims to investigate resilience traits across different breeds of beef cattle, not to assess which breed is more immune competent, but rather, to identify attributes of a particular breed which make them better able to cope with specific challenges and investigate if such attributes can be targeted in other breeds to improve their resilience. Data generated from this project will improve our understanding of the distribution of performance for IC traits across and within breeds and will help identify genetic aspects of IC that are common to all breeds. Those of which may prove suitable candidate markers for the generation of multibreed genomic estimated breeding values (GEBVs).

2. Objectives

The following objectives have been achieved.

- Conduct immune competence testing, at weaning, of approximately 3,000 heifer and steer progeny from two cohorts of the Southern Multibreed (SMB) project using the phenotyping methodology developed in MLA project B.STU.0244.
    - Achieved. Immune competence phenotypes were collected from a total of 3,183 cohort S and T Southern Multibreed project calves.
- Investigate associations between resilience traits both within and between breeds.
    - Achieved. Phenotypic correlation of resilience traits (including temperament, weight change and immune competence) was assessed. Summary statistics of resilience traits have been determined for the entire population and by breed.
- Assess the potential of using 'weight change over weaning' as an indirect measure of stress-coping ability by correlating to immune competence measures. Conduct a pilot trial using walk over weighing (WOW) technology to generate weight change data (initially a single unit incorporated into a weaning yard at two separate location). Correlate WOW data with periodic weight data collected using traditional methods to assess accuracy and reliability of weight measures.
    - Achieved. Pilot trial to assess weight change over weaning as measured by static scales and walk over weighing scales was completed. Weight data measured by the two systems has been correlated and the accuracy assessed.
- Investigate breed specific attributes associated with their improved ability to cope with specific environmental challenges.
    - Partially achieved. The summary statistics of resilience traits has been reported by breed. The ability to correlate these to health related outcomes has been limited by the unavailability of health records.
- Calculate phenotypic associations between resilience traits (immune competence, flight time and weight change over weaning) and production traits.
    - Achieved. Phenotypic associations between resilience traits and productivity traits (weight and carcase) have been reported.
- Report on the distribution of performance for immune competence traits within and across breeds and breed specific genetic parameter estimates associated with immune competence and resilience related traits.
- Achieved. The adjusted phenotypes across 15 traits were analysed in a series of 105 bivariate GBLUP models (i.e. all possible bi-variates) to obtain estimates of variance components, heritability values, genetic and residual correlations. Identify breed specific genetic markers associated with improved resilience and genomic estimated breeding values (GEBVs) for resilience-related traits within breeds generated.
    - Achieved. A multibreed genomic prediction equation for immune competence has been produced. See section 4.6 for a detailed rationale behind the decision to change from breed specific to a multibreed genomic prediction equation.

3. Methodology

3.1 Animal Ethics Approval

Animal ethics applications, that cover all experimental activities undertaken as part of this project, have been approved by the CSIRO Chiswick Animal Ethics committee. AEC reference approvals designated (ARA 22/02) and (ARA 22/28) Immune Competence Testing – Southern Multibreed Project.

Reciprocal approvals were also granted by the NSW DPI Orange Animal Ethics Committee to cover activities performed on DPI sites (OAEC-0283 Immune competence testing - Southern Multibreed Project).

3.2 Resilience Testing

An animal's immune competence, stress responsiveness and temperament all strongly influence their inherent resilience. To improve understanding of how each of these components of resilience contribute to the variation seen in the ability of different breeds to resist certain diseases in their production environment, calves were resilience tested. Testing occurred during the yard weaning period and calves were then followed through backgrounding, feedlot finishing (steers) and growing out on pasture (heifers) to collect detailed health and performance data. Data collected by CSIRO is shown in Tables 1 and 2. Data collected by NSW DPI is shown in Table 12.

Measures of an animal's ability to mount both cell-mediated and antibody-mediated responses to an immune challenge, delivered on the day of weaning, was used to assess immune competence. Weight change over the weaning period was used as an indirect measure of stress-responsiveness to routine management procedures and flight time and crush score (measured both in the current project and/or in broader SMB project) was used in combination to assess temperament.

Table 1. Timetable for resilience testing procedures conducted on farm. All calves within a given contemporary group at a given research station were tested on the same day.

Day	Operation
Day 0	Weaning Liveweight recording Vaccinate with clostridial vaccine (Ultravac 7in1, Zoetis) Flight time testing Crush score assessment
Day 8, 9 or 10	Liveweight recording Injections for DTH skin test Flight time testing
Day 10, 11 or 12	Liveweight recording Measure response to DTH skin test Collect blood sample Flight time testing

3.3 Immune Competence Assessment

Animals received a clostridial vaccination (Ultravac 7in1, Zoetis) at marking, approx. 4-6 weeks post-marking and again on the day of weaning (Day 0) to induce measurable immune responses. All vaccinations were administered subcutaneously behind the ear as per manufacturer's instructions. A flow diagram describing the various steps involved in determining each individual animal's immune competence phenotype is presented below (Figure 1). To improve theability to identify resilient animals, immune competence testing coincided with weaning so that the immune competence of animals could be assessed whilst animals were under the influence of management induced stress imposed by weaning.

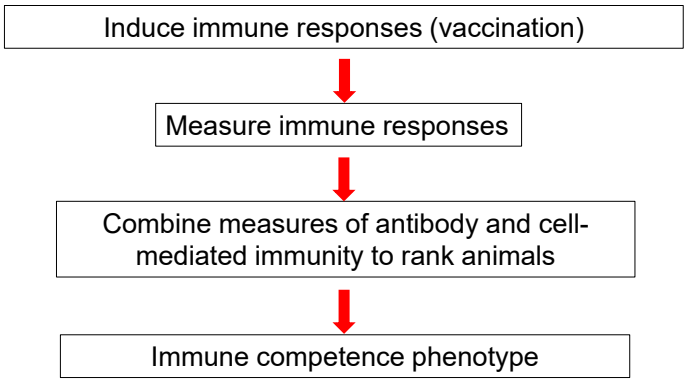

Figure 1. Flow diagram describing the steps involved in determining the immune competence phenotype of individual animals.

3.3.1 Assessing Cell-Mediated Immune Responses

The magnitude of delayed-type hypersensitivity (DTH) skin reactions to vaccine components was used to assess the cell-mediated immune responsiveness of individual animals. To elicit DTH responses, a test (vaccine) or control (saline) solution was injected intradermally in opposing caudal folds of the tail using an insulin syringe with a 30G needle. Prior to injection, skin thickness measurements were taken with calipers, at each respective injection site, to provide a baseline skin thickness. At 48 hours post-injection, changes in skin thickness at each injection site (test and control) were again assessed using calipers (Figure 2A). All animals received a total of 2 intradermal injections as part of the testing procedure, an injection of clostridial vaccine (test reaction, Ultravac 7in1 0.1mL) and an injection of saline (control reaction, 0.1mL), in opposing caudal fold sites on each side of tail. Increases in skin fold thickness at 48hrs post-injection (relative to changes at the control site) were used to assess the magnitude of cell-mediated immune responses. A typical test reaction response is shown below (Figure 3B).

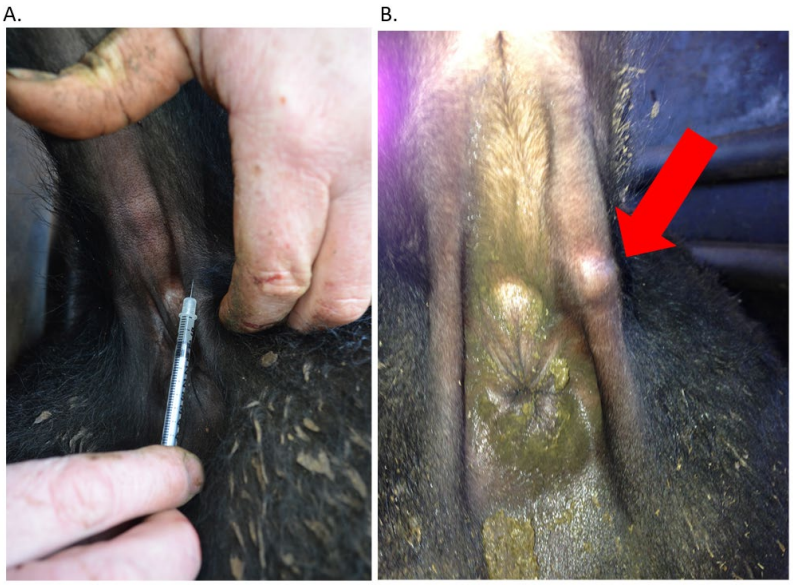

Figure 2. Intradermal injection of vaccine solution into the caudal fold as part of delayed-type hypersensitivity (DTH) testing to assess cell-mediated immune responsiveness (A). A typical DTH response to injected vaccine observed 48Hrs post-injection (B).

