Final report

Quantifying the benefits of breeding for immune competence in high disease risk feedlots

Project code: L.GEN.1817

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Commonwealth Scientific & Industrial Research Organisation

Date published: 19 August 2022

PUBLISHED BY
Meat & Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Selection for production traits with little or no emphasis on health-related traits has led to an increase in the incidence of disease in many production animal systems. Further, ever-changing climatic conditions experienced in production environments are seeing animals exposed to disease challenges not previously encountered. Therefore, we have developed a testing procedure to assess the immune competence of beef cattle on-farm which we expect will allow producers to select animals in their herds that are both highly productive and have an enhanced ability to cope with general disease challenges. In the current project we aimed to further validate the benefits of selecting beef cattle for immune competence, realised through reduced health associated disease and mortalities, in Australian commercial feedlot environments.

A total of 1661 steers were immune competence tested at weaning on three commercial co- operator farms and performance recorded, both in terms of health and productivity, of 1324 of these steers was monitored during feedlot finishing. A significant favourable association between immune competence phenotype and mortalities during feedlot finishing was observed. Enhanced immune competence was also associated with a reduced “pull rate” during feedlot finishing; however, the effect was not significant. Reducing disease incidence and associated reliance on antibiotics in feedlots, while maintaining optimal animal welfare standards, will be key to maintaining consumer confidence in Australian beef products moving forward and ensuring the industries continued social licence to operate.

Executive summary

Background

Cattle face a variety of challenges in the feedlot environment including exposure to infectious agents, climatic extremes, social stressors caused by mixing with unfamiliar animals, and management induced stressors imposed by standard husbandry procedures and practices. 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 with environmental challenges.

Selection for production traits with little or no emphasis on health-related traits has led to an increase in the incidence of disease in many production animal systems. Further, ever-changing climatic conditions experienced in production environments are seeing animals exposed to disease challenges not previously encountered. Therefore, we have developed a testing procedure to assess the immune competence of beef cattle on-farm which we expect will allow producers to select animals in their herds that are both highly productive and have an enhanced ability to cope with general disease challenges.

The Australian cattle feedlot sector is actively seeking strategies which will allow them to reduce their reliance on antibiotics to treat disease while maintaining their existing high standards of animal welfare. We expect selection for enhanced immune competence to be such a strategy. Here we further validate the benefits of selecting beef cattle for immune competence, realised through reduced health associated disease and mortalities, in Australian commercial feedlot environments.

Objectives

The main objective of this project was to further validate the benefits of selecting beef cattle for immune competence in Australian commercial feedlot environments.

Specific key objectives of the project were:

- To further assess the benefits of genetic selection for immune competence, realised through reduced health associated disease and mortalities, in higher disease risk environments than animals were exposed to in the previous MLA Project, B.STU.0244.
- To generate additional immune competence phenotype data to be used in future studies to improve the accuracy of genetic parameter estimates for immune competence traits in beef cattle and inform future GWAS to identify major genes associated with enhanced immune competence in beef cattle.
- To further explore relationships between immune competence, other resilience related traits including stress responsiveness and temperament, growth and carcase traits in beef cattle.
- To develop a pen-side test to replace the laboratory test currently used to assess antibody- mediated immune responses as part of immune competence phenotype testing.

Methodology

A total of 1661 steers were immune competence tested at weaning on three commercial co-operator farms. Of these steers a total of 1324 were inducted into commercial feedlots and their performance during feedlot finishing, including monitoring health and productivity. Following feedlot finishing steers were processed at various processing plants and detailed data on carcase traits, offal defects and lung lesion scores were collected (where possible).

Immune competence phenotypes were calculated for individual steers by combining measures of their ability to mount both antibody-mediated and cell-mediated immune responses. The ability of immune competence phenotypes to predict health outcomes at the feedlot and associations between immune competence and other resilience traits (including stress responsiveness and temperament), productivity traits (including average daily gain weaning to feedlot induction and during feedlot finishing) and carcase traits (including hot carcase weight and MSA traits) was then evaluated.

Methodology to allow 'pen-side' assessment of antibody responses, required as part of immune competence phenotype testing, was investigated.

Results/key findings

- A significant favourable association between immune competence phenotype and mortalities during feedlot finishing was observed, with steers below average for immune competence being three times more likely to die during feedlot finishing than steers above average for immune competence.
- Enhanced immune competence was associated with a reduced "pull rate" during feedlot finishing; however, the effect was not significant. The average immune competence rank of steers with no health issues recorded at the feedlot was 659 versus 690 for steers with ≥1 health issue recorded.
- Immune competence phenotype was not significantly associated with number of offal defects or lung lesion scores observed at processing. However, when the number of offal defects observed was treated as a binary trait (zero or ≥1 defect detected) a trend suggesting that immune competence was unfavourably associated with offal defects being detected at processing. It is noteworthy that this trend was no longer evident when offal defects were treated as an ordinal trait or when the offal defect category 'kidney other' for steers from a single processing plant was excluded from analysis on the basis that 69% of all steers processed were recorded to have this defect which was considered very unlikely to be accurate.
- There was strong evidence to support the notion that a favourable association between immune competence phenotype and weight change over weaning: however, the observed relationship was not strong
- Immune competence phenotype was not associated with growth traits, suggesting selection for immune competence will not compromise productivity.
- Immune competence phenotype was not associated with carcase traits assessed in the current study, suggesting selection for immune competence will not compromise

- carcase characteristics or eating quality.
- A 'pen-side' assay which can be used to assess antibody-mediated immune responses as part of immune competence phenotype testing was developed. This development will allow immune competence phenotype testing to be conducted in 'real time' into the future.

Benefits to industry

Results from the current study further validate the benefits of selecting beef cattle for immune competence, realised through reduced health associated disease and mortalities, in Australian commercial feedlot environments. Genetic strategies aimed at improving the inherent ability of animals to cope with disease challenges experienced in their production environment, used in conjunction with effective vaccination strategies and targeted management practices to reduce exposure to pathogens, have the potential to significantly reduce disease incidence, and subsequent reliance on antibiotics to treat disease, in Australian feedlots. Reducing reliance on antibiotics in feedlots, while maintaining optimal animal welfare standards, will be key to maintaining consumer confidence in Australian beef products moving forward and ensuring the industries continued social licence to operate.

Future research and recommendations

Future research should aim to:

- Further validate the benefits of selecting for immune competence in both commercial grass- fed and grain-fed production systems.
- Continue to refine immune competence testing procedures to reduce testing costs and improve practicality.
- Continue to investigate additional measures of immune competence which could be incorporated into testing procedures to improve the ability of the phenotype to predict favourable health outcomes.
- Genotype animals enrolled in the current study to a) enable immune competence phenotype data collected here to contribute to the identification of genetic markers, associated with improved immune competence in beef cattle, and improve the accuracy of genomic predictions for the trait through inclusion of study animals in relevant reference populations and b) allow genetic associations (based on genomic estimated breeding values) between immune competence and other traits to be investigated.

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1. Background

1.1 Project Background

Cattle face a variety of challenges in the feedlot environment including exposure to infectious agents, climatic extremes, social stressors caused by mixing with unfamiliar animals, and management induced stressors imposed by standard husbandry procedures and practices. 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 with environmental challenges.

Selection for production traits with little or no emphasis on health-related traits has led to an increase in the incidence of disease in many production animal species. Therefore, we have developed testing procedures to assess the immune competence of beef cattle, dairy cattle and sheep on-farm which we expect will allow producers to select animals in their herds/flocks that are both highly productive and have an enhanced ability to cope with disease challenges (Hine et al., 2014).

1.1.1 The Immune System

The immune system is composed of tissues, cells and molecules which work together to protect the host animal against disease. Effective host defence is reliant on the immune system's ability to detect a wide variety of agents, to distinguish whether such agents are part of the body or foreign (self versus non-self), to determine whether non-self agents are commensals or threats, and to eliminate the potentially infectious agents or pathogens. Livestock, with the exception of those raised in specialised facilities, are exposed to a myriad of pathogens on a regular basis. Such pathogens possess the inherent ability to evolve rapidly, and as a consequence, adapt quickly to changes in the environment, and continually develop new strategies to avoid detection and elimination by the host's immune system. To detect and eliminate pathogens, the immune system has developed a diverse range of defensive responses that work together and which can be broadly categorised as either innate or adaptive responses. When a pathogen is first encountered, the innate immune system is activated. In the initial phases of the innate response, pre-formed anti-microbial substances, present in body fluids and secretions, begin to weaken and kill the pathogen while sending signals to alert the adaptive immune system of impending danger. As these responses advance, innate effector cells recognising common molecule structures described as pathogen-associated signatures become activated, setting in motion a signalling cascade that triggers defence mechanisms aimed at eliminating the pathogen. Should a pathogen breach these initial lines of defence and damage the host, mechanisms are in place to trigger adaptive immune responses. In contrast to innate responses which are largely non-specific, fast acting and not substantially enhanced by repeated exposure to the same pathogen, adaptive responses are highly pathogen-specific, slower to develop and continually refined upon repeated exposure to the same pathogen. Adaptive responses have an important memory component, which enables the effector functions of the adaptive immune system to be deployed more rapidly and with increasing specificity upon re-exposure to a pathogen.

The immune system is the body's main defence against disease, however some commonly used terms describing an individual's response to disease should be considered. Different disciplines and

research studies use the related terms of disease resistance, tolerance, resilience and robustness in slightly different ways and therefore the precise relationship between these terms may be context specific. For the purpose of this report the following distinctions will be made between these separate, yet related, terms as they pertain to disease. Disease resistance is considered as the host's ability to limit or eliminate pathogens using a variety of host defence reactions including physiological, behavioural and immunological responses (Colditz, 2008). Morphological traits can also make an important contribution to disease resistance as evidenced by the relationship between breech conformation and resistance to flystrike in Merino sheep (Greeff et al., 2014). These various defence mechanisms work in conjunction to block pathogen invasion or to destroy the invader. However, the host can also defend itself by limiting the damage caused by the pathogen using mechanisms that prevent self-harm or modulate escalating immune responses (Schneider and Ayres, 2008). This is termed disease tolerance, or in other words, an ability to minimise the effects of infection at a given level. This terminology can be further refined by identifying individuals that maintain productivity in the face of a disease challenge. This is generally referred to as disease resilience (Bishop and Morris, 2007). A key difference between disease tolerance and disease resilience is that disease tolerance often implies a permanent state of infection where repeated exposure to a particular pathogen reduces sensitivity to its effects, whereas disease resilience is generally considered a more transient state of infection where the host eventually clears the infection with little or no effect on production. Finally, the term robustness is defined as the ability of the individual to maintain its functions in the face of internal and external challenges (Kitano, 2007). Robustness therefore is quantified by performance of various traits, such as growth, fertility, and carcass characteristics, as well as response to disease.

Both the ability to resist infection and the ability to tolerate the effects of disease are likely contributors to an animal's ability to maintain productivity when faced with a disease challenge. Therefore, disease resistance and disease tolerance can both be considered to contribute to disease resilience (Bishop, 2012). In considering whether to target, disease resistance or disease tolerance, as the basis for improving animal health in selective breeding programs, there are no simple answers. It is important however to realize that disease resistance and disease tolerance are generally negatively correlated, and are based on different underlying host mechanisms and different genes, and have different impacts on the evolving pathogen (Simm and Triplett, 1994). Because disease resistance and disease tolerance are often negatively genetically correlated, individuals identified as susceptible to disease tend to be more tolerant. Conversely, individuals with resistant genotypes tend to be less tolerant. The implication of these factors is outside the scope of this discussion; however, it highlights the importance of considering the preferred final outcomes for both the host and pathogen when establishing selection strategies to improve animal health. The research described here focuses on general disease resistance because in many cases of infectious disease it is critical to eliminate the causal agent in order to prevent mortality and unintended pathogen transmission to the environment or to other hosts. Furthermore, animals identified using appropriate strategies as having enhanced general disease resistance are likely to be resistant to a wide-range of pathological agents.

When developing strategies aimed at improving animal health, it is important to recognise that disease resilience is just one component of general resilience. Just as disease resilience can be considered as the ability of an animal to maintain productivity in the face of disease challenge, general resilience can be considered as the ability of an animal to maintain productivity in the face

of diverse environmental challenges. Livestock are exposed to a variety of environmental challenges in their production environment including abiotic extremes, social and management-induced stressors and disease challenges (Hine et al., 2014). The contribution of immune competence to general resilience will be discussed in further detail later in the report.

1.1.2 Immune Competence

Immune competence can be considered as 'the ability of the body to produce an appropriate and effective immune response when exposed to a variety of pathogens' (Wilkie and Mallard, 1999). Weak responses may allow pathogens to persist or overcome host defences leading to morbidity and mortality. Inappropriate responses to self antigens (an antigen being any substance that provokes an adaptive immune response) can lead to autoimmune diseases, while inappropriate responses to harmless antigens can lead to allergic responses. It is also critical that when faced with a pathogen challenge, the body mounts the most effective type of response to control that pathogen. Some pathogens have devised means by which they enter cells of the body (intracellular pathogens) while others remain in the environment external to cells (extracellular pathogens). Elimination of intracellular pathogens generally requires that infected cells be destroyed. This job is carried out by phagocytes, which are specialised cells with the ability to ingest harmful agents and infected cells, and by cytotoxic cells, which are capable of inducing programmed cell death in infected target cells. Collectively, the actions these host defence cells are described as 'cell-mediated immune responses'. In contrast, extracellular pathogens and soluble antigens are more effectively controlled by 'antibody- mediated immune responses'. Antibodies bind to pathogens and soluble antigens in the extracellular environment, preventing them from damaging or entering cells and tagging them for destruction by immune cells. As the immune system is constantly challenged by both intracellular and extracellular pathogens it is critical that individuals have a balanced ability to mount both cell-mediated and antibody-mediated immune responses (Hine et al., 2014). Equally important is the fact that responses must be of a magnitude that effectively eliminates pathogens without causing self-harm.

1.1.3 Immune Competence – An Important Selection Trait

Selection for production traits with little or no emphasis on health and fitness traits has led to an increase in the incidence of disease in many production animal species. Antagonistic or unfavourable genetic correlations exist between production traits and the incidence of many common diseases in livestock (Rauw et al., 1998). For example, the genetic correlation between milk production and the incidence of mastitis in dairy cows has been estimated at between 0.15 to 0.37 (Lyons et al., 1991; Uribe et al., 1995; Van Dorp et al., 1998). Therefore, progeny of parents with high genetic potential for milk production generally have a higher incidence of mastitis than do progeny of parents with low genetic potential for milk production. In pigs, selection focussed on high productivity has led to an increase in susceptibility to stress and disease (Prunier et al., 2010). In sheep, recent production focussed breeding has been achieved in an environment where chemicals have been available to control the major pathogens, gastrointestinal nematodes. A comparison of progeny sired by contemporary rams or from semen collected over 30 years ago shows advances in many productivity traits during this time however natural resistance to nematodes has declined significantly (Shaw et al., 2012). Such findings suggest that continued selection based on productivity alone will result in further increases in the incidence of disease in production animals. The production animal industries are aware of this issue and are actively

seeking solutions to the problem.

Changes in community attitudes are also contributing to a renewed focus on breeding production animals that have an enhanced natural ability to resist disease. Consumer awareness of practices that impact the health and welfare of food-producing animals is increasing, as is concern regarding the use of antimicrobials to control disease in livestock and the potential food contamination issues that arise from their misuse. Strategies which can reduce reliance on antimicrobials to prevent/treat disease while maintaining the highest standards of animal welfare are urgently required. However, it must also be acknowledged that selection for increased productivity remains a key profit driver for our livestock industries. It is therefore proposed that a possible genetic solution is to combine production traits and immune competence traits into a weighted selection index with the aim of breeding high-producing animals with enhanced general immune competence (Mallard et al., 1998a; Wilkie and Mallard, 1999).

1.1.4 Selecting for Resistance to Specific Diseases versus Selection for General Disease Resistance

Breeding strategies targeted at increasing resistance to specific diseases in livestock have proven very successful. Such strategies include breeding sheep with enhanced resistance to specific internal parasites (Le Jambre et al., 1971), dairy cattle with enhanced resistance to mastitis (Heringstad et al., 2000) and beef cattle with increased resistance to brucellosis (Adams and Templeton, 1993) and to cattle ticks (Frisch et al., 1998). Based on the knowledge that the host immune system tailors' responses to the type of pathogen encountered, it could be expected that selection of animals based on their resistance to a specific disease may inadvertently increase their susceptibility to other diseases. For example, selection of animals based on their resistance to an extracellular pathogen, largely controlled by an antibody-mediated immune response, might inadvertently increase their susceptibility to intracellular pathogens, largely controlled by cell- mediated immune responses. Indeed, it has been reported that cell-mediated and antibody mediated immune responses are negatively genetically correlated in dairy cattle even though these immune responses work at the phenotypic level in a coordinated manner to protect the host (Hernandez et al., 2006; Thompson-Crispi et al., 2012b). More research is required to assess the long-term effects of selection for resistance to a specific disease on susceptibility to other diseases in livestock. We hypothesise that long term benefits can be expected from adopting breeding strategies based on enhancing general disease resistance of livestock, as an alternative to or in conjunction with, enhancing resistance to specific diseases of significant economic importance to the livestock industries.

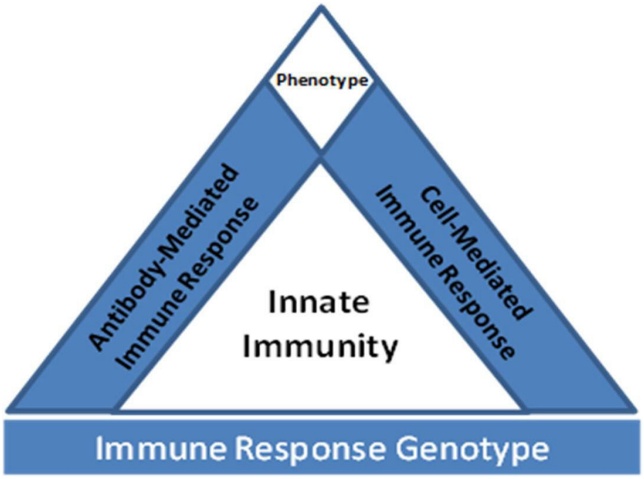
1.1.5 Assessing Immune Competence

Genetic variation in the ability to resist disease is due to a large number of additive genetic effects which together regulate innate and adaptive immune responses (Wilkie and Mallard, 1999). It has been estimated that greater than 7% of all known genes in the mammalian genome are involved in immune function (Kelly et al., 2005). Although the underlying genotype involves complex interactions between many genes, by inducing immune responses and objectively measuring such responses in livestock, general immune responsiveness of individual animals can be assessed (Wilkie and Mallard, 1999) (Fig 1.). This was first demonstrated amongst production species in Yorkshire pigs, where measures of innate and adaptive immunity (both antibody and cell-mediated) were combined to generate estimated breeding values (EBVs) for general immune

responsiveness and to rank boars and gilts as high, intermediate and low immune responder (IR) phenotypes for use in future breeding programs (Mallard et al., 1992). This strategy aimed to simultaneously improve the ability of animals to mount both antibody and cell-mediated responses, and as a consequence, enhance general disease resistance. Following the inbreeding of high, intermediate and low IR phenotype pigs for several generations it was found that high IR pigs had superior antibody responses to test antigens and several commercial vaccines (Wilkie and Mallard, 1999), a lower frequency of non-responders when vaccinated with inactivated influenza vaccine (Wilkie and Mallard, 1998) and higher antibody avidity, a measure of the strength of the antibody-antigen interaction (Appleyard et al., 1992), than their intermediate and low IR counterparts. Although such findings provide overwhelming evidence to suggest that selection successfully enhanced general immune responsiveness in high IR pigs, when challenged with Mycoplasma hyorhinis, these pigs displayed more severe arthritis than LR pigs, suggesting that high IR phenotype pigs may be more prone to generating inflammatory responses (Magnusson et al., 1998). However, in the same study, high IR pigs were found to have less severe peritonitis, less severe pleuritis and produced serum antibody against M. hyorhinis both earlier and to a higher level than did their low IR counterparts and therefore survived better. Thus, the trade-off between lameness and survival may be defensible in this case.

More recently, research efforts have been focussed on developing protocols to assess general immune responsiveness in dairy cattle, similar to those used in pigs, and on investigating associations between immune responsiveness phenotypes and the incidence of disease in large-scale commercial dairy farms. This strategy involves immunising animals with antigens that stimulate either strong antibody or cell-mediated immune responses, and then measuring both types of response. The responses are then used in combination to rank animals for general immune responsiveness (Heriazon et al., 2009a; Heriazon et al. 2009b). Although this ranking strategy does not incorporate measures of innate immunity, in contrast to the strategy used in pigs, it is acknowledged that strong adaptive immune responses are underpinned by strong innate immune responses (Figure 1.). In fact, macrophage function, including both phagocytosis and nitrous oxide production, seems to be stronger in high responder dairy cows (B.A. Mallard, pers. comm.) as does TLR2 expression, a receptor involved in the recognition of a wide array of microbial molecules (Wagter-Lesperance et al., 2014). Therefore such a strategy can still be expected to identify animals with enhanced general immune responsiveness and, as a consequence, general disease resistance. Researchers have utilised this testing strategy to investigate the influence of hybrid vigour on general immune responsiveness in purebred and crossbreed dairy cattle (Begley et al., 2009, Cartwright et al., 2012), the influence of age and pregnancy status on general immune responsiveness in dairy heifers (Hine et al., 2011), leukocyte (white blood cell) populations in high and low IR dairy heifers (Hine et al., 2012) and the influence of geographical location on immune response profiles of Canadian dairy cattle (Thompson-Crispi et al., 2012a).

Figure 1. Genetic variation in the ability to resist disease is due to a large number of additive genetic effects which together regulate innate and adaptive immune responses (Source: adapted from Wilkie and Mallard 1999)



1.1.6 Immune Competence in Beef Cattle

In a previous MLA co-funded project, MLA Project (Ian Colditz – Mentor for Postdoctoral Fellow, MLA Project Code B.STU.0244) we developed methodology, modified from that used previously in dairy cattle, to assess the immune competence of commercial beef cattle which is both practical to apply on-farm and does not restrict the future sale of tested animals. The specific test antigens used previously to assess immune competence in North American dairy cattle would require registration with the APVMA before they could be used in commercial beef cattle in Australia. Therefore, we use a commercially available vaccine, rather than specific test antigens, to stimulate measurable immune responses in our modified methodology. Further, as part of our methodology, immune competence is assessed while cattle are being exposed to the stress of weaning. It is well recognised in production animal species that high disease incidence risk periods are often associated with stressful events, as such events can suppress immune system function. By assessing the immune competence of cattle when exposed to an industry relevant stressor, we expect to be able to identify animals which can mount a strong immune response to a disease challenge when under stress.

In project B.STU.0244, the immune competence methodology developed was used to investigate associations between the resilience traits of immune competence, stress-responsiveness and temperament in 1,149 Performance Recorded Angus calves assessed 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 low reactivity to stressors and a calm temperament (Hine et al. 2019). In that study, cattle were classified into one of three categories:- high, average or low immune responders, based on their immune competence phenotype that was calculated from a combination of antibody and cellular immune response parameters. Animals with high immune competence demonstrated a strong ability to deal effectively with disease, with no recorded mortalities and incurred health costs of only $4 per head during feedlot finishing. Conversely, the low immune competence group had a mortality rate of

6.1% and incurred substantially higher health associated costs during feedlot finishing of $103 per head. Animals classified into the average immune competence group displayed intermediate values with a mortality rate of 1.2% and incurred health associated costs of $28 per head. Collectively these results suggests that selection for immune competence could potentially have significant health & welfare benefits for feedlot cattle and significant economic benefits for feedlot operators (Hine et al. 2021).

1.1.7 Feedlot Health

Animals with average to high general immune competence are expected to exhibit broad-based resistance to diseases commonly encountered in the feedlot environment. Of these diseases, bovine respiratory disease (BRD) is the most common disease encountered in Australian feedlots, causing significant economic losses and animal welfare issues. It has been estimated that BRD costs the Australian feedlot sector in excess of $40 million annually, with losses across all animals estimated at $20 per head (Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep production, MLA Project Code AHW.087). BRD is a complex, multi-factorial disease caused by a variety of infectious agents and is most prevalent in cattle during periods of heightened stress such as the initial six weeks spent acclimatising to the feedlot environment. Commercial vaccines have been developed to protect cattle against particular agents which contribute to the BRD disease complex, however providing blanket protection against the full complement of potential BRD causing agents through vaccination is difficult to achieve. Therefore, reducing the incidence of BRD (and other common feedlot diseases) is likely to require a combinatorial approach involving strategic management practices, improved genetic ability to resist disease and targeted vaccination strategies. Selection for immune competence is such an approach. This approach is expected to play a critical role in improving the inherent ability of animals to resist disease and to also enhance the efficacy of vaccination programs employed in Australian feedlots to reduce the incidence of disease in Australian feedlots by identifying animals with an enhanced ability to mount an immune response when under stress.

The cattle studied in MLA project, B.STU.0244 were considered to have a below average risk of contracting BRD at the feedlot due to the calves having been a) yard-weaned and acclimatised to handling, b) pre-vaccinated against BRD causing agents prior to feedlot entry and c) exposed to minimal mixing with unfamiliar animals during feedlot finishing. Therefore, the outcomes of B.STU.0244 can be seen as being representative of the potential economic and health and welfare benefits of selecting for immune competence in a low disease risk feedlot environment. On this basis, it could be expected that the benefits of selection for immune competence could be greater in a higher disease risk feedlot environment. Therefore, in this study we aimed to quantify the benefits of breeding for immune competence in commercial Australian feedlots under higher disease risk conditions than animals in previous project work.

The expected outcomes of this project are closely aligned with key priorities areas identified in relevant industry strategic plans (MISP 2020, MLA 2020, SISP 2020, BISP 2020). Improving the immune competence of Australian beef cattle is expected to significantly improve animal health and welfare outcomes for animals in all beef production enterprises, enhance community and consumer support for the beef industry and result in significant productivity gains for beef producers both on-farm and at the feedlot.

2. Objectives

A previous project (B.STU.0244) estimated genetic parameters and genetic and phenotypic associations between immune competence and feedlot health and performance. This project aimed to extend and validate the work conducted in B.STU.0244 (and work with industry partners at Angus Australia), to quantify the benefits of breeding for immune competence in commercial Australian feedlots under higher disease risk conditions than animals were exposed to in the previous project work.

This project aimed to quantify the benefits of selecting for immune competence in Australian commercial Australian feedlots.

Specific aims of the project were:

Part I

- To assess the benefits of genetic selection for immune competence, realised through reduced health associated disease and mortalities, in higher disease risk environments.
- To generate additional immune competence phenotype data to be used in future studies to improve the accuracy of genetic parameter estimates for immune competence traits and to assess the potential for generating EBVs for immune competence traits in beef cattle.
- To generate phenotypic data to be used to inform future GWAS to test whether major genes are associated with enhanced immune competence in beef cattle. Future genotypic data also has the potential to be used in the development of a genomic test (gEBVs) for immune competence in beef cattle.
- To further determine the ability of immune competence phenotypes to mitigate disease incidence and severity in commercially relevant feedlot environments.
- To incorporate metrics and physiological parameters for individual animals, collected as part of resilience testing at yard weaning, into disease risk assessment models to improve model predictions
- To collect detailed disease incidence data through close monitoring of cattle in feedlot pens and
    1. physically scoring animals for symptoms of BRD using a standardised scoring system,
    2. lung function scoring using an electronic stethoscope (whisper veterinary stethoscope) where animals are pulled from pens and
    3. monitoring feed intake (where GrowSafe systems are available).
- Lung lesion scores will also be collected on individual animals at slaughter where possible. Data collected as part of BRD detection in feedlot pens and lung lesion scoring at slaughter will provide critical data to validate:
    1. the ability of immune competence phenotyping to predict feedlot health and performance,
    2. the ability of BRD risk assessment models to predict BRD risk in feedlot environments and
    3. the effectiveness of 'whisper' electronic stethoscope to accurately diagnose BRD in the

Australian feedlot environment.

- To provide data to support the implementation of best practice objective scoring for BRD in feedlot pens.
- To more accurately measure relationships between immune competence, stress responsiveness and temperament in beef cattle.

Part II

We also identified a need for resilience phenotyping methods to be refined in future studies, improving the practicality of testing large numbers of animals on farm, improving compatibility of testing procedures with current husbandry procedures used in industry and reducing testing costs. Central to this refinement is the need for development of pen-side tests to replace labour intensive laboratory tests, and in so doing, remove the logistical issues associated with sample transportation. Therefore, additional specific aims of the project were:

- To refine the current on-farm resilience phenotype testing protocol through:
  1. reducing the number of farm visits required,
  2. development of pen-side tests to replace laboratory tests removing the need for samples collected on farm to be stored and transported frozen to the laboratory for analysis and
  3. investigating if alternative, easier to measure immune parameters, may be indicative of general immune competence including total (non-specific) antibody levels and thermal imaging of skin test reactions.

3. Methodology

3.1 Study Animals

An animal ethics application, to cover all experimental activities to be undertaken as part of this project, was approved by the CSIRO Chiswick Animal Ethics committee (ARA 19/25). All animals enrolled in the study (n=1,661) were commercial purebred Angus or crossbred Angus x Hereford steers. Steers enrolled were from three co-operator herds located in NSW at Barraba (Herd 1), Walcha (Herd 2) and Gloucester (Herd 3). It was deemed important to enrol co-operator herds located across different geographical locations to ensure results from the study were not location specific. All steers were resilience tested at weaning. Steers were yard-weaning for a minimum of 7 to maximum of 15 days. During yard weaning, steers were fed high quality hay ad libitum, supplemented with weaner pellets or a feedlot starter ration and had free access to clean drinking water. Where yard-weaning concluded prior to completion of resilience testing, steers were released into small holding paddocks and re-mustered to yards for testing procedures. Details of the number of steers enrolled from each co-operator herd and the resilience testing cohorts employed within each herd are presented in Table 1. Birth dates were not available for individual steers, however based on joining dates of their dams, steers were calculated to be 4-9 months of age at weaning and steers within each testing cohort were expected to differ in age by a maximum of approximately 10 weeks. All steers, except for steers in testing cohort 3B, were born in severe drought conditions which continued until the steers had reached approximately yearling

age. As a consequence, these steers were extensively supplementary fed during the first year of their life. Post weaning, all steers were backgrounded on pasture at co- operator herd farms with supplementation as required until reaching feedlot entry weights.

Table 1. Testing cohort details for resilience testing.

Co-operator Herd	Testing Cohort	Number of Animals
Herd 1	1A	183
	1B	184
	1C	162
	1D	174
	1E	162
Herd 2	2A	82
	2B	95
	2C	160
	2D	154
	2E	163
Herd 3	3A	67
	3B	75
TOTAL		**1661**

3.2 Resilience Testing

The immune competence, stress responsiveness and temperament of all steers (n=1661) was assessed at weaning using the methodologies described below. A timetable of testing procedures which occurred during the weaning period is presented in Table 2.

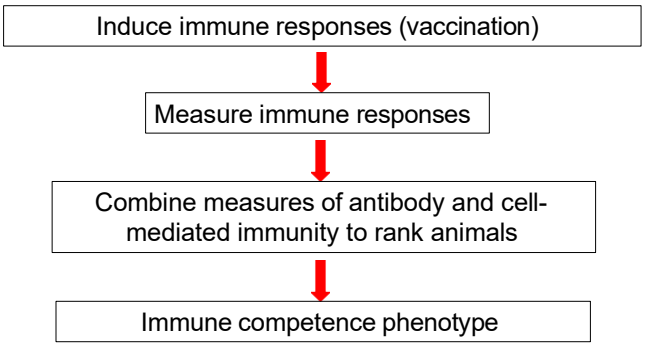
Table 2. Timetable for resilience testing procedures conducted on farm. All calves within a given herd testing cohort were tested on the same day. Responses to delayed-type hypersensitivity (DTH) testing were always assessed 2 days post injection (eg. operation were either undertaken on day 0, 12 & 14 or, alternatively on day 0, 13 & 15).

Day	Operation
Day 0	Weaning Liveweight recording Vaccinate with clostridial vaccine (Ultravac 7in1, Zoetis) Flight speed testing Crush score assessment
Day 12 or 13	Liveweight recording Injections for DTH skin test
Day 14 or 15	Liveweight recording Measure response to DTH skin test Collect blood sample Flight speed testing Bleeding score assessment

3.2.1 Assessing Immune Competence

Animals received a clostridial vaccination (Ultravac 7in1, Zoetis) at marking (2-3 months of age) and again on the day of weaning (Day 0) to induce measurable immune responses (Table 2). All vaccinations were administered subcutaneously at the base of 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 2). To improve our ability 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 2. Flow diagram describing the steps involved in determining the immune competence phenotype of individual animals.



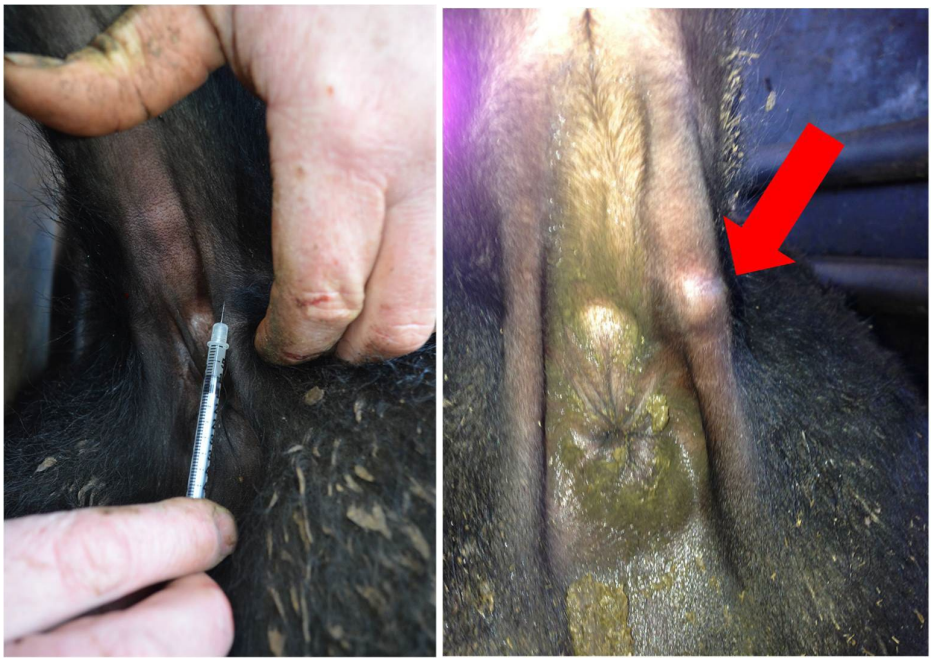
Assessing Cell-Mediated Immune Responses

The magnitude of delayed-type hypersensitivity (DTH) skin reactions to vaccine components was used to assess the cell-mediated immune responses (CMIR) 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 fitted 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 48Hrs post-injection, changes in skin thickness at each injection site (test and control) were again assessed using calipers (Figure 3A). 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).

The magnitude of DTH responses were calculated as the log of (double skin fold thickness (DSFT) at test site / DSFT at control site) at 48 hours post-injection (T48). For analysis, the log of (DSFT at test site / DSFT at control site) at T0 was fitted as a covariate in statistical models (see section 3.6).

Figure 3. 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).

A. B.



Assessing Antibody-Mediated Immune Responses

Production of antibody, more specifically anti-tetanus toxoid serum IgG1, in response to vaccination was used to assess antibody-mediated immune responses (AMIR). The clostridial vaccination administered to animals at 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 using jugular venepuncture post-vaccination (Table 2). Optimal timing of collection, to coincide with peak antibody response, has been investigated previously. 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.

As all steers enrolled in the study had already received a clostridial vaccination prior to when immune competence testing commenced at weaning, baseline antibody levels on Day 0 were not assessed. The rationale for this decision was based on the following factors:-

1. Based on previous studies, circulating antibody produced in response to the previous vaccination administered at marking may still be detectable in serum at the start of testing at weaning and therefore adjusting post-testing antibody level values (assessed on day 14 or 15 of testing) based on pre-testing antibody level values (assessed on day 0 of testing) was expected to disadvantage those animals that had responded strongly to previous vaccination at marking.
2. The clostridial vaccination history of calves in each herd cohort was identical and therefore the response assessed during testing at weaning represents a cumulative response to the vaccination given at marking and at Day 0 of weaning.
3. As calves were between 4 and 9 months of age at weaning (depending on herd) and the

half- life of maternal antibody in the calf being approximately 10-22 days (Cervenak and Kacskovics, 2009), any influence of maternal antibody on responses to vaccination during testing were expected to be minimal.

To assess AMIR, serum IgG1 antibody against tetanus toxoid antigen (kindly provided by Zoetis, Australia) was determined using an in-house developed indirect ELISA method as described previously (Hine et al., 2019). All test and control samples were assayed in quadruplicate. The co-efficient of variation (CV) of quadruplicate and combinations of triplicate values were calculated and the value for the combination with the lowest CV recorded. Where selected sample values had a CV>10%, samples were repeated. Pooled pre- and post-vaccination serum samples were used as negative and positive controls, respectively. Mean optical density (OD) values for replicates were corrected based on the mean OD value of a positive control serum sample assayed on all plates (Mallard et al., 1997). Antigen- specific IgG1 was detected using affinity purified sheep anti-bovine IgG1 conjugated to alkaline phosphatase (AbD, Serotec, Product No. AAI21AB). For analysis, adjusted OD values were square root transformed to improve normality (see section 3.6).

All steps of the ELISA procedure were optimised to maximise signal while minimising background. A subset of samples randomly selected from each testing cohort (see Table 2) were serially diluted and tested to determine the appropriate sample dilutions for each individual testing cohort to be used in the assay.

Immune Competence Phenotypes

Measures of AMIR and CMIR were combined to generate immune competence phenotype values for individual steers enrolled in the study. By combining measures of AMIR and CMIR, steers with a high immune competence phenotype value were expected to exhibit improved general disease resistance.

Specifically, steers were ranked for AMIR and CMIR based on model residual (observed minus predicted) values for each respective trait. Residuals for ranking were generated from the models described in the statistical analysis section 3.6 and were standardised, by dividing each residual value by the standard deviation of all residual values for that trait. Rankings were then used to calculate immune competence phenotype (IC Comb) values for individual steers using the formula shown below, as previously reported by Reverter et al (2021a). This approach considers the correlation (r) between standardised residual values for AMIR (Z_AB) and CMIR (Z_CELL) as well as the difference in ranking (dRank) of individuals for each metric and uses them as weights in the averaging.

$$IC\_Comb = [Z\_CELL + (1-|r|)\, Z\_AB\,](1-|dRank|/(n-1)).$$

3.2.2 Assessing Stress Responsiveness

Average daily weight gain during the yard weaning period (ADGW) 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 as a result of separation anxiety and potentially mixing with new herd mates and stress associated with exposure to a new environment and diet. All calves tested were weighed three times during the weaning period, at

the commencement of weaning (Day 0), at the time when DTH injections were administered (Day 12 or 13) and again at the time when the magnitude of DTH reactions were assessed at the end of weaning (Day 14 or 15). Timing of weighing was consistent within each testing cohort. WtGain was calculated as the mean of average daily gain recorded between each weighing event.

3.2.3 Assessing Temperament

The temperament of individual calves was assessed using flight speed (FS) testing, crush scores (CS) and bleeding scores (BS). Crush scores were assessed by a trained observer 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 (see Table 3). Bleeding score is a novel temperament measure we are currently investigating. Bleeding scores were assessed by trained personnel on day 14 or 15 (coinciding with blood collection) by scoring the behaviour of animals in response to being restrained for blood collection (holding the head to the side to allow access to the jugular vein). Scores, on a scale from 1 to 5, were given by the handler using a standardised scoring system (see Table 4). Flight time was assessed on the day of weaning (Day 0), and again at the end of weaning (Day 14 or 15). To measure flight speed, calves were first restrained in the head bale for approx. 30 secs while vaccinated (Day 0) or for approx. 1 min while blood samples were collected (Day 14 or 15), 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. A typical setup for assessing flight speed is shown below (Figure 4).

Figure 4. Flight speed testing setup as used at Co-operator Herd 3 (left) and Herd 1 (right) cattle handling facilities.