3.3.2 Assessing Antibody-Mediated Immune Responses

Production of antibody, more specifically anti-tetanus toxoid serum IgG1, in response to vaccination was be used to assess antibody-mediated immune responsiveness. The clostridial vaccination administered to animals at marking, post-marking and again at the commencement of weaning (Day 0) contains tetanus toxoid antigen. To obtain serum samples for antibody testing, a total of 2*10mL blood samples were collected into serum tubes (red cap) using jugular venepuncture post-vaccination. Optimal timing of collection, to coincide with peak antibody response, was conducted at 14 days post vaccination. Serum was prepared from coagulated blood by centrifugation (700 × *g*, 20 min, RT) and stored in multiple aliquots at −20°C (or -80°C for long-term storage) for subsequent laboratory analysis. Antibody levels were determined using an ELISA procedure, developed previously and reported in detail in (Hine et al 2019). Briefly, total serum IgG1 antibody against tetanus toxoid antigen (Zoetis, Australia) was determined using an indirect ELISA method. All test and control samples were assayed in quadruplicate. The CV of quadruplicate (using all replicates) and all possible combinations of triplicate values (sequentially leaving a single replicate out) were calculated, and the value for the combination with the lowest CV was used in analysis. If a CV value of <10% was not achieved, the sample was re-assayed. Pooled pre- and post-vaccination serum samples, prepared by combining equal volumes of serum from multiple individual calves, were used as negative and positive controls, respectively. Mean OD values for replicates were corrected based on the mean OD value of control serum samples assayed on all plates. Antigen-specific total IgG1 was detected using affinity purified. sheep anti-bovine IgG1 conjugated to alkaline phosphatase (AbD, Serotec, Product No. AAI21AB).

3.3.3 Stress-responsiveness Assessment

Average daily weight gain (ADG) during the yard weaning period was used as an indirect measure of responsiveness to management-induced stress. Yard weaning provides an ideal stressor upon which an animal's ability to cope with management induced stress can be assessed as it involves handling stress associated with human interactions, social stress due to separation anxiety, potentially mixing with new herd mates and stress associated with exposure to a new environment and diet. All cohort 1 calves tested were weighed multiple times during the weaning period, including at the commencement of weaning (Day 0), at the time when DTH injections were administered (Day 8, 9 or 10) and again at the time when the magnitude of DTH reactions were assessed at the end of weaning (Day 10, 11 or 12). Additional weight records during weaning period were collected by NSW DPI staff as part of the broader SMB project. Timing of weighing was consistent within each testing cohort. Weight gain was calculated as the mean of the average daily gain recorded between each weighing event. In Year 2 of the project walk-over weighing (WOW) was incorporated into a weaning yard at two research stations and data from WOW was be compared to weight change determined using periodic weighing on a traditional weighing platform.

3.3.4 Temperament Assessment

The temperament of individual calves was assessed using flight time testing and crush scores. Crush scores were assessed by trained NSW DPI staff on the day of weaning (Day 0) by placing calves in the crush (not restrained in the head bale) for a period of 30 seconds and scoring their behaviour in the crush on a scale from 1 to 5 using a standardised scoring system (conducted as part of the broader SMB project). Flight time was assessed multiple times on the day of weaning (Day 0) (by trained NSW DPI staff) at Day 8, 9 or 10 (by CSIRO staff) and again at Day 10, 11 or 12 (by CSIRO staff). To measure flight time post-weaning, calves were first restrained in the head bale for approx. 1 min while skin injections were administered (Day 8, 9 or 10) or while blood samples were collected (Day 10, 11 or

12), calves were then pushed back into the crush, allowed to settle briefly and then released from the crush and their flight time recorded using electronic equipment as per standard operating procedures (Burrow et al 1988; Hine et al 2019). A typical setup for assessing flight time is shown below (Figure 4). Briefly, for flight time testing, animals were released from the crush/chute following restraint in a head bale for blood collection and their time over a distance of 1.8 m recorded using electronic equipment. Calmer animals are expected to have longer flight times as they exit the crush/chute more slowly.

Figure 3. Standard flight time testing setup.

3.4 Collection of walk over weighing records

Walk over weighing scales were deployed at Glen Innes (Datamars FLEXIDRAFT MOBILE 4000C) and Trangie (Datamars Prime 4000C). Scales were set up as per manufacturers' instructions. See Walk Over Weighing (WOW) systems | Help Centre | Datamars Livestock. The unit was located so as to provide the only means of access to an attractant (water). In seeking water, the livestock walk across, or on to, the weigh platform where a weight is collected, the electronic identification ear tag is scanned, and the animal weight is matched to this. Algorithms calculate the animal's liveweight and adjusts for gutfill. Data is processed and delivered through online software, Datamars Livestock. https://monitoring.livestock.datamars.com/

Figure 4. Walk over weighing scales installed in Glen Innes yards.

3.5 Calculation of Immune Competence Values

Immune competence or ImmuneDEX (IDEX) is calculated as described in Reverter et al (2021). ImmuneDEX considers the correlation (r) between Cell-IR and Ab-IR as well as the difference in ranking (dRank) of individuals for each metric, and uses them as weights in the averaging as follows:

$$\text{IDEX} = [Z_{\text{CELL}} + (1 - |r|)\, Z_{\text{AB}}] \left(1 - \frac{|d\text{Rank}|}{n-1}\right)$$

In agreement with the finding of Dominik et al. (2019), IDEX places greater emphasis on Cell-IR. Neither r nor dRank are affected by the standardization of Cell-IR and Ab-IR into ZCELL and ZAB, respectively. At r = 1.0, dRank = 0, and IDEX = ZCELL. On the other extreme, at r = –1.0, dRank = n/2 on average (where n is the sample size or 2,853 in our case) and IDEX = ZCELL/2 on average. However, also at r = –1.0, ZMEAN is uncorrelated with either Cell-IR or Ab-IR. With complete independence at r = 0, IDEX and ZMEAN were on average identical in value for a function of dRank which can range from 0 to (n – 1).

ZMEAN may be obtained from the average of the 2 individual metrics after subjecting each to a Z-score standardization by subtracting the mean and dividing by the standard deviation:

$$Z_{\text{MEAN}} = \frac{Z_{\text{CELL}} + Z_{\text{AB}}}{2}$$

Where ZCELL and ZAB are the Z-score standardization of Cell-IR and Ab-IR, respectively. Standardizing prior to averaging was done to ensure both metrics were in standard units and to stabilize the variance, otherwise the resulting average would be dominated by the most variable metric. Assuming ZCELL and ZAB are independent, ZMEAN was expected to follow a normal distribution with zero mean and a variance equal to 0.5. Also, ZMEAN was expected to be equally correlated with both ZCELL and ZAB. Finally, ZMEAN corresponds to the function evaluated by Dominik et al. (2019) at weight $\alpha = 0.5$.

3.6 Fixed effects models for the analysis of resilience-based traits and ancillary phenotypes

The original aim of this project was to undertake a comprehensive analysis of the six putative resilience phenotypic traits of: Flight Time (FTime), Liveweight at start of weaning (LWt1 and WWT for weaning weight), Liveweight gain during weaning (WtGain), Cell-mediated Immune Response (Cell_IR), Antibody-mediated Immune Response (Ab_IR), and ImmuneDEX score (IDEX).

Subsequent discussions with MLA and Animal Genetics & Breeding Unit (AGBU), revealed the possibility of adding a further nine phenotypes to the overall analyses as well as genotypes for the same set of cattle. In November 2024, NSW DPI and AGBU made available to CSIRO the following additional nine phenotypes: birth weight (BW), hip height at weaning (HH), 400-day weight (Wt400), carcase weight (CWT), eye muscle area (EMA), subcutaneous fat depth at the rib (RIB) and at the rump (RUMP), MSA marbling score (MSA_MB) and MSA Index (MSA_Index). However, carcase traits were only available for ~640 steers from year drop “S” (ie. 2022). The inclusion of weight and carcase traits will be used in the calculation of phenotypic associations with resilience measures such as immune competence. Low value negative correlations have been observed previously between immune competence and productivity traits and as such it is important to determine if a similar

relationship is observed here. Although the carcase records are only available for a proportion of the test animals the number is sufficient to generate a reliable result.

To analyse the 15 traits and to obtain adjusted phenotypes (required for subsequent genomic analyses), the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used to fit a linear fixed effect model that contained linear covariates and class effects. All models contained the covariates of age at measurement (except for BW) and the first 5 principal components of the genomic relationship matrix (see next sections). In addition, the model for the analyses of immune competence phenotypes (Cell_IR, Ab_IR and IDEX) contained the covariate of change in skin fold thickness (CST). In relation to class effects, all model contained the effect of breed (9 levels) and a contemporary group that included the effects of Sex (2 levels), Property (5 levels) and year of birth (YOB; 2 levels).