Table 3. Description of crush scoring protocol

Crush Score – Scored by observer. Animal in crush but not restrained in head bale	
Score	**Description**
1	Calm, minimal movement
2	Some movement, restless
3	Continuous agitated movement
4	Continuous vigorous movement
5	Violent struggling, rearing, twisting

Table 4. Description of bleeding scoring protocol

Bleeding Score – Scored by handler holding the animals head to side while restrained in the head bale	
Score	**Description**
1	Calm, minimal resistance to being held by handler
2	Restless, some resistance to being held by handler
3	Resistance to being held but handler can control head movement
4	Continuous vigorous head movement, handler finds it difficult to hold animal
5	Violent struggling, handler unable to hold animal

3.2.4 DNA Sample Collection

A DNA sample (buffy coat) was collected from all tested animals and stored for future genotyping. Please note that no allowance for genotyping has been included in the current project. To prepare buffy coat samples, a single 10mL blood samples was collected into EDTA vacutainer tubes using jugular venepuncture and immediately stored on ice. Buffy coats were then prepared from blood by centrifugation (700 × *g*, 20 min, RT) and stored at −20°C for future genotyping.

3.2.5 Summary Description of Traits Assessed at Weaning

Table 5. Description of traits assessed at weaning.

Description	
Immune Competence Traits	
AMIR (OD units)	Antibody-mediated immune response. Assessed by measuring production of anti-tetanus toxoid serum IgG1 antibody in response to vaccination at Day 14 or 15 of weaning.
CMIR (log fold change in skin thickness)	Cell-mediated immune response. Assessed by measuring delayed type hypersensitivity (DTH) response to 7in1 vaccine components at Day 14 or 15 of weaning.
IC_Comb (rank)	Combined immune response. Calculated by combining (with equal weighting) measures of AMIR and CMIR. An indicator of an animals overall immune competence.
Stress Responsiveness Traits	
ADGW (kg/day)	Change in liveweight recorded over the weaning period (coinciding with resilience testing period from Day 0 to Day 14 or 15 of weaning).
Temperament Traits	
FT1 (secs)	Flight time assessed on Day 0 of weaning.
FT2 (secs)	Flight time assessed on Day 14 or 15 of weaning.
CS (score 1-5)	Crush score assessed on Day 0 of weaning.
BS (score 1-5)	Bleeding score assessed on Day 14 or 15 of weaning
Growth Traits	
WWT1 (kgs)	Liveweight recorded on Day 0 of weaning.
WWT2 (kgs)	Liveweight recorded on Day 12 or 13 of weaning
WWT3 (kgs)	Liveweight recorded on Day 14 or 15 of weaning
Health Status Traits	
Condition (score 0 or 1)	Health status assessed during weaning (0 = no health issues, 1 = 1 or more health issues observed including respiratory illness, pinkeye or general ill-thrift)

3.3 Feedlot Performance

3.3.1 Feedlot Induction

A total of 1326 resilience tested steers were inducted into Feedlot 1 in North Western NSW (n=1022), Feedlot 2 in Northern NSW (n=134) or Feedlot 3 in Southern QLD (n=170). For induction group details see Table 6. A description

Please note: We were able to gain approval from both CSIRO (internal approval required for all staff travel due to Covid restrictions) and feedlot operators (approval required for visitors to enter the feedlot due to Covid restrictions) for CSIRO technical staff to be present at the time when steers were inducted into the feedlot, with the exception of two small groups of steers inducted into Feedlot 1 (Ind_1J (n=65) & Ind_1F (n=23)) and steers inducted into Feedlot 3 (Ind_3A (n=64) Ind_3B (n=106)). Approval to be present at feedlot induction was granted on the basis that the data to be collected was critical to the success of the project (a requirement of CSIRO for approval of staff travel) and that appropriate social distancing could be maintained between feedlot and CSIRO staff when collecting data at induction (a requirement of both CSIRO and feedlot operators for approval).

Being in attendance at the feedlot at the time steers were inducted allowed us to a) cross- reference vendor visual ear tags (removed at induction) with feedlot visual ear tags (applied at induction) in the case that an animal losses their electronic NLIS tag at the feedlot, b) access feedlot entry liveweight data, c) record treatments administered to steers at induction and d) identify specific lot/pen numbers in each feedlot's data recording system corresponding to each group of inducted steers so their performance during feedlot finishing could be monitored. The induction procedure also provided us with an opportunity to collect an additional post-weaning flight time measurement on steers in unfamiliar surroundings. As we had observed moderate favourable genetic correlations between immune competence and temperament in previous studies, we wanted to investigate the relationship between flight time measured on animals in familiar versus unfamiliar settings and how these measures may be predictive of feedlot performance in terms of health and productivity. Where CSIRO staff were unable to be present at induction (due to covid restrictions) feedlot induction liveweights and feedlot identification details were provided by feedlot operators; however, flight time measurements at induction were not collected. An example of routine feedlot induction data recorded is presented in Table 7.

Table 6. Induction liveweight and flight time summary statistics for each induction cohort

Co-operator Herd	Induction Cohort	Feedlot	Induction Date	n
Herd 1	Ind_1A	1	13/10/2020	51
Herd 1	Ind_1B	1	21/10/2020	60
Herd 1	Ind_1C	1	28/10/2020	62
Herd 1	Ind_1D	1	1/12/2020	256
Herd 1	Ind_1E	1	16/12/2020	193
Herd 1	Ind_2A	2	20/01/2021	134
Herd 1	Ind_1F	1	9/02/2021	23
Herd 2	Ind_1G	1	13/01/2021	184
Herd 2	Ind_1H	1	17/02/2021	73
Herd 2	Ind_3A	3	19/01/2021	64
Herd 2	Ind_3B	3	13/04/2021	106
Herd 3	Ind_1I	1	12/08/2020	55
Herd 3	Ind_1J	1	4/08/2021	65
TOTAL				**1326**

Table 7. Example of the feedlot induction data recorded.

Feedlot	Induction Cohort	RFID	Visual Tag	Breed	Customer ID	Market Specification	Induction Date	Induction liveweight (kgs)	Induction Pen	Induction treatments
Feedlot 1	Ind_1B	982 xxxxxxxxxx	39023	ANGUS	51	150-260 HGP Free	20/10/2020	450	B5	HGP Free induction + Bovilis IBR + Easy Dose
Feedlot 1	Ind_1B	982 xxxxxxxxxx	39044	ANGUS	51	150-260 HGP Free	20/10/2020	456	B5	HGP Free induction + Bovilis IBR + Easy Dose
Feedlot 1	Ind_1B	982 xxxxxxxxxx	39038	ANGUS	51	150-260 HGP Free	20/10/2020	466	B5	HGP Free induction + Bovilis IBR + Easy Dose
Feedlot 1	Ind_1B	982 xxxxxxxxxx	39027	ANGUS	51	150-260 HGP Free	20/10/2020	480	B5	HGP Free induction + Bovilis IBR + Easy Dose
Feedlot 1	Ind_1B	982 xxxxxxxxxx	39022	ANGUS	51	150-260 HGP Free	20/10/2020	493	B5	HGP Free induction + Bovilis IBR + Easy Dose

3.3.2 Summary Description of Traits Assessed at Feedlot Induction

Table 8. Description of traits assessed at feedlot induction

	Description
Temperament Traits	
IndFT (secs)	Flight time assessed at feedlot induction.
Growth Traits	
IndWT (kgs)	Liveweight recorded at feedlot induction
ADGI (kg/day)	Change in liveweight recorded from weaning to feedlot induction.

3.3.3 Feedlot Health and Production Performance Monitoring

Dr Tony Batterham (Bovine Dynamics) provides Veterinary Consultation services to Feedlot 1 where the majority of steers (n=1022) enrolled in the project were feedlot finished. Dr Batterham was contracted as a consultant on this project to provide advice on project design and to facilitate access to performance data, both in terms of health and productivity, recorded on steers enrolled in the project during feedlot finishing and at slaughter. Bovine Dynamics operates the Animal Health Data (AHD) system (see details below) in which all disease incidence (including pull date and disease diagnosis), health treatments, health-related mortalities (including cause of death) and disease and defect data collected at slaughter are recorded for feedlot cattle. Data was automatically uploaded from the feedlot/abattoir to the AHD system in real time. Similar health and performance data is routinely collected at both Feedlot 2 and Feedlot 3 and was made available to the research team.

Please note: We had originally proposed to physically score all steers entering the feedlot during the initial phases of feedlot finishing for symptoms of bovine respiratory disease (BRD) using a standardised scoring system and to assess symptoms, in any steers pulled for suspected BRD, using a whisper stethoscope. However, at the time when the first cohort of steers were scheduled to arrive at the feedlot it was deemed unlikely that it would be possible for CSIRO staff to conduct this regular BRD scoring and whisper stethoscope assessment due to Covid restrictions on staff travel imposed by CSIRO and the frequency at which visitors were permitted at each feedlot imposed by feedlot operators. Therefore, a contingency plan was put in place to ensure that detailed health and productivity data would be collected on all steers during feedlot finishing. As part of the contingency plan, steers were inspected daily, by highly experienced feedlot staff at each respective feedlot and health data was regularly communicated to CSIRO technical staff working on the project to enable them to closely monitor the performance of steers remotely. However, as scoring of animals for symptoms of BRD in feedlot pens and assessment of animals, suspected to be infected with BRD, using the whisper stethoscope are not part of routine disease monitoring and diagnosis undertaken by feedlot staff, data on BRD scores and lung function using the whisper

stethoscope could not be collected on steers during feedlot finishing in the current project as was originally planned.

Therefore, highly experienced feedlot staff inspected steers daily for any signs of ill-thrift or illness. Any steer deemed to require treatment was immediately pulled (transferred from the feedlot pen to the hospital pen) and treated. Diagnosis of disease and appropriate treatment plans were generally determined by experienced feedlot personnel; however, when the cause of disease was unclear the feedlot veterinarian was consulted to confirm disease diagnosis and advise on an appropriate treatment plan. All disease incidence (including pull date and disease diagnosis), health treatments and health-related mortalities (including cause of death) was recorded and uploaded in real time to the AHD system (Feedlot 1) or an equivalent recording system (Feedlot 2 and Feedlot 3). Necropsies were completed on all animals which died at the feedlot (where in a fit state), including collection of appropriate tissue samples for pathology, to determine cause of death. Necropsies were undertaken by either the feedlot veterinarian or, when the feedlot veterinarian was not available, by trained feedlot personnel. Where feedlot personal conducted necroscopies, outcomes were discussed with the feedlot veterinarian to confirm cause of death. Health reports summarising the above information and associated information were extracted from the AHD system (or equivalent) database on a regular basis and sent to CSIRO technical staff to ensure that detailed health data was captured on all steers enrolled in the project throughout the feedlot finishing process. An example of health data records available from the AHD system for individual steers is shown in Table 9. Similar health data was available for steers finished at Feedlot 2 and Feedlot 3.

Animal Health Data (AHD) System Description

About	Animal	Health	Data

(Source: https://www.quirindivetclinic.com.au/OurServices/FeedlotServices.aspx)

The Animal Health Data (AHD) system is an online industry benchmarking and feedlot health and production analysis system used by feedlots.

AHD has been collecting and benchmarking feedlot data for more than 10 years. There are more than 15 million individual animal health and performance records covering from feedlot induction through to carcase grading. At any point in time there is in the order of 750,000 head of cattle on feed that are being benchmarked by the AHD system.

The AHD system works by importing data files from feedlots and where authorised grading data from the MSA program, slaughter feedback data files and disease and defect data files from processing plants.

This collection of data allows the AHD system to conduct analysis that generates reporting covering:

- Feedlot health performance and benchmarking.
- Feedlot production performance and benchmarking.
- Vendor health and production performance and benchmarking (feedlot,

slaughter and whole of life).

- Processing plant yield, offal loss and grading performance and benchmarking.

Table 9. Example of the health data accessed from the Animal Health Data (AHD) system.

RFID	Pull date	Pull instance	Action instance	Liveweight (kgs)	DOF	Pull reason	Body temperature	Hospital pen no.	Final Diagnosis	Treatment
982 xxxxxxxxxx	20201123	FIRST PULL	ACTION 1	444	34	BRD	39.2	B6	BRD	1 x Draxxin
982 xxxxxxxxxx	20210430	FIRST PULL	ACTION 1	700	192	Foot Abscess		TC4	Foot Abscess	1 x PROPERCILLIN
982 xxxxxxxxxx	20201225	FIRST PULL	ACTION 1	583	66	Foot Abscess		TC4	Foot Abscess	1 x PROPERCILLIN
982 xxxxxxxxxx	20201023	FIRST PULL	ACTION 1	474	3	Foot Abscess		TC4	Foot Abscess	1 x PROPERCILLIN
982 xxxxxxxxxx	20201123	FIRST PULL	ACTION 1	572	34	BRD	39.1	B6	BRD	1 x Draxxin
982 xxxxxxxxxx	20201225	FIRST PULL	ACTION 1	615	66	Big Legs		TC6	Laminitis	1 x VITAMIN A D & E

3.3.4 Summary Description of Traits Assessed During Feedlot Finishing

Table 10. Description of traits assessed during feedlot finishing.

	Description
Health Traits	
PullNo (number of incidents)	Number of times an individual steer was pulled (removed from home pen to hospital pen) for health reasons during feedlot finishing
Mortalities (score 0 or 1)	Fate at feedlot, 0 = completed feedlot finishing, 1 = died during feedlot finishing
Growth Traits	
ADGF (kg/day)	Change in liveweight recorded during feedlot finishing (feedlot exit liveweight estimated based on HCWT being a standard 60% of feedlot exit liveweight).

3.4 Offal and Carcase Assessment at Processing

Steers from Herd 1 and Herd 2 were European Union (EU) accredited and entered an EU finishing program upon arrival at the feedlot, whereas steers from Herd 1 were mostly non-EU accredited (with exception of Induction Cohort Ind_1F) and entered a non-EU finishing program. EU accreditation influenced the abattoir at which steers were processed. Details of kill dates and abattoirs at which steers were processed from each induction cohort are provided in Table 10.

3.4.1 Offal Inspection

Offal defects were recorded by appropriately trained QA officers who carefully inspected offal from individual steers and recorded any signs of present or past disease as per routine offal inspection procedures implemented at the processing plant.

Please Note: No offal data was available for lots Ind_1C, Ind_1E, Ind_2A, Ind_3A and Ind_3B as the health terminal at which offal defects are routinely recorded was not operating at the processing plant at the time steers were processed due to labour shortages resulting from covid.

Where CSIRO technical staff were able to be present at the abattoir when trial steers were being processed, In addition, appropriately trained CSIRO technical staff inspected lungs and recorded detailed lung lesion scores for the right and left lung of individual steers. Where CSIRO staff were unable to be present at the abattoir when steers were being processed (due to covid restrictions on staff movements imposed by CSIRO, restrictions on access imposed by processors and/or state border restrictions) offal defect were recorded by appropriately trained QA officers using routine offal inspection procedures implemented at processing plant and data collected retrospectively (where available).

Table 11. Processing plant details.

Induction Cohort	Kill date	Vendor	EU accredited	Processing plant
Ind_1I	18/01/2021	Herd 3	Yes	Plant 1
Ind_1A	22/04/2021	Herd 1	No	Plant 2
Ind_1B	23/04/2021	Herd 1	No	Plant 2
Ind_1C	13/04/2021	Herd 1	No	Plant 1
	20/04/2021	Herd 1	No	Plant 3
Ind_1D	19/04/2021	Herd 1	No	Plant 4
	26/04/2021	Herd 1	No	Plant 4
Ind_1E	3/05/2021	Herd 1	No	Plant 4
Ind_1E$^{\pm}$	Various	Herd 1	No	Various
Ind_1G	21/07/2021	Herd 2	Yes	Plant 1
Ind_1F	11/08/2021	Herd 1	Yes	Plant 1
Ind_1H	16/08/2021	Herd 2	Yes	Plant 1
Ind_2A	25/10/2021	Herd 1	No	Plant 5

Ind_3A	12/08/2021	Herd 2	Yes	Plant 5
Ind_3B	18/11/2021	Herd 2	Yes	Plant 5
Ind_1J	17/01/2022	Herd 3	Yes	Plant 1
	24/01/2022	Herd 3	Yes	Plant 1

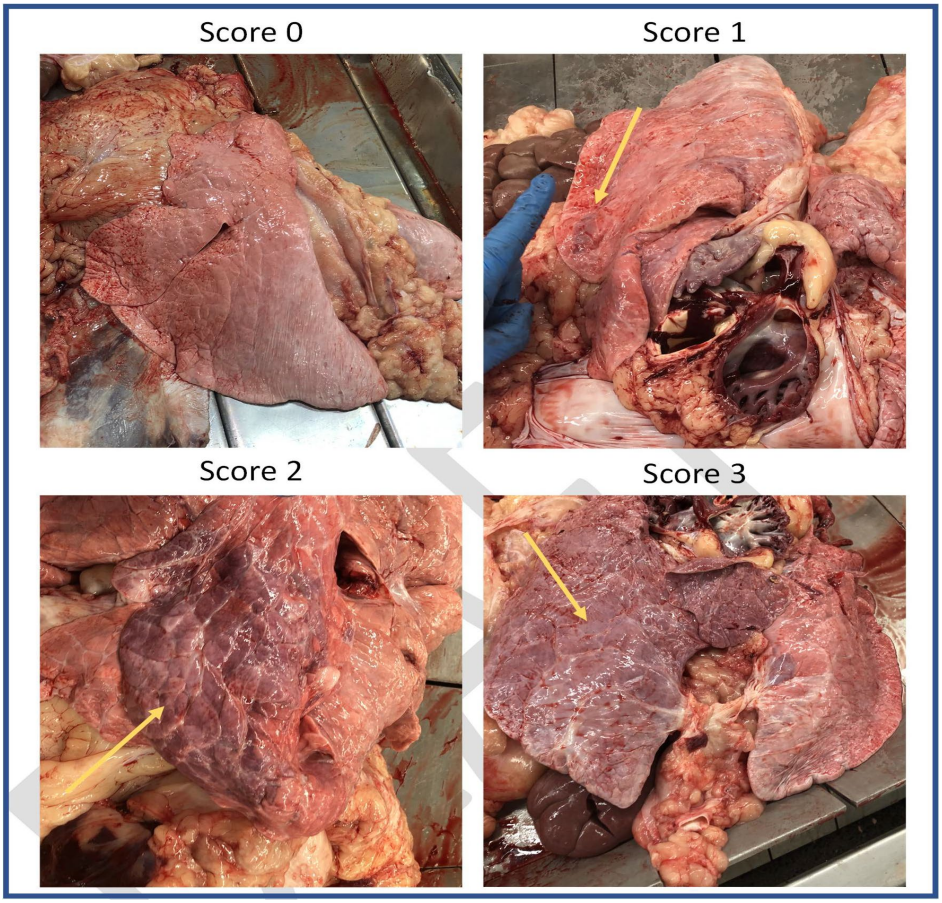
[±]Steers in Induction Cohort Ind_1E entered a short fed program and were subsequently slaughtered at various processing plants once reaching target weights

A simplified lung lesion (consolidation) scoring guide suitable to identify the presence and extent of lung damage on the processing chain was developed in consultation with an experienced veterinarian (see Table 11). Images depicting lung lesion scoring examples are shown in Figure 5. Pleurisy and lung abscesses were recorded separately as part of routine offal inspection.

Table 12. Lung lesion (consolidation) scoring system

Lung Lesion Score	Classification	% Lung Affected
0	Absent	0
1	Minor	1-10
2	Moderate	11-49
3	Severe	≥ 50

Figure 5. Lung lesion scoring images (yellow arrow indicates areas of lung damage)



Please note: Detailed lung lesion data could not be recorded for Induction Cohorts Ind_1B, Ind_1C, Ind_1F, Ind_1G, Ind_1H, Ind_1J, Ind_2A, Ind_3A and Ind_3B as covid restrictions prevented access for CSIRO technical staff to enter the plant at the time steers were processed. However, for Induction Cohorts Ind_1B, Ind_1F, Ind_1G, Ind_1H and Ind_1J lungs from steers were inspected for signs disease by appropriately trained QA staff as part of routine offal inspection procedures implemented at processing plant.

3.4.2 Carcase Assessment

Carcase traits were assessed on individual steers at slaughter by experienced MSA accredited graders and included hot carcase weight (HCWT), Dentition (Dent), P8 fat cover (P8), eye muscle area (EMA), AUSMEAT marbling score (AUSMarb), Meat Standards Australia (MSA) marbling score (MSAMarb), meat colour (MeatCol), fat colour (FatCol), rib fat (RibFat), ultimate pH (pH), loin temperature (LoinTemp), hump height (Hump) and ossification (Oss). A detailed description of each trait measured on animals in the study is presented in Table 12.

Please Note: Carcase trait data was kindly supplied by co-operating processing plants directly or through the AHD system. Therefore, carcase trait data was only available for traits which were routinely measured at each respective processing plant and data for all traits was not available for all steers which were processed.

Table 13. Description of carcase traits measured on animals in the study including details of groups of animals each trait was measured on.

	Description[#]
Carcass Traits	
HCWT (kgs)	Weight of hot standard carcase
DENT (score 0 or ≥2)	Dentition – Number of permanent incisors erupted
P8 (mm)	Fat depth assessed at the P8 site
EMA (cm^2)	Carcase eye muscle area
AUSMarb (score 0-9 in increments of 1)	AUSMEAT marbling score assessed at the 12th to 13th rib of the carcase on the exposed rib eye
MSAMarb (score 100-1190 in increments of 10)	MSA marbling score assessed at the 12th to 13th rib of the carcase on the exposed rib eye
MeatCol (score 1A, 1B, 2 or ≥3)	AUSMEAT standard meat colour assessed on eye muscle
FatCol (score 0 or ≥1)	AUSMEAT standard fat colour assessed on intermuscular fat lateral to the eye muscle
RibFat (mm)	Subcutaneous fat depth assessed at the quartering site
pH (pH units)	Ultimate pH assessed at loin site
LoinTemp (°C)	Loin temperature taken at assessment site when measuring pH
Hump (mm in 5mm increments)	Hump height
Oss (score 100-590 in increments of 10	Ossification assessed in the cartilage within the vertebral spinous processes

NoOffalDef (cumulative score)	Cumulative number of offal defects observed at processing
NoOffalDefBIN (zero or non-zero)	Number of offal defects observed at processing
LungScore (cumulative score)	Total lung lesion score (both lobes) observed at processing
LungScoreBIN (zero or non-zero)	Lung lesion score (both lobes) observed at processing

3.5 Pen-Side Antibody Assay Development

The testing protocol we have developed to assess immune competence phenotypes of beef cattle involves measuring the ability of animals to mount both CMIR and AMIR. These measures are then combined to generate an immune competence phenotype index. Currently, cell-mediated immune responses are assessed using a skin test which is conducted pen-side. In contrast, antibody- mediated responses are assessed in the laboratory by measuring antibody production in response to vaccination using in-house developed enzyme-linked immunosorbent assay (ELISA) (see section 3.2.1). The assay requires that blood be processed shortly after collection on-farm to prepare serum which is then frozen and transported back to the laboratory for subsequent analysis. Development of a pen- side test to assess antibody-mediated immune responses would remove the need to process samples to prepare serum and to transport frozen serum samples back to the laboratory for analysis, greatly improving the practicality of immune competence testing. Therefore, this component of the project was aimed at investigating the potential to develop a dipstick-based ELISA protocol which would allow pen-side testing of AMIR as part of our standard immune competence testing protocol.

To develop a dipstick-based ELISA test which can be conducted pen-side. To be practical to apply on farm the test must meet the following criteria:

- Does not require the use of specialist equipment
- Requires minimal sample preparation
- Has sufficiently high throughput to allow ‘same day’ analysis of samples
- Is accurate and precise (as validated against existing laboratory-based methods)
- Is repeatable
- Yields semi-quantitative results which allow animals to be ranked effectively
- Is cost effective to facilitate the economical testing of large numbers

<u>Investigating available technologies for development of a dipstick-based ELISA method</u>

A rapid dipstick ELISA test is commercially available to assess tetanus immunity (levels of tetanus toxoid specific antibodies) in human patients, the Tetanos Quick Stick (TQS) test, available through the Nephrotek Laboratory, Rungis, France (Korinek et al., 2008; N’Diaye et al., 2014; Orsi et al., 2015; Hatamabadi et al., 2011). This test is used in emergency departments to rapidly determine the tetanus vaccination status of patients presenting with wounds to determine if a tetanus vaccination should be administered to the patient. A rapid dipstick ELISA test is also commercially available to assess

tetanus immunity in horses, the Fassisi TetaCheck test available through Fassisi^{AT}, Austria (Recknagel, 2015). Similar to the TQS used for human patients, the Fassisi TetaCheck is used to determine the tetanus vaccination status of horses.

Fassisi TetaCheck tests were purchased directly from the supplier, Fassisi^{AT}, in Austria. These tests were rapid and easy to use but were relatively expensive at around 10 EURO per test (including delivery). Further, as the test is designed specifically for use in horses, extensive validation would be required to confirm cross-reactivity to bovine antibodies and the specificity of the test for detecting specific bovine antibody isotypes (eg. IgG1, IgG2 etc). On this basis, it was decided to investigate the potential to develop, from first principles, our own in-house dipstick-based ELISA assay.

Thermo Fisher recently marketed Nunc™ Immuno Sticks which are specifically designed to be used in dipstick-based ELISA assays (https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2FApplication- Notes%2FD19562.pdf). The 'Immuno Sticks' have a paddle which comes pre-coated with the same MaxiSorp™ coating that is applied to the 96 well plates currently used in our laboratory plate-based assay (see Figure 6). Immuno Sticks are relatively inexpensive, easy to use and do not require specialised equipment (such as multi-channel pipettes) to load. Therefore, it was decided to explore the potential of using Immune Sticks to develop a dipstick-based ELISA protocol. This proved a successful approach, and we report here the development and validation of an in-house dipstick- based ELISA assay for detecting levels of tetanus-toxoid specific bovine IgG1 in serum and whole blood which can be conducted 'pen side' based on the use of Nunc™ Immuno Sticks.

Figure 6. Nunc™ Immuno Sticks

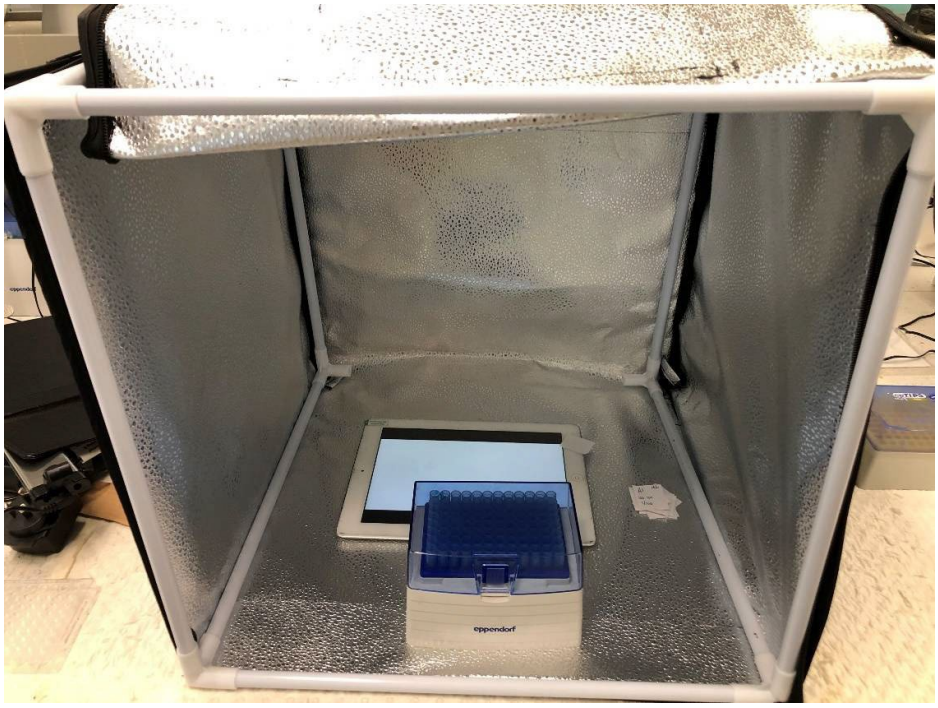
Dipstick-based ELISA Assay Development

Modification to existing plate-based ELISA protocol

Several modifications to the existing ELISA protocol were required to convert the protocol from a plate-based assay to a dipstick-based assay. These included modifications to reagent/sample volumes, washing steps, sample and reagent loading procedures and colour development detection. In the existing plate-based assay, wells are coated with 100uL of coating solution, blocked with 250uL of blocking solution and then 100uL of samples, conjugated antibody and substrate are sequentially added. Reagent volumes were increased 4-fold for the dipstick-based ELISA, 400uL of coating solution, 1000uL of blocking solution and then 400uL of sample, conjugated antibody and substrate. These volumes were required to ensure adequate coverage of the paddle with each reagent and complete coverage of the paddle with blocking solution (see figure 7). In the existing protocol plates are washed with a plate washer which automatically washes all wells on each plate simultaneously with 250uL of wash solution. For the dipstick-based ELISA assay, washing was conducted by hand using a squeeze bottle (1mL per dipstick). In the plate-based ELISA samples and reagents are loaded using an automated multi-channel pipette. For the dipstick-based ELISA, samples and reagents were loaded using a manual single channel pipette (a graduated disposable transfer pipette could also be used). In the current plate-based ELISA protocol, colour development in wells following the addition of substrate (the readout for the assay) is measured with a specialised plate reader which collects readings from all wells on the plate simultaneously. Colour development in the dipstick-based ELISA was either assessed visually (in dipstick tubes) against a colour gradient chart (semi-quantitative) or by transferring solutions from dipstick tubes to a low-binding plate and quantifying pixel intensity in images of the plate collected with the phone App (ProCam8) using the specialised free software, ImageJ. Transferring solutions from individual dipstick tubes to a low binding plate allowed for multiple assay results to be captured in a single image. To ensure appropriate image quality, images were captured on a white background in a collapsible photo cube (see Figure 8) to minimise reflection and glare.

Figure 7. Dipstick paddle coverage when 400uL and 1000uL added to tubes

Figure 8. Collapsible photo cube



Optimisation and validation of the dipstick-based ELISA protocol

When developing an ELISA, it is important to a) ensure minimum non-specific binding occurs in all steps of the assay to confirm assay specificity and minimise background interference, 2) optimise all steps of the assay to maximise the signal:background ratio and 3) maximise the dynamic range of the assay. Further, once the assay has been developed and optimised it is then important to evaluate assay performance in terms of intra- and inter-assay variation. Therefore, a series of experiments were undertaken to evaluate the above with results presented below. To optimise and validate the dipstick-based ELISA, solutions were transferred from dipstick tubes to a low-binding plate following colour development in the final step of the assay and the optical density of each solution measured using a plate reader. Although, we do not intend to use a plate reader to assess colour development in the dipstick-based ELISA protocol when conducted 'pen-side' (to avoid the need for specialised equipment), a plate reader was used to quantify results during assay development where highly accurate quantification of assay results were required.

Sample preparation

An important criterion for development of a pen-side ELISA test is the requirement for minimal sample processing prior to being assayed. As part of our current testing protocol, serum is prepared from blood using centrifugation, aliquoted and frozen on the day of collection. Serum samples are then transported back to the laboratory frozen and upon arrival are stored for subsequent analyses. Centrifugation requires the use of specialised equipment and the transportation of frozen samples can be logistically challenging. Therefore, we explored the potential of using whole blood or serum prepared from clotted blood by decanting serum (without centrifugation), as an

alternative to using serum prepared by centrifugation, in the dipstick-based ELISA protocol.

Incubation times

Binding of antibodies to their target antigens forms the basis of the ELISA test. Therefore, ELISA protocols involve a series of incubation steps to facilitate binding of antibody to antigen. Required time to facilitate binding is temperature dependant with increased temperatures reducing required times for binding. In the existing plate-based ELISA protocol, incubations (post addition of block) are conducted at room temperature for 1 hour. Incubation at 37°C was expected to reduce required incubation times, allowing the assay to be completed in less time. Therefore, we investigated the potential for incubation at 37°C to shorten the time required to complete the dipstick-based ELISA protocol. Although incubating at 37°C does require access to a small incubator/oven, these are relatively inexpensive, transportable, and easy to use. Further, when an incubator/oven is not available, the dipstick-based ELISA test could be conducted with incubation at room temperature providing incubation times are extended to 1 hour

3.6 Statistical Analyses

Immune Competence Traits

The immune competence traits, AMIR and CMIR were analysed using ANOVA linear models (SAS9.4 statistical analysis software) with relevant fixed effects and covariates fitted as described in Table 13. After fitting models, residuals were obtained for each trait and standardised. Standardised residuals were then used to calculate Immune competence phenotypes (IC_Comb) as described in section 3.2.1.

Phenotypic Correlations

Resilience, growth and carcase traits were analysed using ANOVA linear models (SAS9.4 statistical analysis software) with relevant fixed effects and covariates fitted as described in Table 13. After fitting models, residuals were obtained for each trait. Residuals were then treated as adjusted phenotypes for each respective trait and used to compute a 25 by 25 trait correlation matrix (see appendix).

Offal Defect and Lung Lesion Score Data

Offal defect and lung lesion score data were analysed using binomial linear regression models (binomial data) or ordinal regression models (ordinal data) (R studio) with relevant fixed effects and covariates fitted as described in Table 13.

Health Traits

Given the small sample size of animals that died at the feedlot (n=17) or were 'pulled' due to health issues observed during feedlot finishing (n=99), traditional statistical tests such as ANOVA and t-tests were deemed inappropriate. Therefore a non-parametric permutation test was applied to the data in which 10,000 random samples of size 17 were obtained and, for each sample, their average IC_Comb rank recorded and compared against the average IC_Comb rank of the 17 animals that died (Mortalities,)

or 10,000 random samples of size 99 were obtained and, for each sample, their average IC_Comb rank recorded and compared against the average IC_Comb rank of the 99 animals that were 'pulled' at the feedlot (PullNo, converted to a binary trait 0 = not 'pulled' or 1 = 'pulled 1 or more times).

Table 14.

Trait	Transformation	Fixed effects[±]	Covariates
AMIR	None	Herd*CGMark*CGWean, Condition	WWT1
CMIR	Log	Herd*CGMark*CGWean, Condition	WWT1, Log (DSFT at test site / DSFT at control site) at T0
ADGW	None	Herd*CGMark*CGWean, Condition	WWT1
FT1	None	Herd*CGMark*CGWean, Condition	WWT1
FT2	None	Herd*CGMark*CGWean, Condition	WWT1
CS	None	Herd*CGMark*CGWean, Condition	WWT1
ADGI	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort	WWT1
IndFT	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort	Nil
IndWT	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort	WWT1
ADGF	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort	IndWT
PullNo	Permutation test used to analyse trait		
Mortalities	Permutation test used to analyse trait		
HCWT	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
P8	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
EMA	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
AUSMarb	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
MSAMarb	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
MeatCol	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
FatCol	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
RibFat	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
pH	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
LoinTemp	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
Hump	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
Oss	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir, Dentition	Nil
NoOffalDef	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir	Nil
NoOffalDefBIN	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir	Nil
LungScore	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir	Nil
LungScoreBIN	None	Herd*CGMark*CGWean, Condition, Feedlot*Induction Cohort*Abattoir	Nil

[±]CGMark = Management group from birth to marking, CGWean = Management group from marking to weaning

4. Results

4.1 Summary Statistics

4.1.1 Traits Assessed at Weaning

Summary statistics for field data collected during resilience testing at weaning is provided for growth-related traits in Table 14, temperament-related traits in Table 15 and DTH tests to assess CMIR in Table 16. Although the distance over which flight time was measured was standardised (1.8m), flight time testing setups differed between co-operator herd farms (and different yards at the same farm) due to differences in the design of cattle handling facilities. Summary statistics for the immune competence-related traits AMIR, CMIR and IC_Comb are presented in Table 17. The variation in CMIR and AMIR observed across testing cohorts is presented graphically in Figures 9 and 10, respectively. A scatterplot depicting the relationship between measures of CMIR and AMIR in individual steers is shown in Figure 11.

Results demonstrated that significant variation in ability to mount both AMIR and CMIR exists between individual animals within management groups in each herd (Figures 9 & 10). Genetics and management (eg. differences in prior pathogen exposure and timing between primary and secondary vaccination between management groups) are both expected to have contributed to the variation in mean responses observed between both management groups within each herd and between different herds. As significant between animal variation was observed in all herds tested, results suggest that variation is likely to exist in the broader Australian beef cattle population. This finding, combined with the moderate heritability estimates of 0.33 (±0.06) for AMIR and 0.25 (±0.04) for CMIR reported previously (Reverter et al., 2021b), suggest there is potential to select beef cattle for immune competence and that a reasonable rate of genetic gain can be expected when selecting for the trait.

A positive phenotypic correlation (r_p 0.163, $P<0.001$) was observed between AMIR and CMIR in the current study (Figure 11, Table 29), supporting previous findings where a positive phenotypic (0.15
± 0.03) and genetic (0.51 ± 0.18) correlation between AMIR and CMIR in Australian Angus cattle was reported (MLA project, B.STU.0244; Hine et al., 2019). These findings are in contrast to those observed in North American dairy cattle where weak to moderate negative genetic correlations between AMIR and CMIR (-0.13 ± 0.37 and -0.45 ± 0.32, depending on timing of measuring AMIR) were reported (Thompson et al., 2012). However, in another study in North American dairy cattle, Hernandez et al. (2006) reported a weak positive genetic correlation between AMIR and CMIR when using one antigen to induce CMIR (0.309) and a weak negative genetic correlation when inducing CMIR with a different antigen (-0.295). Regardless, results from the current and previous studies suggest that selecting for AMIR in beef cattle will simultaneously improve the ability of animals to mount CMIR and vice versa. On this basis it is tempting to suggest that measuring just CMIR or AMIR (but not both) is all that is required to improve the general disease resistance of your herd. However, it is important to consider that even when AMIR and CMIR are strongly positively genetically correlated,

when selection is based on only AMIR or CMIR that a proportion of animals will be low responders for the other trait. Further, as the immune system is constantly challenged by both intracellular and extracellular pathogens it is critical that selection strategies aimed at improving general disease resistance are based on selecting individuals which have a balanced ability to mount both cell-mediated and antibody-mediated immune responses. Therefore, we propose that selection based on direct measures of an animal's ability to mount both AMIR and CMIR remains the most efficient and sustainable means of improving general disease resistance in beef cattle.

Table 15. Growth trait summary statistics.

Testing Cohort	WWT1				WWT2				WWT3				ADGW			
	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev
1A	127.4	193.5	42.0	22.1	133.6	200.0	49.0	24.4	137.2	210.0	52.0	24.2	0.56	1.74	-0.66	0.43
1B	124.8	170.5	61.0	20.9	136.3	186.0	66.5	22.4	137.7	184.0	65.0	22.4	0.92	1.95	-0.65	0.52
1C	130.4	189.0	58.0	26.0	141.0	198.0	65.5	27.5	141.4	200.0	67.5	27.7	0.84	1.95	-0.71	0.43
1D	105.0	180.0	60.0	21.3	107.6	186.5	68.0	22.0	107.9	186.0	69.5	21.6	0.19	1.38	-0.66	0.34
1E	104.4	167.0	55.0	23.8	116.3	191.0	68.5	27.1	117.4	188.5	71.5	27.2	0.76	1.87	-0.61	0.42
2A	112.7	147.0	69.5	16.6	117.9	162.5	65.5	17.8	114.5	149.5	66.0	17.2	0.26	0.74	-0.41	0.24
2B	122.8	185.5	87.5	17.6	127.5	190.0	87.0	18.5	123.7	183.5	86.5	17.9	0.23	0.70	-0.43	0.25
2C	107.2	179.0	57.0	20.7	108.9	178.0	54.0	21.5	108.2	176.0	55.0	21.8	0.12	1.31	-0.69	0.29
2D	103.6	175.5	55.0	18.2	108.4	180.5	58.0	18.9	105.6	174.5	52.5	18.5	0.27	1.10	-0.50	0.30
2E	98.4	146.5	55.5	16.7	102.9	153.5	49.0	17.9	102.4	145.5	54.5	18.0	0.27	1.01	-0.40	0.27
3A	183.7	270.0	90.5	42.2	185.4	281.0	92.0	40.9	185.1	278.0	89.5	41.4	0.14	1.19	-0.86	0.39
3B	224.4	295.0	114.5	37.9	235.5	309.0	118.0	38.2	239.7	310.0	123.0	37.4	0.97	1.86	-1.0	0.46
All Steers	**121.4**	**295.0**	**42.0**	**36.6**	**128.0**	**309.0**	**49.0**	**38.3**	**128.2**	**310.0**	**52.0**	**39.1**	**0.48**	**1.95**	**-1.0**	**0.51**

Table 16. Temperament trait summary statistics. (Note: Bleeding Scores were not collected for Testing Cohort 3A as this additional novel measure of temperament was not added to the resilience testing protocol until these steers had been tested).