3.7 Genotypes, genomic relationship matrix (GRM) and principal component analyses (PCA)

In November 2024, the project received from AGBU the raw genotypes for 3,133 cattle (or 98.7% of the 3,175 with immune competence phenotypes) from three different SNP chips. These chips included the Illumina 100K chip (N=885 cattle), the Illumina 50K Indicus chip (N=344 cattle) and the Zoetis 65K chip (N=1,904). The observed SNP overlap among chips implied that 158,591 unique SNP were represented.

Because of time constraints and the lack of a suitable reference population, genotypes were not imputed to a single platform (eg. 100K map). Instead SNP with minor allele frequency (MAF) > 5% were selected and observed in at least 25% of cattle and from at least 2 of the 3 chips. These editing criteria resulted in 42,772 high-quality SNP to be used for further analyses.

This panel of 43K SNP was employed to generate a multi-breed genomic relationship matrix (GRM) among all 3,133 cattle and the PCA of the GRM revealed clusters consistent with their breed denomination. The first 5 PC were fitted as covariates in the analytical models and explained over 20% of the variation in genomic relationships and with PC1 to PC5 explaining, respectively, 6.95%, 5.75%, 3.65%, 2.31%, and 1.43%.

3.8 Multibreed genomic BLUP (GBLUP) and genomic breeding values (GEBVs)

The adjusted phenotypes across the 15 traits were analysed in a series of 105 bivariate GBLUP models (i.e. all possible bi-variates) to obtain estimates of variance components, heritability values, genetic and residual correlations. Reported heritability values for each trait correspond to the average estimate obtained across the 14 bi-variate models where that trait was represented. Similarly, trait GEBVs for the 3,133 animals in the GBLUP models were the average obtained across the 14 bi-variate models where that trait was represented.

All genomic analyses were undertaken using CSIRO-owned UNIX scripts and FORTRAN source codes. Variance components in GBLUP were estimated using a CSIRO-owned version of Qxpak 5.0 software and specifically compiled for Petrichor, a CSIRO high-performance computing cluster of over 400 server nodes with more than 25,000 cores and 235 TB of system memory.

4. Results

4.1 Traits collected

Table 2 contains the list of traits collected from the Multibreed Cohort S (2022) and T (2023) calves that were analysed in this report. This data is stored in the CSIRO database and is available on request.

Table 2. Traits measured on Cohort S and Cohort T calves.

Trait	Number of Records	Description	Indicator For	Timing
FTime (sec)	3164	Flight Time	Temperament	Day 8, 9 or 10
LWT1 (kgs)	3155	Liveweight at start of weaning	Growth	Day 8, 9 or 10
WtGain	2955	Liveweight gain during weaning	Resilience	Liveweight at end of weaning – Liveweight at start of weaning
CST (mm)	3175	Change in Skin Thickness	Cell_IR control	End of weaning (Day 10, 11 or 12)
Cell_IR (mm)	3175	Cell-mediated Immune Response	Immune Competence	End of weaning (Day 10, 11 or 12)
Ab_IR (OD)	3175	Antibody-mediated Immune Response	Immune Competence	End of weaning (Day 10, 11 or 12)
IDEX	3175	ImmuneDEX score	Immune Competence	Combination of Ab_IR and Cell_IR

4.2 Summary of cattle sampled by year, property, breed and sex.

Tables 3 and 4 contain a summary of the property, sex and number of cattle tested from the Southern Multibreed project. Data is presented by year / cohort.

Table 3. Number of cohort 1 (S) weaners that were immune competence tested in 2022 by Property and Sex.

DPI Property	Sex	Number of Animals
Grafton	Heifers	179
Grafton	Steers	182
Trangie	Heifers	92
Trangie	Steers	83
Tocal	Heifers	121
Tocal	Steers	101
Glen Innes	Heifers	97
Glen Innes	Steers	86
EMAI	Heifers	212
EMAI	Steers	253
TOTAL		**1408**

Table 4. Description of cohort 2 (T) weaners that were immune competence tested in 2023 by Property and Sex.

DPI Property	Sex	Number of Animals
Grafton	Heifers	222
Grafton	Steers	211
Trangie	Heifers	215
Trangie	Steers	192
Tocal	Heifers	106
Tocal	Steers	110
Glen Innes	Heifers	117
Glen Innes	Steers	153
EMAI	Heifers	239
EMAI	Steers	210
TOTAL		**1775**

The number of immune competence records collected and presented by breed across the 2022 and 2023 S and T cohorts stands at 983 Angus (AA), 112 Brahman (BB), 337 Charolais (CC), 679 Hereford (HH), 412 Simmental (SS), 415 Wagyu (WY), and 237 Crossbreed (AABB, BBAA, BBHH, HHBB). Results are shown in Table 5.

Table 5. Number of cattle immune competence tested by breed.

Breed	Number of Animals Immune Competence Tested		
	Cohort S 2022	Cohort T 2023	Total
AABB	20	28	48
AA	406	577	983
BBAA	32	38	70
BBHH	29	37	66
BB	61	51	112
CC	148	189	337
HHBB	35	18	53
HH	313	366	679
SS	185	227	412
WY	174	241	415

4.3 Resilience measures and immune competence summary statistics

In this section the full set of resilience traits measured on the Multibreed cohorts S and T cattle are reported on.

Weight gain across the weaning period (Wt Gain) may be indicative of an ability to thrive in the face of a period of stress that occurs due to separation from the dam. As such this trait may be a novel indicator of resilience. Flight Time (FTime), or the average time taken to exit the cattle crush on three occasions may be an indicator of animal temperament, with calmer animals taking longer to exit than cattle that are highly stimulated. Conversely, exceptionally long flight times may also be indicative of timid or non-co-operative individuals. This temperament trait may be useful in assessing animal resilience to management events and handling.

Immune competence is calculated from the two component traits, the antibody mediated immune response (Ab_IR) and the cell mediated immune response (Cell_IR). The cell mediated immune response is calculated after accounting for control skin thickness (CST).

Summary values for all resilience traits, immune competence and the respective component traits (Cell_IR and Ab-IR) are presented in Table 6. Walk over weighing (WOW) scales were deployed at Trangie and Glen Innes for the 2023 Cohort T calves. Only the Glen Innes deployment returned usable data. Variation between weights recorded by static and WOW scales has been observed. This data is reported in detail in milestone 5 report of this project. The manufacturer of the WOW scales has since recommended that the WOW scales not be deployed for a period of less than 1 month, making this technology not fit for purpose for measurement of weight change during the weaning period, that takes approximately 2 weeks. Variation between weights recorded by static and WOW scales has been observed. Due to concerns about the reliability of the WOW data it has not been included in further analysis here but weight change over weaning as measured by static scales has been included.

Table 6. Summary statistics of traits collected from Cohort S and T calves.

Variable	N	Mean	Std Dev	Min	Max
LWt1	3155	252.24	54.31	88.00	426.00
WtGain	2955	0.22	0.92	-5.07	7.03
FTime	3164	0.98	0.51	0.21	7.04
CST	3175	-0.01	0.04	-0.20	0.16
Cell_IR	3175	31.71	9.82	-0.45	71.72
Ab_IR	3175	86.41	17.64	0.00	127.60
IDEX	3175	-0.01	1.14	-5.96	4.19

Table 7 contains the immune competence values for the purebred cattle tested from Cohorts S and T. The value includes measures of both the cellular and antibody based immune responses. A negative value indicates relatively lower immune competence, and a positive value indicates relatively higher immune competence, within this population of animals. The calculation of immune competence centres the distribution of the ImmuneDEX values around zero with the result being that half the animals will have a negative immune competence value and half a positive value (See Figure 5). A negative value is not an indication of any functional consequence but merely that the animal sits in the lower half of the distribution. It is tempting to compare the breed mean values but, again, these means should not be interpreted directly as an indication of functional difference. All breeds show overlapping distributions (as seen by the minimum and maximum scores presented in Table 6 and frequency distribution shown in Figure 6). The range of the distribution is likely to be influenced by the number of animals tested, with breeds having higher numbers of tested animals likely to cover a wider distribution of values. Equally, it is important to note that a threshold of immune competence has yet to be defined. By this, we mean a level of immune competence below which an animal is likely to have an ineffective or low functioning immune system. The values are most appropriately used as a means of including immune competence in a breeding objective aimed at moving the population mean by reducing the number of animals at the lower end of the distribution.

Table 7. Immune competence values by breed and year tested.

Breed and Year Tested	Immune Competence				
	Number Tested	Raw Mean	Standard Deviation	Minimum	Maximum
AA__2022	406	-0.0237448	1.19083	-4.78428	3.68504
AA__2023	577	-0.0244153	1.18113	-5.96106	3.66155
BB__2022	61	-0.554985	0.920339	-3.89646	1.44912
BB__2023	51	-0.440203	1.26418	-4.81164	2.63188
CC__2022	148	0.110497	1.12201	-3.57425	2.77093
CC__2023	189	0.376801	1.02482	-2.48525	4.18932
HH__2022	313	0.116108	1.10779	-4.48262	3.04509
HH__2023	366	-0.12296	1.0971	-3.58744	3.128
SS__2022	185	-0.114495	1.05094	-3.35053	3.09103
SS__2023	227	0.177775	1.08354	-3.46209	3.6833
WY__2022	174	0.212589	1.10057	-2.61664	3.0357
WY__2023	241	0.210992	0.969889	-3.80474	3.50976

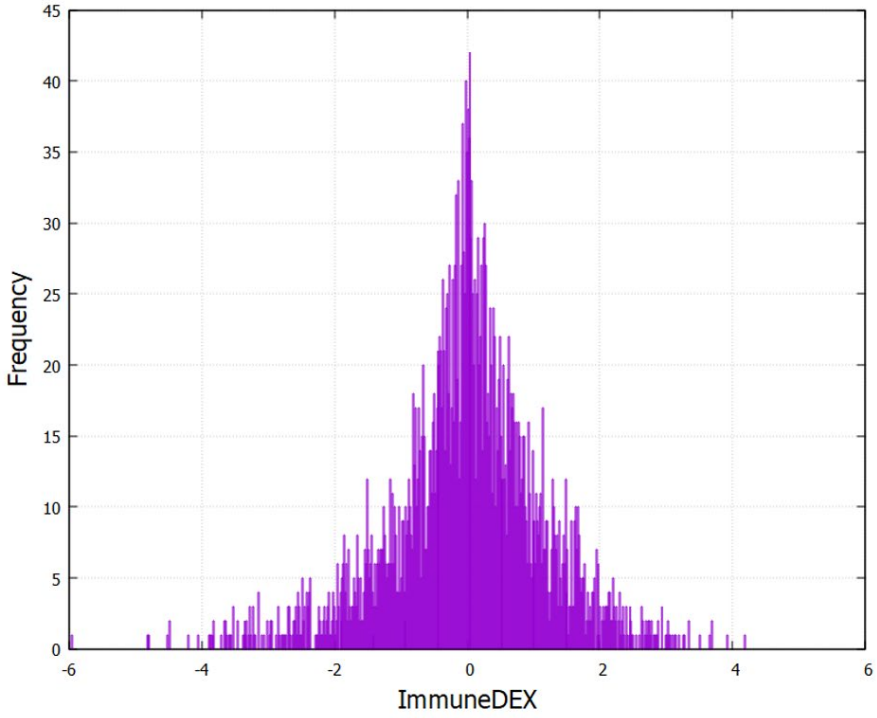

Figure 5. Frequency distribution of Immune Competence scores across the Cohort S and T calves.

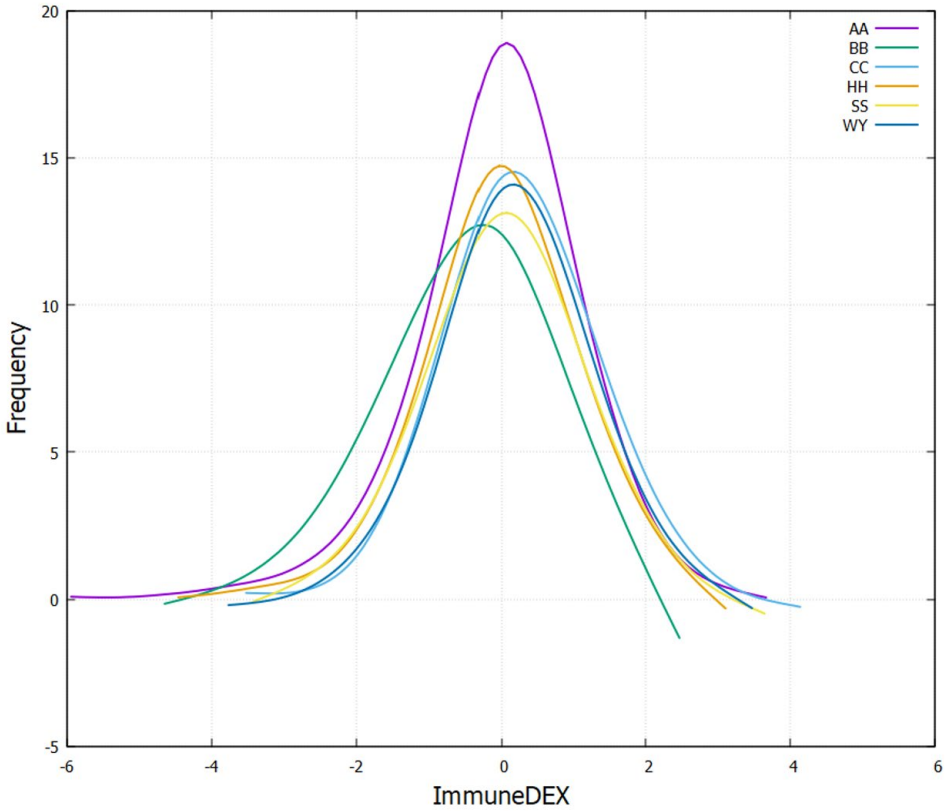

Figure 6. Frequency distribution of immune competence scores by breed.

Table 8 contains the immune competence values for the crossbred cattle tested from Cohorts S and T. The nomenclature represents the sire and dam of each animal, where AABB indicates an Angus Sire and Brahman Dam whereas BBAA indicates a Brahman sire and Angus Dam. Here again the distributions of immune competence values are overlapping and are likely to be highly influenced by the relatively low numbers of animals tested. This data could be useful for determining paternal or maternal influences on the inheritance of immune competence, but many more records (in the order of 1,000 records for each mating option of Brahman sire x Angus Dam and Angus sire x Brahman Dam) are required for this data to be robustly interpreted.

Table 8. Immune competence values for crossbreed cattle.

Breed and Year Tested	Immune Competence				
	Number Tested	Mean	Standard Deviation	Minimum	Maximum
AABB__2022	20	-0.323652	1.12893	-3.2804	0.860727
AABB__2023	28	-0.879515	1.36689	-3.86589	2.03383
BBAA__2022	32	-0.425406	0.787447	-1.80295	1.20106
BBAA__2023	38	-0.775265	1.39524	-3.6544	2.1183
BBHH__2022	29	-0.287001	0.700805	-1.94687	1.63497
BBHH__2023	37	-1.06404	1.30772	-4.80249	1.44427
HHBB__2022	35	-0.331589	0.964544	-4.19496	1.18207
HHBB__2023	18	-0.309681	1.24476	-2.97509	2.24158

4.4 Trait Correlations

A correlation analysis was completed for all measured traits to determine if any relationship exists between these traits, and the results are presented in Table 9. The strongest correlations observed (by design) were between Immune Competence (IDEX) and its two component traits (Cell_IR and Ab_IR). The lack of correlation between traits, such as flight time, weight change over weaning and immune competence (IDEX), confirms that these traits need to be individually selected in a breeding program. A further option could be the development of a resilience index to account for a range of stressors such as disease (IDEX), temperament (FTime) and social (WtGain). A weak negative genetic correlation between Ab_IR and WtGain was observed, and this is consistent with previous reports that demonstrated weak negative associations between growth related productivity traits and immune competence values.

Table 9. Correlations of collected resilience traits.

Pearson Correlation Coefficients*							
Trait	**LWt1**	**WtGain**	**FTime**	**CST**	**Cell_IR**	**Ab_IR**	**IDEX**
LWt1	1.000	-0.035	0.111	0.090	0.057	0.064	0.066
WtGain	-0.035	1.000	0.018	0.012	0.130	-0.060	0.042
FTime	0.111	0.018	1.000	0.013	0.047	0.001	0.033
CST	0.090	0.012	0.013	1.000	0.172	-0.011	0.107
Cell_IR	0.057	0.130	0.047	0.172	1.000	0.111	0.749
Ab_IR	0.064	-0.060	0.001	-0.011	0.111	1.000	0.693
IDEX	0.066	0.042	0.033	0.107	0.749	0.693	1.000

* Colour spans Bright Red (indicating strong correlation) to bright green (no correlation)

4.5 Trait LSMean values by year, property, breed and sex.

For comprehensive analysis of the six phenotypic traits, a linear model was fitted containing the main fixed effects of Breed (6 levels), Sex (2 levels), Property (5 levels) and Year of Birth / YOB (2 levels). The model for the analysis of immune competence traits also contained CST as a linear covariate. Breed and property were highly significant for all six traits (P<0.001; Table 10). After fitting the model, least-square means and their standard errors were computed (Tables 11 and 12).