Testing Cohort	FS1				FS2				CS				BS			
	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev
1A	0.89	2.24	0.38	0.38	0.72	2.22	0.38	0.31	1.6	4	1	0.74	2.2	4	1	0.8
1B	0.55	1.19	0.26	0.15	0.52	1.89	0.31	0.18	1.8	4	1	0.76	2.6	5	1	0.8
1C	0.64	2.33	0.33	0.27	0.54	1.18	0.35	0.12	1.6	4	1	0.70	2.2	4	1	0.7
1D	0.90	4.20	0.40	0.43	0.86	3.05	0.43	0.43	2.0	5	1	0.89	1.8	4	1	0.7
1E	0.86	3.21	0.35	0.44	0.75	2.24	0.37	0.32	1.8	4	1	0.84	2.2	4	1	0.7
2A	1.01	2.20	0.32	0.41	0.79	1.64	0.39	0.24	1.8	4	1	0.75	2.4	5	1	1.2
2B	0.66	1.60	0.38	0.23	0.86	2.17	0.35	0.34	1.9	4	1	0.78	2.3	5	1	1.0
2C	0.73	2.08	0.30	0.34	0.70	1.86	0.31	0.28	1.6	4	1	0.70	1.9	4	1	0.9
2D	0.74	2.33	0.28	0.31	0.81	3.06	0.40	0.42	1.7	4	1	0.75	2.2	5	1	1.0
2E	0.93	3.10	0.43	0.42	0.82	2.68	0.37	0.39	2.0	4	1	0.70	2.0	4	1	0.8
3A	0.61	1.10	0.41	0.16	0.64	1.64	0.35	0.23	1.3	3	1	0.6	NA	NA	NA	NA
3B	0.71	2.13	0.38	0.31	0.50	1.3	0.32	0.13	1.75	4	1	0.90	1.6	5	1	0.9
All Steers	**0.77**	**4.20**	**0.26**	**0.37**	**0.71**	**3.06**	**0.31**	**0.33**	**1.76**	**5**	**1**	**0.79**	**2.15**	**5**	**1**	**0.89**

Table 17. Cell-mediated immune response trait summary statistics.

Testing Cohort	DTH Control Site Baseline (0Hrs)				DTH Control Site (48Hrs)				DTH Test Site Baseline (0Hrs)				DTH Test Site (48Hrs)			
	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev	Mean	Max	Min	StdDev
1A	3.9	5.7	2.5	0.5	4.1	6.3	2.5	0.6	3.8	5.1	2.6	0.5	7.8	15.0	3.8	1.6
1B	4.0	5.7	2.6	0.5	4.2	6.1	2.9	0.6	3.8	5.3	2.6	0.5	7.7	12.0	4.2	1.5
1C	4.2	5.7	2.9	0.6	4.2	5.6	2.7	0.6	4.2	5.3	3.0	0.5	7.4	11.4	3.9	1.4
1D	3.6	5.1	2.5	0.5	3.6	6.1	2.1	0.5	3.5	5.3	2.3	0.5	7.1	14.7	4.0	1.6
1E	3.6	5.2	2.5	0.6	3.7	5.3	2.4	0.6	3.5	5.1	2.5	0.5	7.3	13.4	2.9	1.7
2A	3.8	5.1	3.0	0.5	3.8	5.4	3.1	0.5	3.7	5.7	2.9	0.5	8.1	12.3	5.0	1.4
2B	3.9	5.4	3.0	0.5	3.9	5.3	2.7	0.5	3.8	5.4	2.8	0.4	7.4	10.8	4.8	1.3
2C	3.7	5.3	2.5	0.6	3.7	5.3	2.5	0.6	3.5	5.0	2.1	0.5	6.5	10.5	3.9	1.2
2D	3.7	5.2	2.7	0.4	3.6	5.1	2.5	0.5	3.5	4.9	2.5	0.4	7.3	11.4	3.7	1.4
2E	3.5	4.9	2.2	0.5	3.6	5.3	2.5	0.5	3.4	4.7	2.2	0.4	7.9	12.0	4.6	1.5
3A	4.3	5.9	2.3	0.8	4.3	6.3	2.3	0.8	4.1	6.1	2.3	0.8	8.7	16.6	4.2	2.1
3B	4.8	9.1	3.0	0.9	4.4	7.2	3.2	0.7	4.8	9.4	3.0	0.8	9.0	13.6	6.0	1.7
All Steers	**3.9**	**9.1**	**2.2**	**0.6**	**3.7**	**7.2**	**2.1**	**0.6**	**3.9**	**9.4**	**2.1**	**0.7**	**7.6**	**16.6**	**2.9**	**1.6**

Table 18. Immune competence trait summary statistics.

Testing Cohort	n	CMIR (Change in skin fold thickness, mm)				AMIR (OD)				IC_Comb			
		Mean	Min	Max	StDev	Mean	Min	Max	StDev	Mean	Min	Max	StDev
1A	183	2.02	1.28	3.77	0.37	1.06	0.42	1.66	0.30	-0.02	3.41	-3.28	1.06
1B	184	1.95	1.23	3.30	0.34	0.93	0.22	1.88	0.33	0.00	4.05	-3.10	1.15
1C	162	1.79	0.94	2.63	0.28	0.78	0.22	1.78	0.33	0.00	3.06	-2.56	0.99
1D	174	2.07	1.22	3.40	0.39	1.24	0.44	1.92	0.31	0.02	4.05	-3.31	1.17
1E	162	2.08	1.27	4.02	0.45	1.25	0.37	1.89	0.34	0.00	4.51	-3.45	1.29
2A	82	2.21	1.45	3.34	0.40	1.31	0.50	1.87	0.27	0.02	2.67	-3.26	1.21
2B	95	1.98	1.28	3.18	0.34	1.30	0.67	1.80	0.25	-0.01	2.89	-3.30	1.04
2C	160	1.87	1.23	3.08	0.35	1.25	0.56	1.89	0.33	0.02	3.56	-2.59	1.13
2D	154	2.12	1.33	3.35	0.39	1.12	0.38	1.79	0.31	-0.02	2.97	-3.24	1.03
2E	163	2.27	1.35	3.85	0.44	1.28	0.60	1.89	0.31	0.03	3.69	-3.08	1.23
3A	67	2.12	1.42	3.30	0.42	0.94	0.26	1.81	0.43	0.05	4.41	-2.65	1.28
3B	75	2.04	1.47	3.44	0.40	0.97	0.14	1.90	0.45	-0.04	3.80	-3.65	1.35
All Steers	**1661**	**2.03**	**0.94**	**4.02**	**0.40**	**1.12**	**0.14**	**1.92**	**0.37**	**0.00**	**4.51**	**-3.65**	**1.15**

Figure 9. Cell-mediated immune responses (CMIR) observed across testing cohorts.

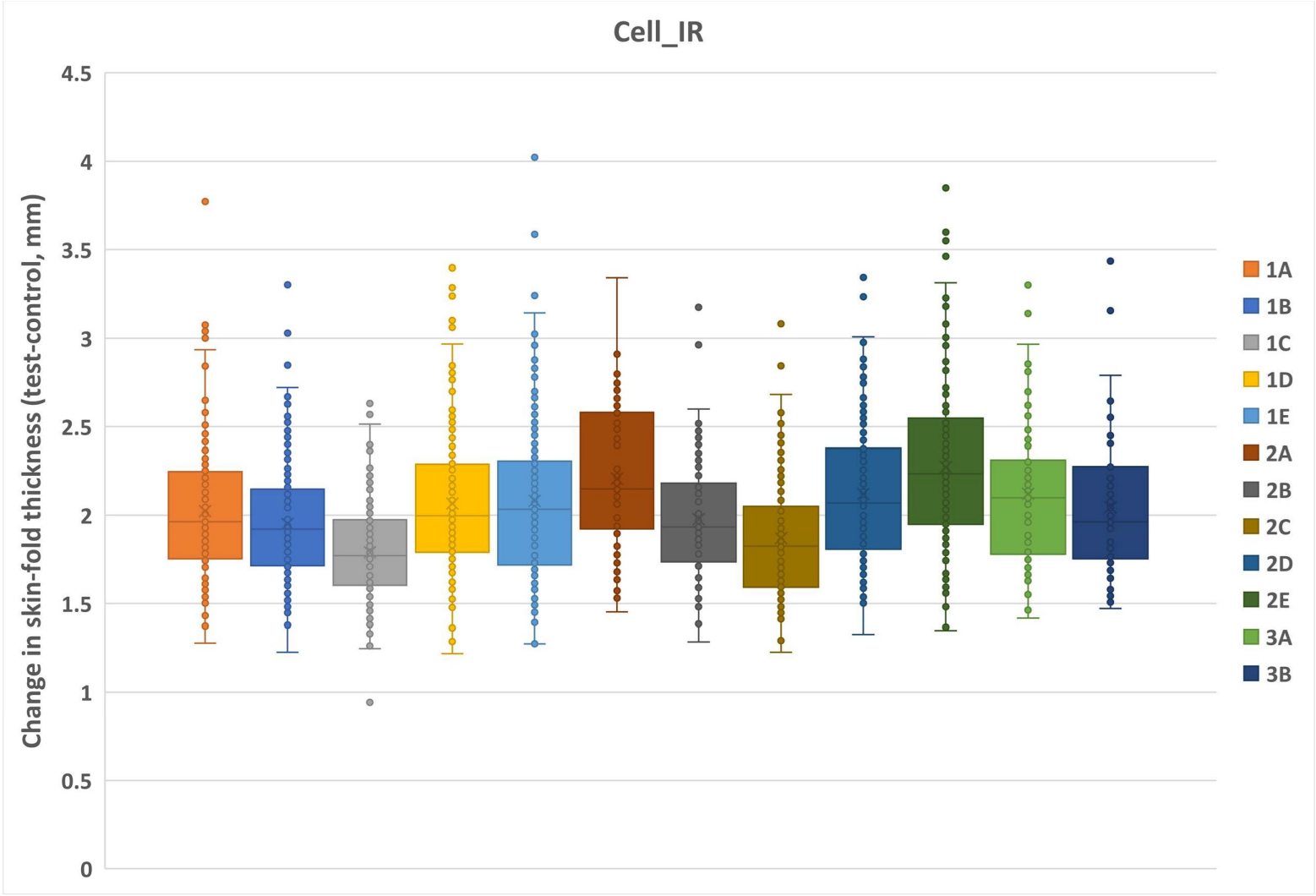


Figure 10. Antibody-mediated immune responses (AMIR) observed across testing cohorts.

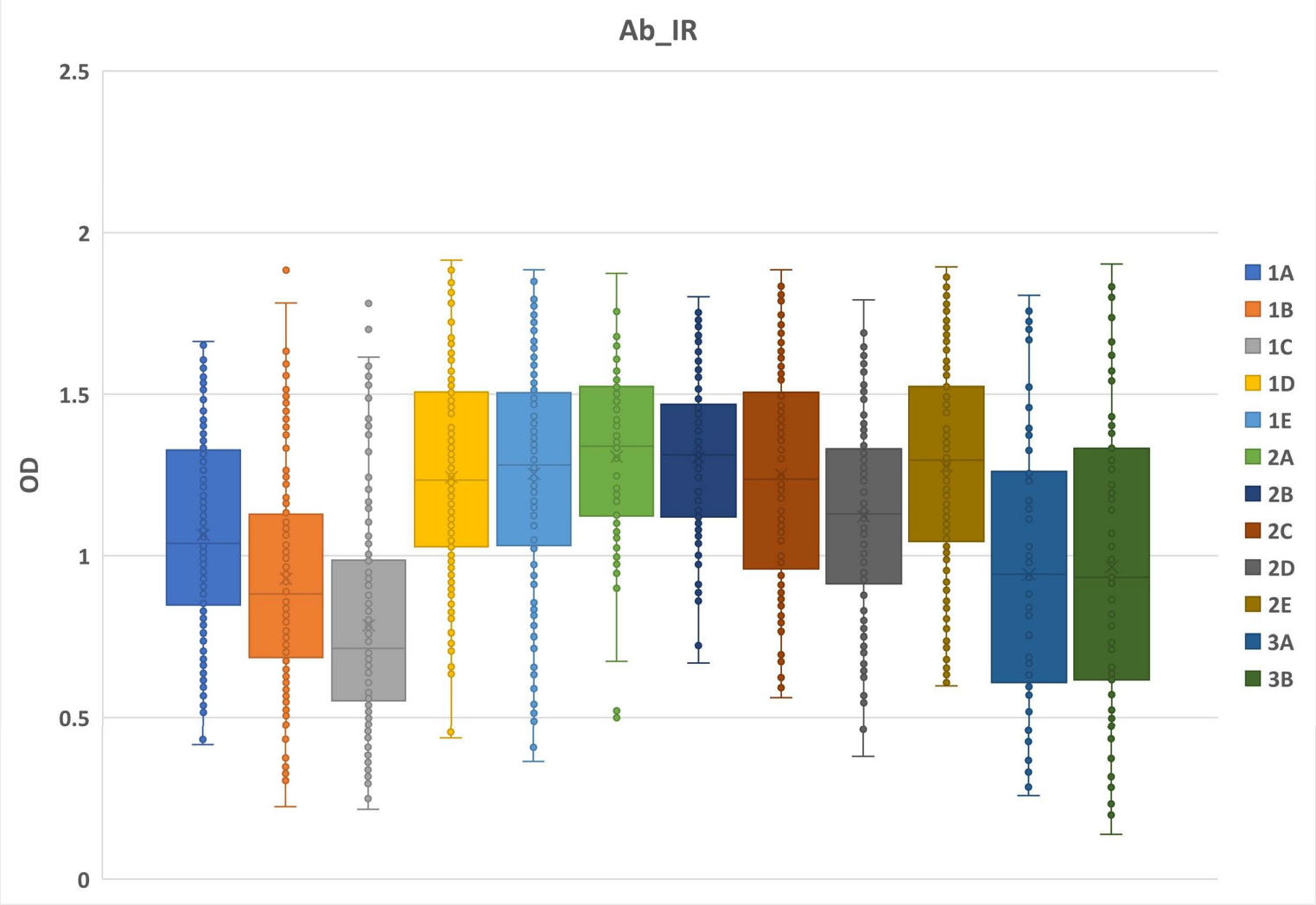
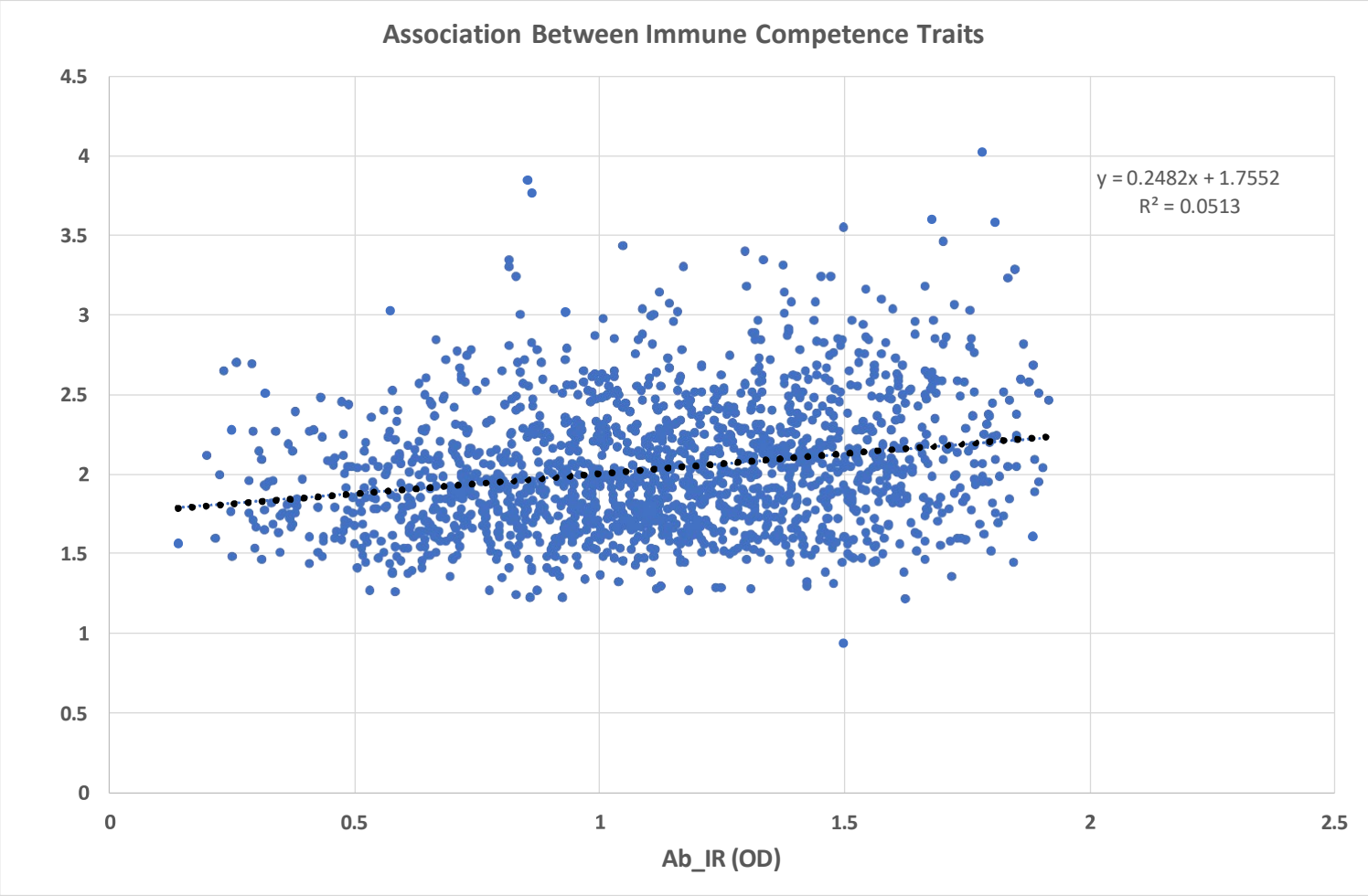


Figure 11. Association between cell-mediated (Cell_IR) and antibody-mediated immune responses (Ab_IR) in all steers tested (NOTE: raw unadjusted values presented)



4.1.2 Traits Assessed at Feedlot Induction

Summary statistics for field data collected at feedlot induction including induction weight and induction flight time are presented in Table 18. Induction weight data was collected on all steers entering the feedlot. Collection of flight time data at induction was not possible for all steers due to covid restrictions on staff movements and/or feedlot access. Although the distance over which flight time was measured was standardised (1.8m), flight time testing setups differed between feedlots due to differences in the design of cattle handling facilities.

Table 19. Induction liveweight and flight time summary statistics for each induction cohort

Induction Cohort	Feedlot	n	IndWT (kgs)				IndFT (secs)			
			Mean	Min	Max	StDev	Mean	Min	Max	StDev
Ind_1A	Feedlot 1	51	516	482	560	18.63	0.53	0.36	0.92	0.13
Ind_1B	Feedlot 1	60	503	479	531	11.91	0.64	0.32	1.61	0.27
Ind_1C	Feedlot 1	62	484	445	515	16.24	0.65	0.33	1.54	0.25
Ind_1D	Feedlot 1	256	499	463	542	16.21	0.57	0.32	1.87	0.23
Ind_1E	Feedlot 1	193	471	438	500	14.21	0.65	0.30	1.94	0.28
Ind_2A	Feedlot 2	134	456	407	508	17.17	0.67	0.42	1.79	0.24
Ind_1F	Feedlot 1	23	460	446	476	9.46	NA	NA	NA	NA
Ind_1G	Feedlot 1	184	455	399	509	17.35	0.84	0.32	2.62	0.43
Ind_1H	Feedlot 1	73	440	413	485	4.07	0.68	0.28	1.47	0.30
Ind_3A	Feedlot 3	64	501	474	542	16.26	NA	NA	NA	NA
Ind_3B	Feedlot 3	106	488	406	548	33.41	NA	NA	NA	NA
Ind_1I	Feedlot 1	55	458	385	560	41.84	0.51	0.29	1.08	0.16
Ind_1J	Feedlot 1	65	450	374	518	35.08	NA	NA	NA	NA
All Steers		**1326**	**476**	**374**	**560**	**29.9**	**0.66**	**0.28**	**2.62**	**0.30**

4.1.3 Mortality and Disease Incidence Data Recorded During Feedlot Finishing

A summary of disease incidence data recorded for steers finished at Feedlot 1, Feedlot 2 and Feedlot 3 are presented in Tables 19, 20 and 21, respectively. Generally, disease incidence and health- related mortalities in trial steers were low, especially considering steers had not been pre-vaccinated against BRD prior to feedlot entry (as per trial design). Although several factors may have contributed to the low incidence of disease observed in the current study, steers enrolled in the study were exposed to severe drought conditions from birth through to post-weaning and, as a consequence, were confined fed for extended periods of time on-farm. This may have contributed the low disease incidence observed in these steers as confined feeding likely exposed steers to environmental stressors, similar to those experienced at the feedlot following induction, increasing disease risk and pre-conditioning steers to feedlot-like conditions prior to induction at the feedlot.