Table 10. Statistical significance[A] of main effects and percent of variation (R^2) explained by the model for resilience traits.

Trait	Breed	Sex	Property	YOB	R^2, %
LWt1	***	***	***	***	50.1
WtGain	***	**	***	**	6.4
FTime	***	NS	***	NS	19.4
Cell-IR	***	**	***	***	12.4
Ab-IR	***	NS	***	***	14.8
IDEX	**	**	***	NS	9.5

[A]Significance: *** = P<0.0001; ** = P<0.001; * = P<0.01; NS = P > 0.05.

Examination of the breed (AA, BB, CC, HH, SS, WY) trait means shown in Tables 11 and 12 revealed some highly significant (P<0.0001) differences between values. For the weight gain over weaning (WtGain) trait the Angus cattle had a significantly higher gain than Charolais or Simmental cattle. For flight time (FTime) the Hereford cattle had a higher value than Angus cattle. Both Angus and Charolais cattle had a higher flight time mean value than did Simmental and Wagyu cattle.

Comparison of immune competence and component trait values show several significant differences. Note the functional significance of these differences is yet to be determined. Brahman cattle yielded a lower mean Cell_IR score than Angus, Hereford and Simmental cattle. However, Brahman cattle returned a higher mean antibody level score than Angus cattle. It is possible that the Indicine breed cattle have a stronger emphasis on antibody response, over cellular response, when compared to many European breeds of cattle. This difference is visualised in Figure 7 where a circled cluster of Brahman individuals can be seen in the upper left quadrant, representing individuals with a low Cell_IR and high Ab_IR. Charolais cattle also returned a high mean Ab_IR level that was significantly different from Angus, Hereford, Simmental and Wagyu cattle. For the immune competence mean values the only significant difference observed was between Charolais and Angus cattle, with Charolais cattle having a higher immune competence score.

Table 11. Liveweight at start of weaning, weight gain over weaning and flight time by breed.

		LWt1		WtGain		FTime	
		LSMean	SE	LSMean	SE	LSMean	SE
Breed	**AA**	277.10	1.24	0.407	0.030	1.045	0.015
	AABB	295.58	5.87	0.571	0.165	1.124	0.070
	BB	243.81	4.06	0.158	0.106	1.021	0.049
	BBAA	290.88	4.95	0.266	0.126	0.860	0.059
	BBHH	275.31	5.08	0.047	0.140	0.909	0.060
	CC	276.44	2.27	0.010	0.053	1.102	0.027
	HH	257.71	1.56	0.146	0.038	1.205	0.019
	HHBB	286.59	5.67	0.429	0.140	1.060	0.067
	SS	251.08	2.08	0.282	0.049	0.881	0.025
	WY	180.23	2.01	0.215	0.047	0.894	0.024
Sex	**F**	257.68	1.55	0.198	0.040	1.000	0.018
	S	269.26	1.57	0.308	0.040	1.020	0.019
Property	**EMAI**	249.56	1.99	0.036	0.050	0.891	0.024
	Glen_Innes	288.80	2.34	0.262	0.058	1.279	0.028
	Grafton	233.10	1.51	0.480	0.041	0.717	0.018
	TARC	310.26	2.17	0.271	0.054	0.942	0.026
	Tocal	235.64	2.51	0.217	0.062	1.221	0.030
YOB	**2021**	272.80	1.57	0.307	0.040	1.024	0.019
	2022	254.15	1.56	0.199	0.040	0.996	0.018

Colour indicates magnitude of value with Red as maximum value and green as lowest.

Table 12. Cell_IR, Antibody_IR and ImmuneDex values by breed.

		Cell_IR		Ab_IR		IDEX	
		LSMean	SE	LSMean	SE	LSMean	SE
Breed	AA	33.200	0.296	83.768	0.524	-0.017	0.035
	AABB	31.032	1.394	83.648	2.471	-0.224	0.164
	BB	27.771	0.966	90.910	1.712	-0.086	0.114
	BBAA	29.845	1.178	85.912	2.088	-0.201	0.139
	BBHH	29.390	1.208	84.078	2.142	-0.298	0.142
	CC	30.612	0.543	95.780	0.962	0.267	0.064
	HH	31.892	0.373	87.751	0.661	0.053	0.044
	HHBB	30.684	1.335	91.491	2.366	0.082	0.157
	SS	33.590	0.498	84.854	0.882	0.059	0.059
	WY	31.944	0.480	89.565	0.850	0.115	0.057
Sex	F	31.562	0.369	88.097	0.654	0.036	0.043
	S	30.430	0.373	87.455	0.662	-0.086	0.044
Property	EMAI	29.149	0.474	87.440	0.840	-0.176	0.056
	Glen_Innes	33.245	0.560	86.932	0.993	0.117	0.066
	Grafton	28.138	0.361	83.357	0.641	-0.430	0.043
	TARC	33.052	0.519	93.739	0.920	0.369	0.061
	Tocal	31.395	0.599	87.412	1.062	-0.004	0.071
YOB	2021	30.275	0.375	92.467	0.665	0.007	0.044
	2022	31.717	0.370	83.085	0.655	-0.057	0.044

Colour indicates magnitude of value with Red as maximum value and green as lowest.

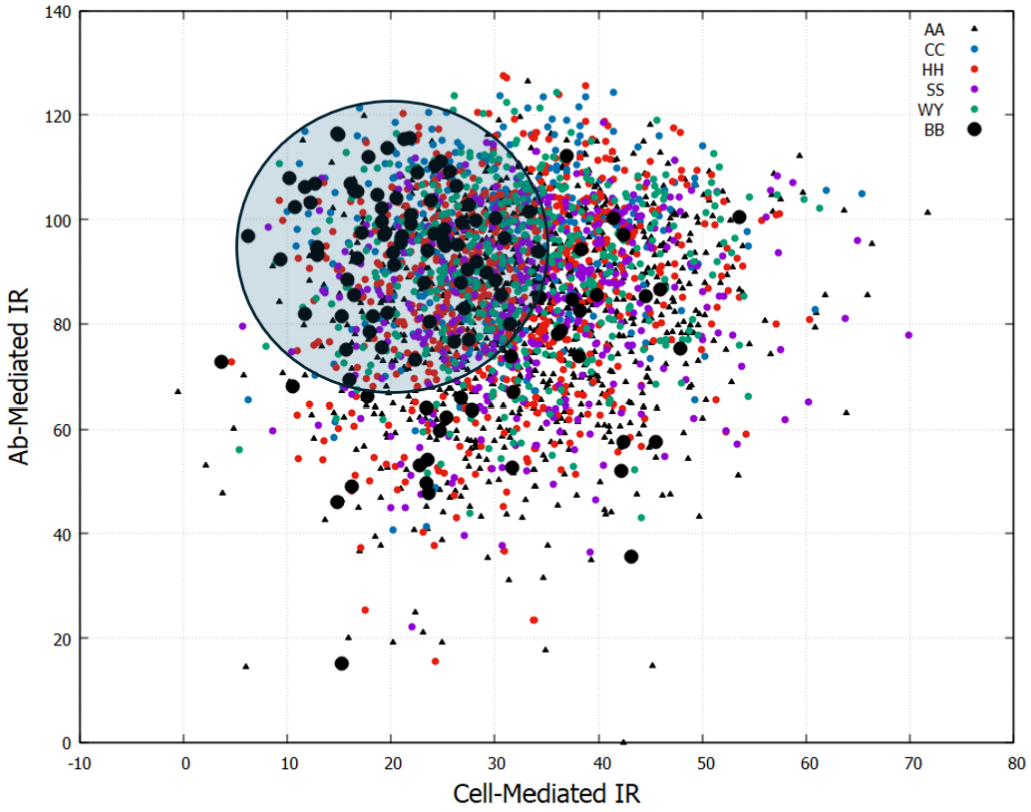

Figure 7. Plot of antibody mediated against cell mediated immune response. A circled cluster of low Cell_IR and high Ab_IR Brahman cattle is highlighted.

4.6 Genomic Relationship Matrix for Immune Competence tested cattle

As shown in Table 6, Immune Competence and other resilience measures (Weight Gain over Weaning (Wt Gain), Flight Time (FTime), Cell-mediated Immune Response (Cell_IR), Antibody-mediated Immune Response (Ab_IR), ImmuneDEX score (IDEX)) were available for 3,175 cattle born in 2021 (N=1,473) and 2022 (N=1,771) from the Southern Multibreed (SMB) project. This figure of 3,175 represents ten cattle breeds / crossbreeds (Angus (AA), Angus x Brahman (AABB), Brahman (BB), Brahman x Angus (BBAA), Brahman x Hereford (BBHH), Charolais (CC), Hereford (HH), Hereford x Brahman (HHBB), Simmental (SS), Wagyu (WY)) and five properties (EMAI, Glen Innes, Grafton, Trangie (TARC) and Tocal).

Following conversations with the NSW DPI SMB management team CSIRO was provided with data for a further 10 traits and the genotypes for the majority of these 3,175 individuals. The additional trait data represents four further growth measurements (Birth Weight (BW), Weaning Weight (WWT), Hip Height (HH), Weight at 400 days (WT400)) for 3,133 of these cattle, as well as six carcase measurements (Carcase weight (CWT), Eye Muscle Area (EMA), Subcutaneous Rib Fat (RIB), Subcutaneous Rump Fat (RUMP), MSA Marbling (MSA_MB), MSA Eating Quality Index (MSA_INDEX)) for 692 steers from the 2021 cohort.

The genotypes were also provided for these 3,133 cattle (or 98.7% of the 3,175) that had been determined from three different SNP chip platforms, The Illumina 100K chip (N=885 cattle), the

Illumina 50K Indicus chip (N=344 cattle) and the Zoetis 65K chip (N=1,904). The observed SNP overlap among chips implied that 158,591 unique SNP were represented.

Due to time constraints and the lack of a suitable reference population, genotypes were not imputed to a single platform (eg. 100K map) and instead chose to base analysis on a selected set of SNP with minor allele frequency (MAF) > 5%, observed in at least 25% of cattle and represented on at least 2 of the 3 chips. These editing criteria resulted in 42,772 high-quality SNP to be used for further analyses.

This panel of 43K SNP was employed to generate the genomic relationship matrix (GRM) among all 3,133 cattle and the PCA of the GRM revealed clusters consistent with their breed denomination, as shown in Figures 8 and 9. Some anomalies are present with, as an example, Wagyu (red) or Hereford (green) datapoints seen in the grey cluster of Angus individuals. This data could be checked using tools developed by CSIRO in a future project, as recommended in the future work section.

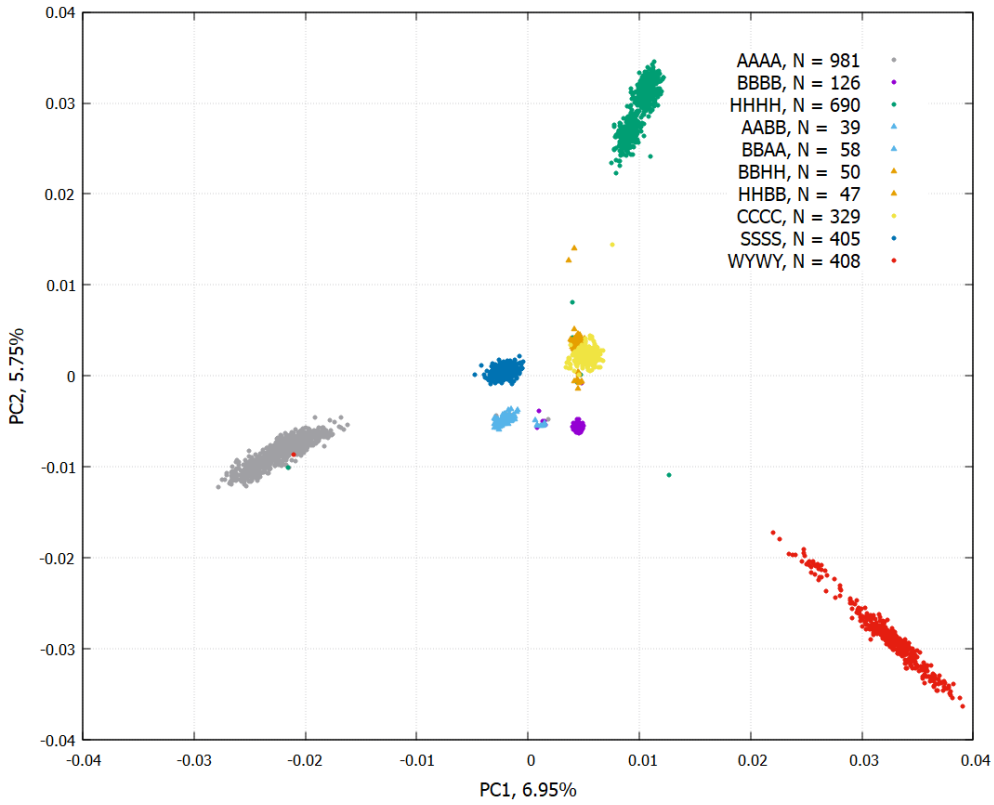

Figure 8. Animal genotype plotted by principal components 1 and 2 for the 3,133 cattle across ten breed categories.

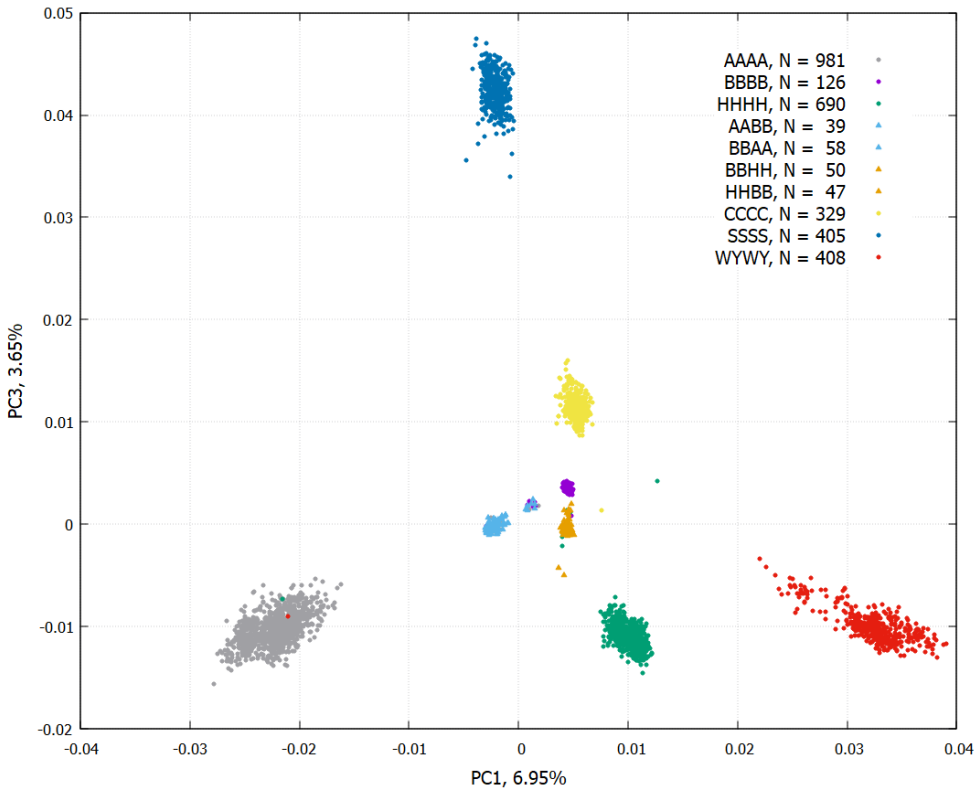

Figure 9. Animal genotype plotted by principal components 1 and 3 for the 3,133 cattle across ten breed categories.

The first five Principal Components (PC) accounted for 20.1% of the variation in genomic relationships, and each of these PC accounted for > 1% variation. These 5 PC were included as covariates in the GBLUP models which also included the fixed effects of breed, sex, property and YOB. Also, noting that date of sampling was confounded with property.

Individuals originating from a cross-breeding event (AABB, BBAA, BBHH, HHBB) also present as distinct clusters that are well separated from the purebred animals. Interestingly the clusters of crossbred individuals are distinct based on the sire breed, with AABB and BBAA individuals presenting as clearly differentiated from each other, and also for BBHH and HHBB.

A total of 15 traits were analysed in a series of 105 bivariate GBLUP models to obtain estimates of variance components, heritability values, along with genetic and residual correlations. The summary statistics for the 15 traits and their covariates used in developing the GBLUP model are shown in Table 13. The values presented include the mean, standard deviation, minimum and maximum values for each of the measured traits and these are well within the expected range for commercially raised beef cattle. One set of values that stands out is the low R^2 observed for the IC traits (IDEX, Ab-IR and Cell_IR) relative to the values obtained for other traits. This result indicates that IC measures are less affected by fixed effects (eg. sex, herd) and covariates (eg. age) than the other traits, such as body weights. One interpretation of this result is that certain factors influencing the immune system are pre-programmed in animals at birth and are not unduly influenced by the environmental influences observed in the present trial or parameters included in the GBLUP model. A key consideration here is that all animals were raised without nutritional deficit, and it is likely that this could influence immune responsiveness.