Table 20. Summary of disease incidence data recorded on individual steers finished at feedlot 1.

Induction Cohort	Induction date	Property of origin	Number IC tested	Kill date	DOF	Respiratory /pneumonia	Laminitis	Tracheal oedema	Foot /lame	Other	Unknown	Deaths
Ind_1I	12/08/2020	Herd 3	55	18/01/2021	159	14,(1),[0]	0,(0),[0]	0,(0),[0]	0,(0),[0]	0,(0),[0]	0,(0),[0]	0
Ind_1A	13/10/2020	Herd 1	51	22/04/2021	191	1,(0),[1]	3,(0),[0]	0,(0),[0]	5,(0),[0]	0,(0),[0]	0,(0),[0]	1
Ind_1B	21/10/2020	Herd 1	60	23/04/2021	184	0,(0),[0]	2,(0),[0]	0,(0),[0]	3,(0),[0]	0,(0),[0]	0,(0),[1]	1
Ind_1C	28/10/2020	Herd 1	62	13/04/2021 or 20/04/2021	184	0,(0),[0]	0,(0),[0]	0,(0),[0]	0,(0),[0]	0,(0),[0]	0,(0),[0]	0
Ind_1D	1/12/2020	Herd 1	256	19/04/2021 or 26/04/2021	139 or 146	0,(0),[1]	1,(0),[0]	0,(0),[0]	5,(0),[0]	0,(0),[1]	0,(0),[1]	3
Ind_1E	16/12/2020	Herd 1	176	3/05/2021	138	0,(0),[0]	0,(0),[0]	0,(0),[0]	5,(1),[0]	0,(0),[0]	0,(0),[1]	1
Ind_1E[±]	16/12/2020	Herd 1	17	Various	Various	1,(0),[0]	0,(0),[0]	0,(0),[0]	1,(0),[0]	0,(0),[0]	0,(0),[0]	0
Ind_1G	13/01/2021	Herd 2	184	21/07/2021	189	2,(0),[0]	0,(0),[0]	1,(0),[0]	3,(0),[0]	0,(0),[0]	0 (0),[0]	0
Ind_1F	9/02/2021	Herd 1	23	11/08/2021	183	2,(0),[0]	0,(0),[0]	0, 0),[0]	0,(0),[0]	0,(0),[0]	0,(0),[0]	0
Ind_1H	17/02/2021	Herd 2	73	16/08/2021	180	4,(0),[0]	1,(0),[0]	1,(0),[0]	0,(0),[0]	1,(0),[0]	0,(0),[0]	0
Ind_1J	4/08/2021	Herd 3	65	17/01/2022 or 24/01/2022	166 or 173	2,(1), [1]	0,(0), [0]	0,(0),[0]	2,(0),[0]	1,(0),[1]	0,(0),[0]	2

[±]Steers in Induction Cohort Ind_1E entered a short fed program and were subsequently slaughtered at various processing plants once reaching target weights.

Table 21. Summary of disease incidence data recorded on individual steers finished at Feedlot 2.

Lot	Induction Date	Property of origin	Number IC tested	Kill date	DOF	Respiratory /pneumonia	Cellulitis	Bloat	Foot/Lame	Abscess in joint	Haematoma	Coccidiosis	Other	Unknown	Deaths
Ind_2A	20/01/2021	Herd 1	134	25/10/2021	270	4,(0),[1]	3,(0),[2]	1,(0),[1]	8,(0),[0]	8,(0),[0]	1,(0),[0]	1,(0),[0]	5,(0),[1]	4,(0),[4]	9

Table 22. Summary of disease incidence data recorded on individual steers finished at Feedlot 3.

Lot	Induction Date	Property of origin	Number IC tested	Kill date	DOF	Respiratory /pneumonia	Tracheal oedema	Bloat	Foot /lame	Deaths
Ind_3A	19/01/2021	Herd 2	64	12/08/2021	205	1,(0),[0]	0,(0),[0]	1,(0),[0]	1,(0),[0]	0
Ind_3B	13/04/2021	Herd 2	106	18/11/2021	219	2,(0),[0]	1,(0),[0]	0,(0),[0]	1,(0),[0]	0

4.1.4 Offal Defect and Lung Lesion Score Data Collected at Processing

A summary of offal defects recorded at the processing plant by appropriately trained QA staff is provided in Table 22. Categories of offal defects recording were aligned with those used in routine offal inspection procedures implemented at each respective processing plant. A summary of lung lesion scores recorded at the processing plant by appropriately trained CSIRO technical staff is provided in Table 23. In line with the low disease incidence and health-related mortalities observed, numbers of offal defects and lung lesion scores were generally low, potentially due to reasons discussed above (section 4.1.3). A significant proportion of steers in induction cohorts Ind_1F (91%), Ind_1G (58%), Ind_1H (85%) and Ind_1J (75%) which were processed at processing plant 1 were identified with the offal defect category “kidney-other” which was considered very unlikely to be accurate. Therefore, offal defect data was analysed both including and excluding this offal defect category.

Table 23. Offal defects recorded during routine offal inspection at the processing plant.

Induction Cohort	Kill date	Offal Defects														
		Pneumonia	Pleurisy	Abscess	Pathology[1]	Fluke	Adhesions	Eosinophilic myositis[2]	Emphysema	Hydatids	Telangiectasis[3]	Retention Cysts	Actino[4]	Pericarditis	Kidney	Other
Ind_1I	18/01/2021	1	0	4	3	1	0	0	0	10	0	1	0	0	1	0
Ind_1A	22/04/2021	0	7	5	0	1	1	0	0	0	0	0	0	6	13	0
Ind_1B	23/04/2022	0	1	4	0	0	1	0	0	0	0	0	0	0	0	
Ind_1C	13/04/2021 & 20/04/2021	No offal data available. Health terminal not operating														
Ind_1D	19/04/2021 & 26/04/2021	36	5	19	0	1	9	0	0	1	4	0	7	5	63	20
Ind_1E	3/05/2021	2	7	2	0	0	15	0	4	21	2	0	7	0	28	6
Ind_1E±	Various	No offal data available. Health terminal not operating														
Ind_1G	21/07/2021	9	4	18	3	0	0	1	0	0	0	0	0	1	108	16
Ind_1F	11/08/2021	2	1	2	0	1	0	0	0	0	0	0	0	0	20	1
Ind_1H	16/08/2021	8	3	8	0	0	0	0	0	4	0	0	0	0	62	5
Ind_2A	25/10/2021	No offal data available. Health terminal not operating														
Ind_3A	12/08/2021	No offal data available. Health terminal not operating														
Ind_3B	18/11/2021	No offal data available. Health terminal not operating														
Ind_1J	17/01/2022 & 24/01/2022	2	2	8	2	2	0	0	0	25	0	0	0	0	47	9

±Some steers in Induction Cohort Ind_1E entered a short fed program and were subsequently slaughtered at various processing plants once reaching target weights.

[1]Pathology - An indetermined lesion or tumour relating to the lung that the operator was not able to identify upon inspection.

[2]Eosinophilic myositis - A collective term used to describe an inflammatory condition grossly characterized by focal, green, muscular lesions in clinically healthy cattle.

[3]Telangiectasis - Bovine liver telangiectasis.

[4]Actino – Wooden tongue / lumpy jaw

Table 24. Lung lesion scores recorded at the processing plant

Induction Cohort	Kill date	Number inspected	Lung lesion scores							
			Lobe 1				Lobe 2			
			0	1	2	3	0	1	2	3
Ind_1I	18/01/2021	48	22	21	3	2	14	27	6	1
Ind_1A	22/04/2021	23	8	13	2	0	8	13	2	0
Ind_1B	23/04/2022	Covid prevented access to plant								
Ind_1C	13/04/2021 & 20/04/2021	Covid prevented access to plant								
Ind_1D	19/04/2021 & 26/04/2021	89	65	20	4	0	70	16	3	0
Ind_1E	3/05/2021	127	73	46	8	0	99	26	2	0
Ind_1E[±]	Various	Covid prevented access to plant								
Ind_1G	21/07/2021	Covid prevented access to plant								
Ind_1F	11/08/2021	Covid prevented access to plant								
Ind_1H	16/08/2021	Covid prevented access to plant								
Ind_2A	25/10/2021	Covid prevented access to plant								
Ind_3A	12/08/2021	Covid prevented access to plant								
Ind_3B	18/11/2021	Covid prevented access to plant								
Ind_1J	17/01/2022 & 24/01/2022	Covid prevented access to plant								

[±]Some steers in Induction Cohort Ind_1E entered a short fed program and were subsequently slaughtered at various processing plants once reaching target weight

4.1.5 Carcase Traits Collected at Processing

A summary of carcase trait data recorded at the processing plant by experienced MSA accredited graders is provided in Tables 24, 25, 26 & 27. Data was available from all processing plants for HCWT and P8, from all processing plants except processing plant 2 for Dent, from processing plants 4 and 5 for EMA, AUSMarb, MSAMarb, MeatCol, FatCol, pH, LoinTemp, Hump and Oss and from processing plant 4 for RibFat.

Table 25. Carcase trait summary statistics A.

Induction Cohort	n	HCWT				Dent				P8				EMA			
		Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev
Ind_1A	51	431	472	354	22	NA	NA	NA	NA	21	40	9	6	NA	NA	NA	NA
Ind_1B	60	439	487	379	23	NA	NA	NA	NA	26	39	9	7	NA	NA	NA	NA
Ind_1C	62	393	423	364	14	0.3	2	0	0.8	18	30	10	5	NA	NA	NA	NA
Ind_1D	256	402	473	339	19	0.5	2	0	0.9	17	39	8	6	86.5	115	68	8.5
Ind_1E	176	398	439	337	20	0.3	2	0	0.7	18	33	8	6	81.1	118	60	8.6
Ind_1F	23	397	429	339	28	1.5	2	0	0.9	25	31	15	6	NA	NA	NA	NA
Ind_1G	184	401	450	336	21	1.1	2	0	1.0	27	31	15	4	NA	NA	NA	NA
Ind_1H	73	382	422	308	20	1.0	2	0	1.0	24	31	13	5	NA	NA	NA	NA
Ind_1I	54	378	440	292	30	0.9	2	0	1.0	20	31	13	4	NA	NA	NA	NA
Ind_1J	63	400	467	325	30	1.0	2	0	1.0	22	31	10	5	NA	NA	NA	NA
Ind_2A[±]	94	454	517	388	25	2.5	4	2	0.9	25	40	12	6	NA	NA	NA	NA
Ind_2A[±]	40	446	490	361	29	2.2	4	2	0.6	22	36	15	6	NA	NA	NA	NA
Ind_3A	64	444	504	378	21	1.3	4	0	1.0	25	36	14	5	77.7	90	58	8.7
Ind_3B	106	446	528	367	29	2.0	4	0	0.6	24	40	15	6	83.3	99	60	8.7
All Steers Assessed		**413**	**528**	**292**	**33**	**1.1**	**4**	**0**	**1.1**	**22**	**40**	**8**	**7**	**83.3**	**118**	**58**	**9.1**

[±]Steers in Induction Cohort Ind_2A were processed in 2 separate lots on the same kill day

Table 26. Carcase trait summary statistics B.

Induction Cohort	n	AUSMarb				MSAMarb				MeatCol				FatCol			
		Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev
Ind_1A	51	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1B	60	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1C	62	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1D	256	3.4	7	1	1.1	584	920	380	100	2.1	4	2	0.3	0.5	2	0	0.5
Ind_1E	176	3.2	8	1	1.2	566	1050	320	115	2.1	3	2	0.3	0.3	1	0	0.5
Ind_1F	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1G	184	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1H	73	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1I	54	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1J	63	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_2A[±]	94	3.4	6	2	0.9	NA	NA	NA	NA	2.2	3	2	0.4	0.4	1	0	0.5
Ind_2A[±]	40	3.5	7	2	1.1	NA	NA	NA	NA	2.0	2	2	0.0	0.6	2	0	0.6
Ind_3A	64	2.2	5	0	1.2	463	720	200	118	2.1	3	2	0.2	0.9	3	0	0.7
Ind_3B	106	3.4	7	1	1.3	568	900	380	117	2.1	3	2	0.3	0.1	2	0	0.4
All Steers Assessed		**3.3**	**8**	**0**	**1.2**	**562**	**1050**	**200**	**116**	**2.1**	**4**	**2**	**0.3**	**0.4**	**3**	**0**	**0.5**

[±]Steers in Induction Cohort Ind_2A were processed in 2 separate lots on the same kill day

Table 27. Carcase trait summary statistics C.

Induction Cohort	n	RibFat				pH				LoinTemp				Hump			
		Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev	Mean	Max	Min	Std Dev
Ind_1A	51	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1B	60	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1C	62	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1D	256	13.0	38	5	4.4	5.51	5.81	5.31	0.08	3.4	6.8	1.5	0.8	73	90	50	8
Ind_1E	176	15.0	28	5	5.3	5.51	5.67	5.20	0.07	4.8	8.8	1.6	1.3	66	100	40	9
Ind_1F	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1G	184	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1H	73	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1I	54	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_1J	63	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_2A[±]	94	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_2A[±]	40	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ind_3A	64	NA	NA	NA	NA	5.55	5.59	5.44	0.04	NA	NA	NA	NA	74	90	60	7
Ind_3B	106	NA	NA	NA	NA	5.55	5.66	5.44	0.02	5.5	6.2	5.0	0.3	75	95	55	10
All Steers Assessed		**13.8**	**38**	**5**	**4.9**	**5.52**	**5.81**	**5.20**	**0.07**	**4.3**	**8.8**	**1.5**	**1.3**	**71**	**100**	**40**	**9**

[±]Steers in Induction Cohort Ind_2A were processed in 2 separate lots on the same kill day

Table 28. Carcase trait summary statistics D.

Induction Cohort	n	Oss			
		Mean	Max	Min	StdDev
Ind_1A	51	NA	NA	NA	NA
Ind_1B	60	NA	NA	NA	NA
Ind_1C	62	NA	NA	NA	NA
Ind_1D	256	153	200	100	21
Ind_1E	176	161	190	100	19
Ind_1F	23	NA	NA	NA	NA
Ind_1G	184	NA	NA	NA	NA
Ind_1H	73	NA	NA	NA	NA
Ind_1I	54	NA	NA	NA	NA
Ind_1J	63	NA	NA	NA	NA
Ind_2A[±]	94	NA	NA	NA	NA
Ind_2A[±]	40	NA	NA	NA	NA
Ind_3A	64	145	180	120	13
Ind_3B	106	178	190	110	16
All Steers Assessed		**159**	**200**	**100**	**21**

[±]Steers in Induction Cohort Ind_2A were processed in 2 separate lots on the same kill day

4.2 Association Between Immune Competence and other Resilience-related Traits

The significance of fixed effects and covariates fitted to statistical models when analysing immune competence and other resilience-related traits are described in Table 28. Phenotypic correlations between immune competence and other resilience-related traits are described in Table 29. Management group (Herd*CGMark*CGWean) had a significant effect on all resilience-related traits analysed. Condition, defined as health status assessed during weaning, had a significant effect on CMIR, ADGW, FT2 and IndFT but did not influence AMIR, FT1 or CS. Condition was used to identify steers with health conditions at the commencement of weaning and/or that developed health conditions during weaning. Several steers were healthy at the commencement of weaning but developed health conditions during the weaning period which may have contributed to the differential effect of condition observed on FS1 versus FS2. Weaning weight (WT1) had a significant effect on AMIR, ADGW, FT1 and CS but did not influence CMIR or FT2. Both induction cohort (Feedlot*Induction Cohort) and induction weight (IndWT) had a significant effect on IndFT.

As per design, both AMIR and CMIR were strongly positively correlated with IC_Comb (r_p=0.720, *P*<0.001; r_p=0.766, *P*<0.001, respectively). A positive phenotypic correlation (r_p=0.163, *P*<0.001) was also observed between AMIR and CMIR. A weak positive phenotypic correlation (r_p=0.15 ± 0.03) and strong positive genetic correlation (r_g=0.51 ± 0.18) between AMIR and CMIR has been reported previously in Australian Angus cattle (MLA project, B.STU.0244; Hine et al., 2019). Weight gain during the weaning period (ADGW) was monitored as an indirect measure of an animal's ability to cope with management-induced stress imposed by yard weaning. A favourable association between immune competence phenotype (IC_Comb) and weight change over weaning (ADGW) was observed in the current study: however, the association was not strong (r_p=0.049, *P*=0.048). In support of these findings, a trend in weight gain differences over the weaning period across immune competence phenotype categories was reported previously with high IC animals having the highest WtGain (0.44 ± 0.11 kg/day) followed by average IC animals (0.34 ± 0.04 kg/day) and low IC animals (0.31 ± 0.10 kg/day) (MLA project, B.STU.0244; Hine et al., 2019). Together these findings suggest that a favourable association may exist between immune competence phenotype and stress-coping ability in beef cattle.

Flight time was recorded on steers at the start of weaning (FT1), end of weaning (FT2) and at feedlot induction (IndFT). Flight time measures were strongly positively correlated, FT1 and FT2 (r_p=0.542, *P*<0.001), FT1 and IndFT (r_p=0.324, *P*<0.001) and FT2 and IndFT (r_p=0.351, *P*<0.001). The strong positive correlation observed between consecutive measures suggests that the ranking of individual animals for temperament based on FT is expected to be reasonably consistent across testing times and across different testing environments. Flight time measures collected at different times were also all favourably correlated with crush score (CS) suggesting

that FT and CS are complimentary temperament measures in beef cattle. Immune competence phenotype (IC_Comb) and temperament traits (both FT and CS) were not phenotypically correlated. However, significant correlations were observed between CMIR and FT1 (r_P=0.049, *P*=0.048) and CMIR and FT2 (r_P=-0.056, *P*=0.024) but associations were not strong and were favourable in one instance (between CMIR and FT1) and unfavourable in the other (between CMIR and FT2). Associations between immune competence and temperament traits have been investigated previously in Australian Angus cattle (MLA project, B.STU.0244; Hine et al., 2019). Results from that study support those reported here with no association between AMIR and FT or CMIR and FT observed at the phenotypic level. Similarly, no phenotypic association between AMIR and CS or between CMIR and CS was observed. However, a strong positive genetic correlation between both AMIR and FT and CMIR and FT was observed suggesting that immune competence is favourably genetically correlated with temperament in Australian Angus cattle. It has been demonstrated that calm animals (high flight time, low crush score) perform better in the feedlot environment as evidenced by their higher average daily weight gains and lower mortality as compared to their nervous (low flight time, high crush score) counterparts (Fell et al., 1999).

Table 29. Significance of fixed effects/covariates fitted to statistical models when analysing immune competence and other resilience-related traits. Numbers in cells represent p values. P values in cells were considered significant are coloured green (P<0.05).