Table 13. Summary Statistics for Traits used in developing the Multibreed GBLUP Model and GEBVs.

Variable	N	Mean	Std Dev	Min	Max	R^2, %
Covariates						
WAGE	2937	236.37249	23.01879	160.00000	307.00000	
ICAGE	3125	236.38306	22.28385	160.00000	307.00000	
CST	3125	-0.00695	0.04250	-0.20456	0.16037	
A400	2923	422.01197	39.63379	302.00000	599.00000	
CAGE	641	679.42434	69.11497	501.00000	875.00000	
PC1	3133	1.8002E-10	0.01787	-0.02784	0.03901	
PC2	3133	-2.702E-10	0.01787	-0.03635	0.03454	
PC3	3133	-3.057E-10	0.01787	-0.01557	0.04748	
PC4	3133	-5.582E-11	0.01787	-0.01401	0.07541	
PC5	3133	-1.776E-10	0.01787	-0.02068	0.06079	
Traits						
1. BW	2937	38.23017	7.70674	17.00000	69.50000	47.6
2. WWT	2937	254.00970	53.98172	88.00000	426.00000	66.6
3. HH	2937	114.52741	5.70763	92.00000	132.00000	50.1
4. WtGain	2910	0.22369	0.91842	-4.23333	7.03000	29.4
5. FTime	3115	0.98437	0.51089	0.21000	7.04000	23.9
6. Cell_IR	3125	31.74232	9.83159	-0.45476	71.72440	17.7
7. Ab_IR	3125	86.42311	17.62056	13.07040	127.60200	21.1
8. IDEX	3125	-0.00824	1.14119	-5.96106	4.18932	14.2
9. WT400	2923	379.51488	74.64537	150.50000	646.00000	77.2
10. CWT	641	369.47114	55.71590	169.20000	630.00000	67.5
11. EMA	641	89.11076	10.86344	54.00000	134.00000	42.2
12. RIB	641	10.47426	5.93978	2.00000	40.00000	72.8
13. RUMP	641	13.52730	7.44015	4.00000	50.00000	82.9
14. MSA_MB	641	389.53198	150.16584	110.00000	1170	81.3
15. MSA_Index	641	62.08139	3.04465	50.92000	69.61000	85.6

The heatmap of the estimated heritability values (diagonals), genetic correlations (above diagonal) and residual correlations (below) is shown in Figure 10. All values are within the range of previous studies, which provides confirmation of the merit of the current results and analysis method. In the case of the Immune competence component traits Ab_IR and Cell_IR, a previous study focused on 1,000 Angus cattle revealed heritability estimates of 0.32 (±0.09) and 0.27 (± 0.08) for the immune competence traits Ab-IR and Cell-IR, respectively (Hine et al 2019). Here, the respective values were determined as 0.35 and 0.33.

Importantly, Figure 10 also clearly shows the small negative genetic correlation between IC and productivity / growth traits such as WT400, MSA Index and EMA. This result has been consistently observed across other studies (Hine et al 2019, Hine et al 2021). It is hypothesised that this result occurs due to prioritisation of resource or energetic budgets. If the body has a finite amount of energetic resource to spend on competing resources such as growth, reproduction and immune responses there must be a consequence when all resources operate simultaneously. The results reported here, and those published previously, suggest that the selective pressure applied by breeding programs to increase animal growth rates and mature size has the potential to inadvertently draw energy away from the immune system response. This result reaffirms a key message that traits such as immune competence need to be included in selective breeding strategies to ensure the immune system is not compromised when selecting for improved productivity. The unfavourable genetic correlation between IC and growth traits are weak suggesting that it is possible to produce cattle that are both fast growing and immune competent, but it is important to include both traits in a selection index.

	BW	WWT	HH	WtGain	FSpeed	Cell_IR	Ab_IR	IDEX	WT400	CWT	EMA	RIB	RUMP	MSA_MB	MSA_Index
BW	***0.5552***	0.6673	0.6866	0.1283	-0.0814	0.0116	-0.0139	-0.0164	0.6153	0.5303	0.2497	-0.1547	0.0725	-0.0022	0.1614
WWT	0.2956	***0.4535***	0.8229	0.0304	-0.0649	-0.0279	-0.0084	-0.0627	0.8701	0.6589	0.3451	0.0348	0.0841	0.0219	0.1760
HH	0.3282	0.6310	***0.5850***	0.0722	-0.0117	-0.0265	-0.0166	-0.1117	0.8321	0.6929	0.2821	-0.1157	-0.0200	0.0257	0.2508
WtGain	-0.0881	-0.1176	-0.0344	***0.2937***	0.0104	0.1007	0.0152	-0.0402	0.1942	0.0433	0.0579	-0.0078	0.0137	0.0326	0.0182
FSpeed	0.0668	0.0280	0.0058	0.0449	***0.4228***	-0.0386	-0.0562	-0.0548	0.0249	-0.0209	-0.0661	-0.0061	-0.0798	-0.0210	0.0276
Cell_IR	0.0362	-0.0091	0.0007	0.1247	-0.0052	***0.3319***	0.1641	0.7604	-0.0637	-0.0691	-0.1209	0.0155	-0.0118	-0.0208	-0.0801
Ab_IR	-0.0042	0.0179	0.0109	0.0230	-0.0113	0.1520	***0.3481***	0.7224	-0.0231	0.0631	-0.0108	-0.0369	0.0106	0.0168	-0.0360
IDEX	0.0138	0.0050	0.0251	0.1125	-0.0094	0.7490	0.7161	***0.3182***	-0.1369	0.0220	-0.0711	0.0012	0.0061	-0.0025	-0.0711
WT400	0.3768	0.6946	0.5283	-0.0272	-0.0027	0.0102	0.0489	0.0547	***0.4663***	0.8162	0.3717	0.0968	0.1808	0.0941	0.3010
CWT	0.2238	0.4608	0.5081	0.0439	0.0032	-0.0635	0.0682	0.0392	0.6102	***0.3858***	0.4037	0.1685	0.1818	0.1283	0.2546
EMA	-0.0165	0.1661	0.0730	0.0489	0.0019	-0.0813	0.0389	-0.0160	0.1689	0.3912	***0.3860***	-0.1263	0.0130	0.0689	0.0517
RIB	0.0164	0.1945	0.2179	0.0141	0.0112	-0.0118	-0.0103	-0.0036	0.1954	0.1541	-0.1044	***0.3582***	0.2487	-0.0692	0.2248
RUMP	-0.1372	0.0798	0.0488	0.0172	-0.0368	-0.0239	-0.0447	-0.0390	0.0299	0.1580	-0.0106	0.2509	***0.3612***	-0.0140	0.0902
MSA_MB	-0.0383	0.0080	-0.0115	-0.0059	-0.0184	-0.0214	0.0257	-0.0047	0.0558	0.0832	0.0350	-0.0608	0.0203	***0.3486***	0.5944
MSA_Index	0.0269	0.0718	0.0634	-0.0138	0.0126	-0.0764	-0.0001	-0.0512	0.1106	0.1734	0.0072	0.2178	0.0924	0.5616	***0.3455***

Figure 10. Heatmap of the estimated heritability values (diagonals), genetic correlations (above diagonal) and residual correlations (below diagonal) for recorded traits. Red indicates a positive relationship and green a negative relationship. The intensity of the colour reflects the magnitude of the relationship

The genomic prediction equation resulting from the GBLUP equation was then applied to all 3,133 cattle with genotypes that had also been previously phenotyped for IC. The GEBV predictions for IC and the component Ab_IR and Cell_IR traits are presented in Table 14. The summary statistics are presented for the entire cohort of cattle and for each of the breeds. Note, the genomic prediction equation is multibreed where breed has been fitted in the model as well as the PC of the GRM. Although the original aim of the study was to produce breed specific prediction equations, given the limitations of the current dataset (a total of 3,183 animals with no breed containing more than 1,000 animals) a multibreed equation was produced as an alternative. This has two main advantages. The number of animals within each breed is currently quite low so the accuracy of breed specific equations would be reduced. For instance, theoretical expectations from Hayes and Goddard (2009) show that a referenced population of 3,000 animals and a trait with 30% heritability, is expected to allow for a genomic prediction with only 40% accuracy. This value drops considerably with smaller reference population and lower heritability traits. Instead, by combining the animals across breed we have a much larger dataset to derive a multibreed equation. Secondly, in generating this multibreed equation we have removed breed as an effect. This means it is more robust to perform a head-to-head comparison of the breeds. As we mention in the report the finding from this is that all breeds have overlapping distributions. With the results presented by breed in Table 14.