Trait	Fixed effects			Covariates		
	Herd*CGMark *CGWean	Condition	Feedlot *Induction Cohort	WT1	IndWT	LogCellT0
AMIR	<0.001	0.359	NA	<0.001	NA	NA
CMIR	<0.001	0.003	NA	0.108	NA	<0.001
ADGW	<0.001	0.008	NA	0.023	NA	NA
FT1	<0.001	0.505	NA	0.091	NA	NA
FT2	<0.001	<0.001	NA	0.482	NA	NA
CS	<0.001	0.850	NA	0.017	NA	NA
BS	Not analysed					
WWT1	Not analysed used to calculate ADGW and as covariate in analysis					
WWT2	Not analysed used to calculate ADGW					
WWT3	Not analysed used to calculate ADGW					
Condition	Not analysed – used as covariate in analysis					
IndFT	<0.001	0.039	<0.001	NA	0.001	NA

Table 30. Phenotypic correlations between immune competence and other resilience-related traits. In each cell the top number represents the Pearson correlation coefficient, the second number represents the P value associated with the correlation and the bottom number represents the number of observations. P values in cells were considered significant are coloured green (P<0.05).

	IC_Comb	AMIR	CMIR	ADGW	FT1	FT2	CS	WWT1	IndFT
IC_Comb	1.00	0.720 <0.001 1659	0.766 <0.001 1659	0.049 0.048 1659	0.038 0.127 1619	-0.035 0.164 1626	-0.004 0.858 1659	-0.004 0.884 1659	-0.030 0.337 1063
AMIR	0.720 <0.001 1659	1.00	0.163 <0.001 1659	-0.085 0.001 1659	0.012 0.630 1619	-0.005 0.828 1626	-0.002 0.950 1659	NA	-0.031 0.319 1063
CMIR	0.766 <0.001 1659	0.163 <0.001 1659	1.00	0.153 <0.001 1659	0.049 0.048 1619	-0.056 0.024 1626	-0.005 0.835 1659	NA	-0.020 0.516 1063
ADGW	0.049 0.048 1659	-0.085 0.001 1659	0.153 <0.001 1659	1.00	-0.003 0.894 1619	-0.075 0.002 1626	-0.015 0.552 1659	NA	-0.028 0.361 1063
FT1	0.038 0.127 1619	0.012 0.630 1619	0.049 0.048 1619	-0.003 0.894 1619	1.00	0.542 <0.001 1599	-0.183 <0.001 1619	NA	0.324 <0.001 1042
FT2	-0.035 0.164 1626	-0.005 0.828 1626	-0.056 0.024 1626	-0.075 0.002 1626	0.542 <0.001 1599	1.00	-0.158 <0.001 1626	NA	0.351 <0.001 1048
CS	-0.004 0.858 1659	-0.002 0.950 1659	-0.005 0.835 1659	-0.015 0.552 1659	-0.183 <0.001 1619	-0.158 <0.001 1626	1.00	NA	-0.178 <0.001 1063
WWT1	-0.004 0.884 1659	NA	NA	NA	NA	NA	NA	1.00	-0.007 0.817 1063
IndFT	-0.030 0.337 1063	-0.031 0.319 1063	-0.020 0.516 1063	-0.028 0.361 1063	0.324 <0.001 1042	0.351 <0.001 1048	-0.178 <0.001 1063	-0.007 0.817 1063	1.00

4.3 Association Between Immune Competence and Growth-related traits

The significance of fixed effects and covariates fitted to statistical models when analysing growth-related traits are described in Table 30. Phenotypic correlations between immune competence and growth-related traits are described in Table 31. Management group (Herd*CGMark*CGWean) had a significant effect on average daily gain from weaning to feedlot induction (ADGI) but not on average daily gain during feedlot finishing (ADGF). Induction cohort (Feedlot*Induction Cohort) had a significant effect on both ADGI and ADGF. Weaning weight (WT1) and feedlot induction weight (IndWT) had a significant effect on ADGI and ADGF, respectively.

It has been hypothesised that resistance to disease in livestock may incur a production cost as a consequence of nutrients being redirected from production to support immune system function. Indeed, selection for production traits, with little or no emphasis on health and fitness traits, has been shown to reduce the ability of production animals to cope with challenges posed by their production environment

including disease challenges (Rauw et al., 1998). However, counter-balancing this cost of resistance is the metabolic cost of disease (reviewed by Colditz 2002; Colditz, 2008). Chronic activation of immune defence pathways during chronic subclinical infection leads to reduced efficiency of production. In the current study, results showed that immune competence phenotype (IR_Comb) was not associated with ADGI (r_P=-0.040, *P*=0.145) or ADGF (r_P=-0.040, *P*=0.201), suggesting that selection for immune competence is expected to have minimal impact on productivity. Previous studies have reported weak to moderate negative genetic correlations between immune competence and growth traits in Australian Angus cattle (MLA project, B.STU.0244). In that same study AMIR was found to be negatively genetically correlated with weaning weight (-0.38 ± 0.26) and 600 day weight (-0.44 ± 0.20) and CMIR negatively genetically correlated with weaning weight (-0.45 ± 0.27) and 600 day weight (-0.23 ± 0.22). These correlations were supported by the slightly reduced 200 day, 400 day and 600 day weights and CWT in high immune competence animals as compared to their average and low immune competence counterparts observed in the study. In support of these findings, Reverter et al. (2019) reported moderate negative genetic correlations between immune competence and the growth traits weaning weight (r_g=-0.377) and yearling weight (r_g=-0.303) in a larger study population of Australian Angus cattle. It is noteworthy however that in the MLA project (B.STU.0244) high, average and low immune phenotype group means were not significantly different for any of the growth traits measured and that productivity losses due to health associated mortalities at the feedlot were not captured when comparing group means. As mortalities during feedlot finishing in the trial were significantly higher in low immune competence as compared to average and high immune competence animals it was concluded that, although genetic correlation estimates suggest that selection for immune competence may incur minor productivity losses, such losses are offset by the reduced health-related mortality rates expected in high immune competence animals. It is also important to recognise, that reported genetic correlations between immune competence and growth traits are moderate to weak, suggesting that animals which are both highly immune competent and highly productive exist in the population, and that genetic progress can be made simultaneously in traits even when those traits are unfavourably genetically correlated.

In previous studies investigating links between immune competence and growth, high immune responder pigs were found to have higher growth rates relative to their average and low immune responder counterparts, significantly reducing the time taken to reach market weight (Mallard et al., 1998). In housed dairy cattle, multiparous high AMIR responder cows were found to have significantly higher milk production compared with their low immune responder counterparts; however, in first- parity cows, milk production was higher in low AMIR responder animals than in average or high immune responder cows (Wagter et al., 2003). While in pasture reared dairy heifers, high and average AMIR responder animals were found to have higher average daily weight gains as compared to their low AMIR responder counterparts (Aleri 2015).

Table 31. Significance of fixed effects/covariates fitted to statistical models when analysing growth-related traits. Numbers in cells represent p values. P values in cells considered significant (P<0.05) are coloured green.

Trait	Fixed effects			Covariates	
	Herd*CGMark *CGWean	Condition	Feedlot *Induction Cohort	WT1	IndWT
IndWT	Not analysed – used as covariate in analysis				
ADGI	<0.001	0.924	<0.001	<0.001	NA
ADGF	0.797	0.445	<0.001	NA	<0.001

Table 32. Phenotypic correlations between immune competence and growth-related traits. In each cell the top number represents the Pearson correlation coefficient, the second number represents the P value associated with the correlation and the bottom number represents the number of observations. P values in cells were considered significant are coloured green (P<0.05).

	IC_Comb	IndWT	ADGI	ADGF
IC_Comb	1.00	-0.006 0.816 1321	-0.040 0.145 1321	-0.040 0.201 1032
IndWT	-0.006 0.816 1321	1.00	0.598 <0.001 1321	NA
ADGI	-0.040 0.145 1321	0.598 <0.001 1321	1.00	-0.050 0.109 1032
ADGF	-0.040 0.201 1032	NA	-0.050 0.109 1032	1.00

4.4 Association Between Immune Competence and Carcase-related traits

The significance of fixed effects and covariates fitted to statistical models when analysing carcase traits are described in Table 32. Phenotypic correlations between immune competence and carcase traits are described in Table 33. Management group (Herd*CGMark*CGWean) had a significant effect on hot carcase weight (HCWT), P8 fat depth (P8) and ossification (OSS). Kill group (Feedlot*Induction Cohort*Abattoir) had a significant effect on all carcase-related traits measured, except for pH. Dentition (Dent) had a significant effect on fat colour (FatCol) but did not influence other carcase-related traits.

No significant associations between immune competence phenotype and any of the carcase- related traits assessed were observed in the current study. In support of these findings, no phenotypic association between AMIR or CMIR and carcase weight, eye muscle area or intra-muscular fat in Australian Angus cattle were reported in a previous study (MLA project, B.STU.0244). However, in that same study, AMIR was

found to be negatively genetically correlated with carcase weight (-0.40 ± 0.19) and eye muscle area (-0.44 ± 0.18) and CMIR positively genetically correlated with intra-muscular fat (0.21 ± 0.20). Reverter et al. (2019) reported weak negative genetic correlations between immune competence phenotype and carcase weight (rg=-0.150), eye muscle area (rg=-0.154), MSA grade (rg=- 0.034) and ossification (rg=-0.088) and a weak positive genetic correlation between immune competence phenotype and rib fat (rg=0.136) in a larger study population of Australian Angus cattle. Genetic correlation estimates suggest that selection for immune competence may incur minor productivity losses in terms of carcase weight; however, we hypothesise that such losses will be offset by the improved health outcomes expected in high immune competence animals during feedlot finishing.

Table 33. Significance of fixed effects/covariates fitted to statistical models when analysing carcase-related traits. Numbers in cells represent p values. P values in cells considered significant (P<0.05) are coloured green.

Trait	Fixed effects			
	Herd*CGMark *CGWean	Condition	Feedlot *Induction Cohort*Abattoir	Dent
HCWT	0.015	0.695	<0.001	0.270
DENT	Not analysed – used as covariate in analysis			
P8	0.001	0.385	<0.001	0.088
EMA	0.840	0.739	<0.001	0.715
AUSMarb	0.928	0.238	<0.001	0.949
MSAMarb	0.877	0.122	<0.001	0.991
MeatCol	0.245	0.781	<0.001	0.313
FatCol	0.604	0.575	<0.001	0.030
RibFat	0.402	0.844	<0.001	0.501
pH	0.626	0.313	0.807	0.991
LoinTemp	0.472	0.628	<0.001	0.400
Hump	0.489	0.587	<0.001	0.439
Oss	0.010	0.452	<0.001	0.446

Table 34. Phenotypic correlations between immune competence and carcase traits. In each cell the top number represents the Pearson correlation coefficient, the second number represents the P value associated with the correlation and the bottom number represents the number of observations. P values in cells were considered significant are coloured green (P<0.05).

	IC_ Comb	HCWT	P8	EMA	AUS Marb	MSA Marb	Meat Col	FatCol	RibFat	pH	Loin Temp	Hump	Oss
IC_ Comb	1.00	-0.048 0.139 943	-0.054 0.095 943	0.047 0.279 522	0.003 0.948 559	0.007 0.873 522	0.048 0.255 559	0.004 0.921 559	-0.092 0.084 354	0.008 0.863 522	0.0585 0.212 458	0.030 0.488 522	0.048 0.271 522
HCWT	-0.048 0.139 943	1.00	0.212 <0.001 943	0.282 <0.001 522	0.047 0.268 559	0.031 0.476 522	-0.081 0.056 559	-0.005 0.912 559	0.125 0.019 354	0.037 0.396 522	0.142 0.002 458	0.328 <0.001 522	0.197 <0.001 522
P8	-0.054 0.095 943	0.212 <0.001 943	1.00	0.049 0.268 522	-0.004 0.920 559	-0.003 0.938 522	-0.018 0.670 559	0.073 0.084 559	0.417 <0.001 354	-0.002 0.969 522	0.223 <0.001 458	0.118 0.008 522	0.052 0.232 522
EMA	0.047 0.279 522	0.282 <0.001 522	0.049 0.268 522	1.00	0.176 <0.001 522	0.176 <0.001 522	-0.023 0.601 522	0.037 0.403 522	-0.133 0.012 354	-0.069 0.114 522	0.114 0.015 458	0.165 <0.001 522	0.245 <0.001 522
AUS Marb	0.003 0.948 559	0.047 0.268 559	-0.004 0.920 559	0.176 <0.001 522	1.00	0.972 <0.001 522	-0.131 0.002 559	-0.077 0.071 559	-0.057 0.289 354	-0.145 <0.001 522	-0.129 0.006 458	-0.047 0.284 522	0.099 0.024 522
MSA Marb	0.007 0.873 522	0.031 0.476 522	-0.003 0.938 522	0.176 <0.001 522	0.972 <0.001 522	1.00	0.128 0.003 522	-0.082 0.062 522	-0.054 0.312 354	-0.132 0.002 522	-0.108 0.021 458	-0.034 0.438 522	0.110 0.012 522
Meat Col	0.048 0.255 559	-0.081 0.056 559	-0.018 0.670 559	-0.023 0.601 522	-0.131 0.002 559	-0.128 0.003 522	1.00	0.018 0.674 559	0.079 0.138 354	0.081 0.065 522	0.057 0.227 458	0.051 0.245 522	-0.037 0.395 522
FatCol	0.004 0.921 559	-0.005 0.912 559	0.073 0.084 559	0.037 0.403 522	-0.077 0.071 559	-0.082 0.062 522	0.018 0.674 559	1.00	-0.007 0.896 354	-0.088 0.044 522	0.143 0.002 458	0.035 0.428 522	0.064 0.143 522
RibFat	-0.092 0.084 354	0.125 0.019 354	0.417 <0.001 354	-0.133 0.012 354	-0.057 0.289 354	-0.054 0.312 354	0.079 0.138 354	-0.007 0.896 354	1.00	0.084 0.113 354	0.194 <0.001 354	-0.016 0.759 354	0.072 0.179 354
pH	0.008 0.863 522	0.037 0.396 522	-0.002 0.969 522	-0.069 0.114 522	-0.145 <0.001 522	-0.132 0.002 522	0.081 0.065 522	-0.088 0.044 522	0.084 0.113 354	1.00	0.092 0.049 458	0.036 0.414 522	0.007 0.869 522
Loin Temp	0.0585 0.212 458	0.142 0.002 458	0.223 <0.001 458	0.114 0.015 458	-0.129 0.006 458	-0.108 0.021 458	0.057 0.227 458	0.143 0.002 458	0.194 <0.001 354	0.092 0.049 458	1.00	0.067 0.153 458	0.080 0.089 458
Hump	0.030 0.488 522	0.328 <0.001 522	0.118 0.008 522	0.165 <0.001 522	-0.047 0.284 522	-0.034 0.438 522	0.051 0.245 522	0.035 0.428 522	-0.016 0.759 354	0.036 0.414 522	0.067 0.153 458	1.00	0.174 <0.001 522
Oss	0.048 0.271 522	0.197 <0.001 522	0.052 0.232 522	0.245 <0.001 522	0.099 0.024 522	0.110 0.012 522	-0.037 0.395 522	0.064 0.143 522	0.072 0.179 354	0.007 0.869 522	0.080 0.089 458	0.174 <0.001 522	1.00

4.5 Associations Between Immune Competence and Health-related traits

The significance of fixed effects and covariates fitted to statistical models when analysing health-related traits are described in Table 34. Management group (Herd*CGMark*CGWean) had a significant effect on the number of offal defects detected but did not influence lung lesion scores and kill group (Feedlot*Induction Cohort*Abattoir) had a significant effect on both number of offal defects detected and lung lesion scores.

Disease Incidence and Mortalities at the Feedlot

Given the small sample size of animals that died at the feedlot (n=17) or were 'pulled' due to health issues observed during feedlot finishing (n=99) (see Tables 19, 20 & 21), traditional statistical tests such as ANOVA and t-tests were deemed inappropriate. Therefore, a non-parametric permutation test was applied to the data in which 10,000 random samples of size 17 were obtained and, for each sample, their average IC_Comb rank recorded and compared against the average IC_Comb rank of the 17 animals that died (Mortalities, Figure 12). Similarly, 10,000 random samples of size 99 were obtained and, for each sample, their average IC_Comb rank recorded and compared against the average IC_Comb rank of the 99 animals that were 'pulled' at the feedlot (PullNo, converted to a binary trait 0 = not 'pulled' or 1 = 'pulled ≥ 1 time).

When steers were ranked based on immune competence phenotype (IC_Comb), steers which died at the feedlot at a lower average rank (lower immune competence) than their counterparts which exited the feedlot. Permutation testing showed that the chance of observing 17 steers with an average rank for immune competence equal to or lower than that observed for the steers that died was 3.9% (equivalent to P = 0.039) suggesting that a significant favourable association between immune competence phenotype and mortalities during feedlot finishing was observed. Similarly, when steers were ranked based on immune competence phenotype (IC_Comb), steers which were 'pulled' at the feedlot at a lower average rank (lower immune competence) than their counterparts which had no recorded health issues during feedlot finishing. Permutation testing showed that the chance of observing 99 steers with an average rank for immune competence equal to or lower than that observed for the steers that were 'pulled' at the feedlot was 20.6% (equivalent to P = 0.206), suggesting that although enhanced immune competence was associated with a reduced "pull rate" during feedlot finishing, the effect was not significant. A favourable association between immune competence phenotype and mortalities during feedlot finishing has been reported previously with the number of mortalities during feedlot finishing highest in low immune competence phenotype animals (6.1%), followed by average immune competence animals (1.2%) and lowest in high immune competence animals where no mortalities were observed (Hine et al., 2021).

Offal Defects and Lung Lesion Scores

The influence of immune competence (IC_Comb) on number of offal defects and lung lesion scores observed for individual steers at processing was investigated and results presented in Table 35. For analysis, both number of offal defects and lung lesion scores were treated as either a binary (zero or ≥1 offal defect detected, or zero or ≥1 cumulative lung lesion score for both lobes) or ordinal trait. A significant proportion of steers in induction cohorts Ind_1F (91%), Ind_1G (58%), Ind_1H (85%) and Ind_1J (75%) which were processed at processing plant 1 were identified with the offal defect category "kidney-other" which was considered very unlikely to be accurate. Therefore, offal defect data was analysed both including and excluding this offal defect category (Table 35). For number of offal defects and lung lesion scores when recorded as ordinal traits, IC_Comb least squares means for each category grouping were calculated and the significance of differences between groups (Tukey adjustments for multiple comparisons applied) is presented in Tables 36 and 37, respectively. Immune competence phenotype was not significantly associated with number of offal defects or lung lesion scores observed at processing. However, when the number of offal defects observed was treated as a binary trait (zero or ≥1 defect detected) a trend suggesting that immune competence was unfavourably associated with offal defects detected at processing. It is noteworthy; however, that this trend was no longer evident when offal defects were treated as an ordinal trait or when the offal defect category 'kidney other' for steers processed at a single plant were excluded from analysis on the basis that 69% of all steers processed at that plant were recorded as having this defect which was considered very unlikely to be accurate.

Table 35. Significance of fixed effects/covariates fitted to statistical models when analysing health-related traits. Numbers in cells represent p values. P values in cells considered significant (P<0.05) are coloured green.

Trait	Fixed effects		
	Herd*CGMark *CGWean	Condition	Feedlot *Induction Cohort*Abattoir
NoOffalDef	<0.001	0.278	<0.001
NoOffalDef (excluding Kidney-Other)	0.107	0.602	<0.001
NoOffalDefBIN	<0.001	0.314	<0.001
NoOffalDefBIN (excluding Kidney-Other)	<0.001	0.709	<0.001
LungScore	0.107	0.602	<0.001
LungScoreBIN	0.129	0.467	<0.001

Table 36. Significance of immune competence (IC_Comb) when analysing health-related traits. Numbers in cells represent p values. P values in cells considered significant (P<0.05) are coloured green.