Effectively, breed effects have been removed (or accounted for) and these genomic predictions can be applied to any animal regardless of their breeds (or of their sex, YOB, herd, etc. for all other effects fitted in the model). The results are reasonable and follow similar trends to the actual phenotypes displayed in Table 7. Note, that the difference between the breed IDEX minimum and maximum values reported in Tables 7 and 14 is expected. The data presented in Table 14 describes values attributable to genotype whereas Table 7 contains the phenotypic values (representing genotypic and environmental effects).

Table 14. Distribution of Immune Competence GEBV in the tested population and within breed.

Population	N	Mean	SD	Min	Max
		Cell_IR			
Whole	3133	0.000	2.687	-9.398	15.840
AAAA	981	0.009	2.983	-9.398	15.840
AABB	39	-0.180	1.641	-5.143	3.093
BBAA	58	0.259	1.516	-2.492	3.710
BBBB	126	-0.149	1.580	-3.073	3.981
BBHH	50	0.203	1.616	-2.957	4.226
CCCC	329	-0.005	2.525	-6.065	8.804
HHBB	47	0.064	1.604	-2.380	3.896
HHHH	690	0.001	2.857	-9.010	9.648
SSSS	405	-0.007	2.661	-7.391	7.349
WYWY	408	-0.019	2.486	-6.448	9.008

		Ab_IR			
Whole	3133	0.000	4.876	-24.735	17.699
AAAA	981	0.046	5.695	-24.735	17.533
AABB	39	-0.292	4.140	-8.061	6.948
BBAA	58	-0.324	3.455	-8.150	7.175
BBBB	126	0.172	3.190	-8.436	6.975
BBHH	50	-0.142	3.643	-10.072	8.940
CCCC	329	0.014	4.186	-14.979	10.360
HHBB	47	0.315	3.763	-11.763	8.057
HHHH	690	-0.042	5.076	-19.574	17.699
SSSS	405	0.000	4.529	-15.286	9.613
WYWY	408	-0.048	4.134	-13.138	10.367
		IDEX			
Whole	3133	0.000	0.308	-1.400	1.500
AAAA	981	0.002	0.362	-1.400	1.500
AABB	39	-0.019	0.253	-0.704	0.513
BBAA	58	0.008	0.187	-0.375	0.582
BBBB	126	-0.004	0.189	-0.511	0.504
BBHH	50	0.009	0.170	-0.466	0.414
CCCC	329	0.000	0.263	-0.858	0.739
HHBB	47	0.012	0.202	-0.617	0.650
HHHH	690	-0.002	0.332	-1.141	1.016
SSSS	405	-0.001	0.284	-0.735	0.973
WYWY	408	-0.003	0.245	-0.771	0.943

5. Conclusion

There would be considerable benefit in collecting further records to improve confidence in correlation predictions and accuracy of genetic parameter estimations, should this option be available. For reference, the number of immune competence records by breed across the 2022 and 2023 S and T cohorts stands at 983 Angus (AA), 112 Brahman (BB), 337 Charolais (CC), 679 Hereford (HH), 412 Simmental (SS), 415 Wagyu (WY), and 237 Crossbreed (AABB, BBAA, BBHH, HHBB).

Weight gain over weaning and flight time records were also collected for many of these cattle. These traits, in combination with IC, may be useful indicators of resilience, providing an indication of an animal's ability to cope with environmental stressors and could be used to develop a resilience index for inclusion in breeding objectives. No significant correlation was observed between these traits suggesting each of the traits needs to be considered if developing a resilience index.

No specific animal health records were available for the analysis reported here. It will be important to explore associations between IC and health outcomes (including both morbidity and mortality) when data is available. One area for consideration might be association of IC with mortalities if cause of death is known and attributable to disease. Differences in animal number were observed between the time of IC phenotyping at weaning and final carcase data. Missing animals may be due to mortalities.

Walk over weighing scales were deployed at Trangie and Glen Innes for the 2023 Cohort T calves. Only the Glen Innes deployment returned usable data. The manufacturer (Datamars) of the WOW scales has recommended that scales not be deployed for a period of less than 1 month, making this technology not fit for purpose for measurement of weight change during the 2-week period of weaning without weights also being collected pre-weaning. Variation between weights recorded by static and WOW scales has been observed.

The distribution of immune competence scores was similar for all breeds tested here suggesting that all breeds have a natural range of scores that spans from low to high immune competence. Few significant differences were found between breed mean values but there is an indication that Indicine cattle may have a stronger antibody response and lower cellular response than do many European cattle breeds.

A functional threshold for immune competence has not been defined and as such the authors warn against making direct comparisons of breed ability to resist disease based on the results reported here. All breeds have high and low performing individuals. The breeding goal should be to ensure immune competence is considered to prevent intensive selection on productivity traits leading to a decline in immune competence as a consequence of negative genetic associations between these traits. As such, preferential use of breeding animals that do not have low (bottom quartile) immune competence values is recommended. The goal is not to be the highest immune competence individual, as there is likely a production cost for this, but rather, to not be in the tail of the distribution. It is likely this low immune competence cohort is an entry point for infectious disease into the herd, acting as a disease reservoir, and increasing pathogen exposure to broader herd incurring increased health-associated costs.

The GBLUP model results revealed that the IC traits (IDEX, Ab-IR and Cell_IR) were less sensitive to the environmental and fixed effects (eg. sex, herd) and covariates (eg. age) included in the model than the other traits, such as body weights. This however may be a limitation of the factors included in the model. For example, factors reported previously to influence immune function such as measures of thermal stress at the time of sample collection are not included. Further, nutritional

deficit was not a variable assessed in this trial, and it is likely that this could influence immune responsiveness.

The results reported here, and those published previously suggest that the selective pressure applied by breeding programs to increase animal growth rates and mature size has the potential to inadvertently draw energy away from the immune response. This result reaffirms a key message that traits such as immune competence need to be included in selective breeding strategies to ensure the immune system is not compromised when selecting for improved productivity. Genetic correlations between IC and growth traits reported here suggest it is possible to produce cattle that are both fast growing and immune competent, but it is important to include both traits in a selection index.

5.1 Key findings

Distribution of the immune competence trait values was assessed by breed and found to be overlapping, and as such all breeds assessed will have individuals that span low to high immune competence. A similar result was observed for crossbred animals. A slight negative genetic relationship was observed between immune competence and growth-related traits confirming that immune competence should be considering in breeding objectives to ensure no inadvertent deterioration of herd immune competence status when selecting for improved productivity. A genomic prediction equation has been produced that can be used to estimate Immune Competence values in Australian beef breeds and crossbreed cattle.

5.2 Benefits to industry

The development of strategies aimed at improving the resilience of Australian beef cattle has the potential to:

- Increase the ability of Australian cattle to cope with environmental challenges posed by an ever-changing environment.
- Improve animal health & welfare.
- Reduce use of antibiotics in the food-chain and costs associated with treating disease.
- Reduce wastage resulting from animal mortality/morbidity.
- Provide an objective means of demonstrating industries commitment to achieving sustainability-based goals.
- Maintain consumer confidence in the Australian beef industry and the products they produce.

6. Future research and recommendations

- Improve the genotype data using imputation to the 100K chip or, if possible, to HD level (ie. 700K).
- Adopt the CSIRO in-house genomic breed composition tool to ascertain the breed composition of these cattle (ie. addressing the issue about some breed miss-classified animals seen in the PCA plots).
- Explore alternative GBLUP models including effect of heterosis and inbreeding.
- Explore alternative GRM including breed specific allele frequencies.
- Cross-validation studies to assess accuracy of resulting GEBV (both within and across breeds).
- Develop genomic prediction equations for IC to identify elite animals for different breeds.
- Include carcase data from remaining cohorts.

- Opportunity to link data to other studies including Angus and Brahman data from existing projects (eg. Repronomics).
- Incorporate sensor-based behavioural data.
- Complete association study after including detailed 'whole of life' health and performance data that has been recorded on all resilience tested progeny. Health data recorded was expected to include disease incidence during backgrounding and feedlot finishing for steers and disease incidence on pasture of retained females. Mortalities. Longitudinal worm egg count (WEC) measures will also be recorded (as part of broader SMB project). Offal damage scores.
- Evaluation of maternal or paternal influence on immune competence
- Consider the development of a resilience index to account for a range of stressors such as disease (IDEX), temperament (FTime) and social anxiety (WtGain).

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