Trait	IC_Comb
NoOffalDef	0.213
NoOffalDef (excluding Kidney-Other)	0.608
NoOffalDefBIN	0.071
NoOffalDefBIN (excluding Kidney-Other)	0.441
LungScore	0.834
LungScoreBIN	0.481

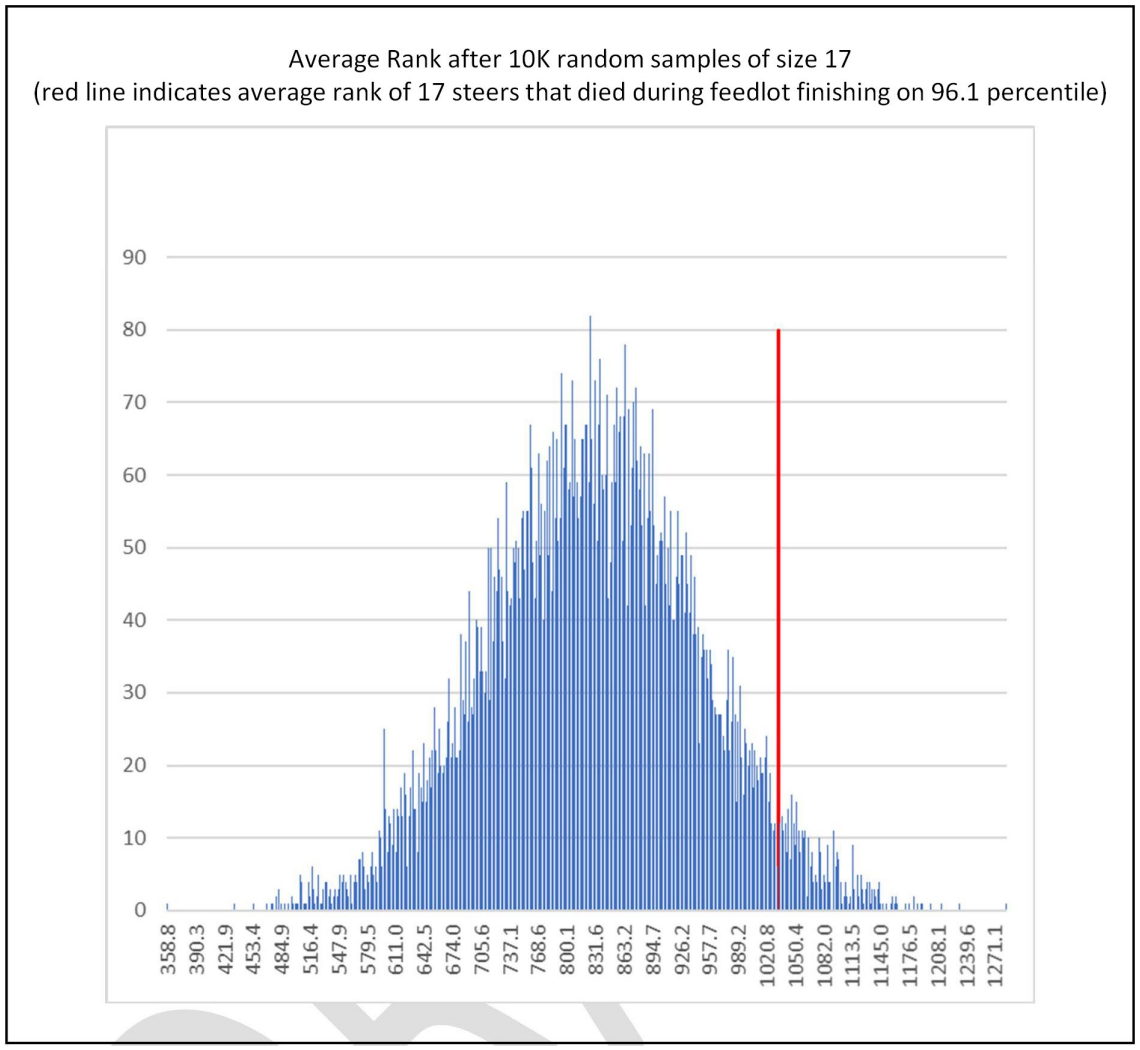
Table 37. Least squares means for immune competence (IC_Comb) within group categories for number of offal defects (diagonal cells). Figures in off-diagonal cells represent the significance of group differences. P values in cells considered significant (P<0.05) are coloured green.

NoOffalDef	0	1	2	3
0	-0.020	0.359	0.863	0.994
1	0.359	0.124	0.958	0.976
2	0.863	0.958	0.067	0.999
3	0.994	0.976	0.999	0.036

Table 38. Least squares means for immune competence (IC_Comb) within group categories for lung lesion score (diagonal cells). Figures in off-diagonal cells represent the significance of group differences. P values in cells considered significant (P<0.05) are coloured green.

LungScore	0	1	2	3	4	5	6
0	0.065	0.792	0.999	1.000	0.951	0.985	1.000
1	0.792	0.248	0.835	0.998	0.857	0.998	1.000
2	0.999	0.835	0.016	0.997	0.969	0.980	1.000
3	1.000	0.998	0.997	0.122	0.941	0.992	1.000
4	0.951	0.857	0.969	0.941	-0.588	0.868	0.949
5	0.985	0.998	0.980	0.992	0.868	0.693	0.997
6	1.000	1.000	1.000	1.000	0.949	0.997	0.167

Figure 12. Permutation test result to assess the probability of observing 17 steers with an average rank for immune competence equal to or lower than that observed for the steers that died at the feedlot.



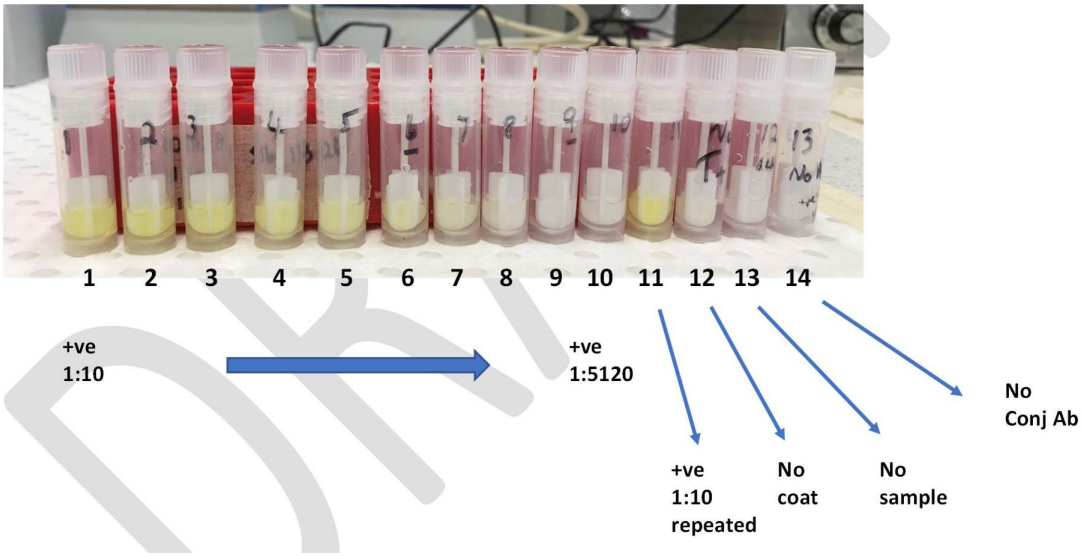
4.6 Pen-Side Antibody Assay Validation

4.6.1 Non-Specific Binding

The extent of non-specific binding in the dipstick-based ELISA test was evaluated by sequentially leaving out individual assay components, a single component at a time. If any one of the assay components, namely coating antigen, sample (containing antibodies) or conjugated secondary antibody are not added in the assay, no signal (above background) should be observed. An assay was conducted in which a positive control serum sample was serially diluted 2-fold from a starting dilution of 1:10 and tested using the standard dipstick-based ELISA protocol (Figure 13, dipsticks 1-10) or the positive control sample diluted 1:10 was assayed using a protocol in which no coating antigen was added but all other reagents added (dipstick 12), no conjugated antibody was added but all other reagents were added (dipstick 14) or no sample but all other reagents were added (dipstick 13). Strong colour development was observed

when the positive control sample diluted 1:10 was assayed using the standard protocol but no colour development was observed when no coating antigen, no sample or no conjugated antibody was added suggesting minimal non-specific binding is detectable in the assay.

Figure 13. Colour development in dipstick tubes



4.6.2 Comparison of Plate-based (existing) and Dipstick-based (new) ELISA Test Results

Assays were run to compare results generated using the plate-based (existing) and dipstick- based (new) ELISA protocol when positive control (Pos Ctrl) and randomly selected test samples were assayed. To compare results, identical samples were simultaneously assayed using the plate-based and dipstick-based ELISA protocols and results from the two assays correlated. Testing was repeated on multiple days using new test samples each day. Representative results from two days of testing (Assay 1 and Assay 2) are shown in Table 39 and correlations between the plate based and dipstick- based ELISA protocols conducted on each day are shown in Figures 14 & 15, respectively. Results were consistently strongly positively correlated ($r^2 > 0.9$) suggesting that the plate-based and dipstick-based ELISA tests yield comparable results.

Table 39. Results generated using the plate-based (existing) and dipstick-based (new) ELISA protocol when positive control (Pos Ctrl) and randomly selected test samples were assayed on two different assay days (Assay 1 & Assay 2)

Assay 1			Assay 2		
Sample	Plate Result (OD units)	Dipstick Result (OD units)	Sample	Plate Result (OD units)	Dipstick Result (OD units)
Pos Ctrl 1	1.81	1.317	Pos Ctrl 1	1.844	1.419
Pos Ctrl 2	1.402	0.788	Pos Ctrl 2	1.453	0.926
50	1.458	0.901	9	0.757	0.359
53	1.644	1.06	47	0.919	0.526
59	1.224	0.735	63	1.570	1.095
65	1.014	0.531	66	1.055	0.525
71	1.683	1.02	67	1.683	1.295
76	1.825	1.375	115	1.683	1.291
106	0.901	0.48	193	1.237	0.722
108	1.493	0.989	202	1.312	0.815
128	1.683	1.265	212	1.132	0.546

Figure 14. Correlation between results generated using the plate-based (existing) and dipstick-based (new) ELISA protocol – Assay 1

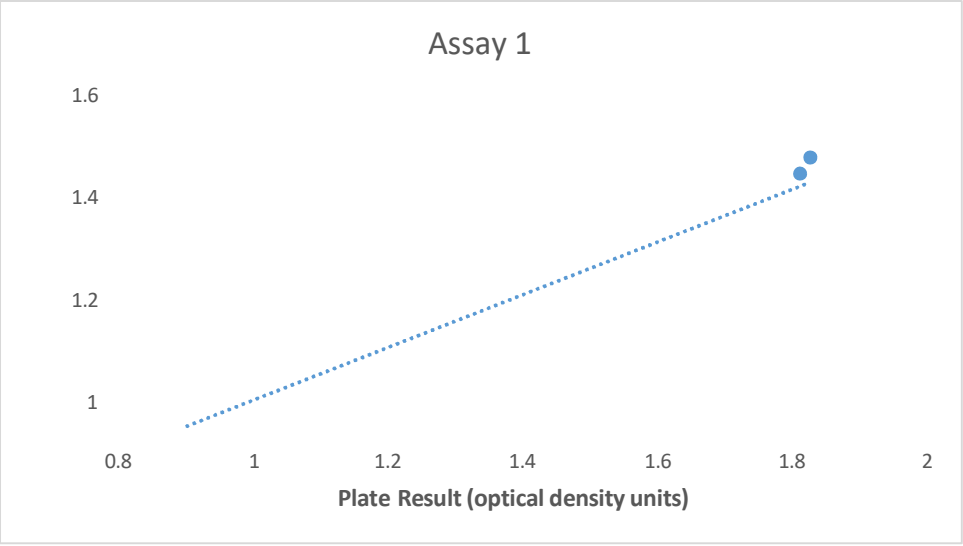
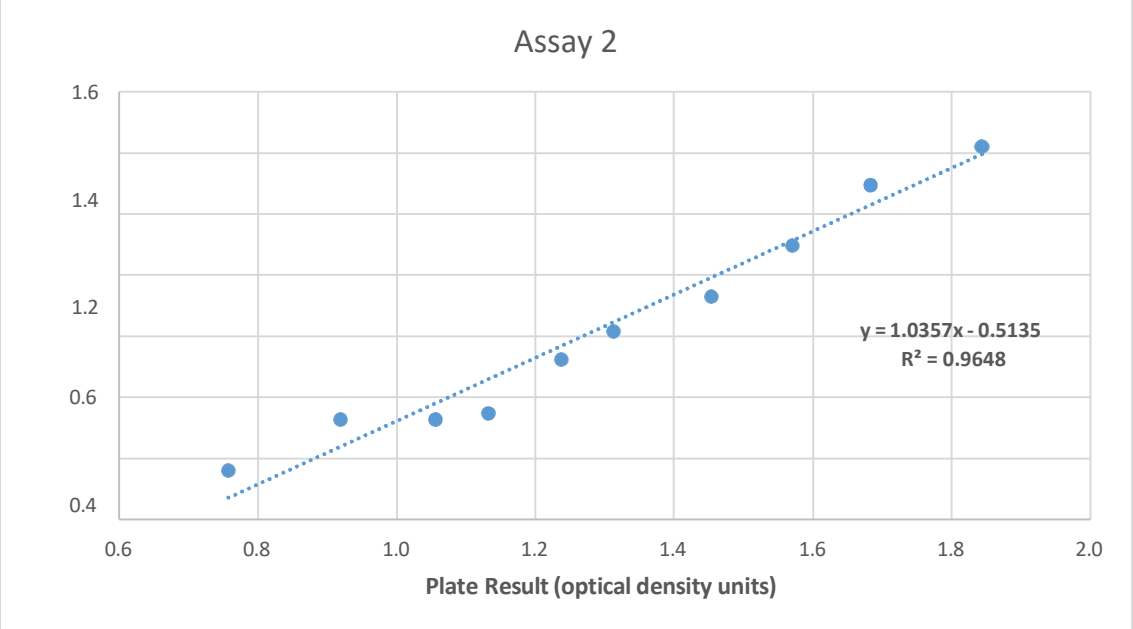


Figure 15. Correlation between results generated using the plate-based (existing) and dipstick-based (new) ELISA protocol – Assay 2



4.6.3 Dynamic Range

The dynamic range of both the plate-based and dipstick-based ELISA methods were compared. When a sample is serially diluted and tested in an ELISA a sigmoidal curve is expected where, at low dilution (high concentration) a point is reached where the signal from the assay is saturated and at a high dilution (low concentration) a point is reached where the signal reaches background levels. Between the saturation point and background level, depicted by flattening at the top and bottom of the sigmoidal curve, respectively, is a linear portion of the curve where the signal from the assay (optical density) is directly proportional to the concentration of the sample (Figure 16). Two positive control samples (Pos Ctrl A & Pos Ctrl B) were serially diluted and tested using the plate-based and dipstick-based ELISA protocols and results are shown in figures 16 and 17, respectively. Results suggested that both assays have a similar dynamic range, as evidenced by the shape of the results curve generated by each assay. The result curve from each of the assays were shifted along the x-axis (dilution factor), but as the shape of the curves were similar, results from each assay are relative to each other (as described above).

Figure 16. Results from the serial dilution of positive control sample A (Pos Ctrl A)

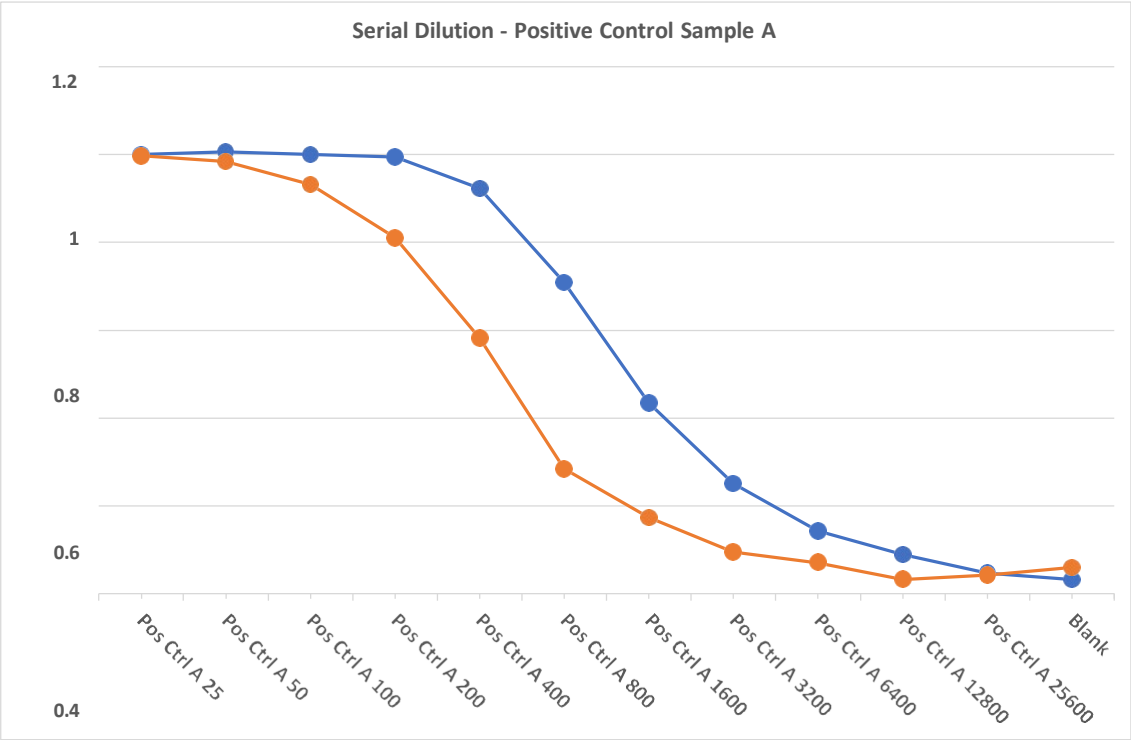
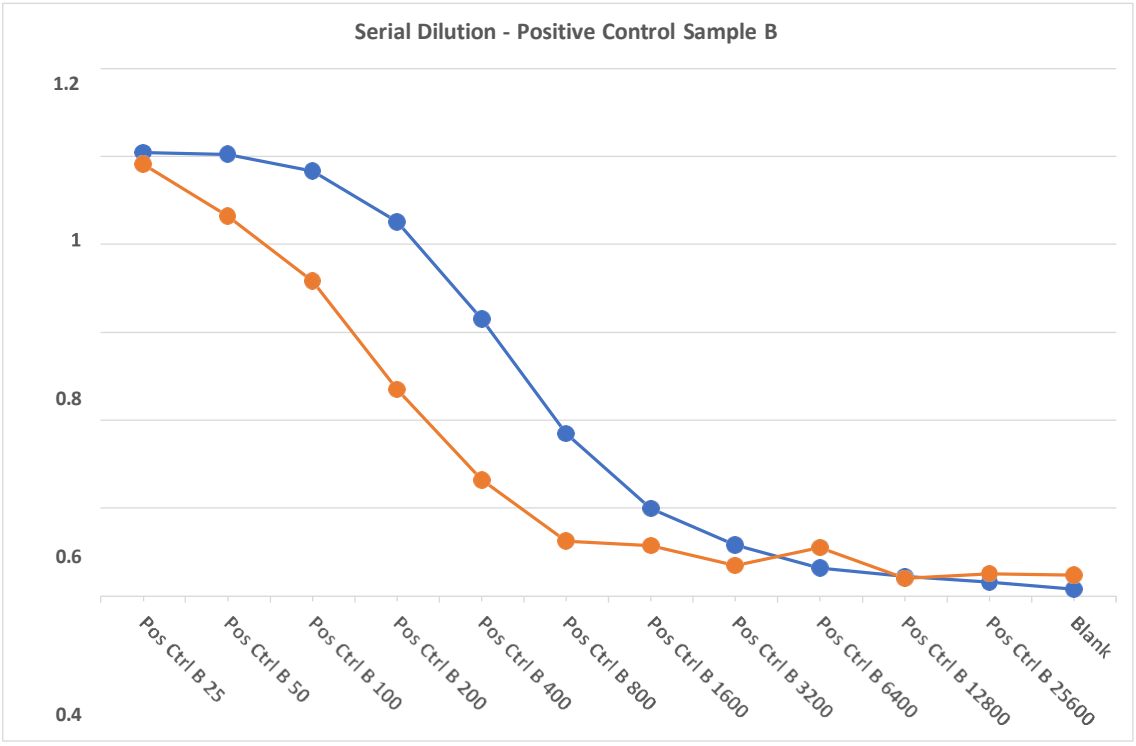


Figure 17. Results from the serial dilution of positive control sample B (Pos Ctrl B)



4.6.4 Comparison of Dipstick-based ELISA Test Results using Different Sample Types

Blood samples were collected from pre-vaccinated cattle and were either not processed (whole blood), were allowed to clot and the serum was poured off (decanted serum) or were centrifuged and serum collected (spun serum). Spun serum is currently used in the existing plate-based ELISA protocol. Whole blood, decanted serum and spun serum from each animal was tested using the dipstick-based ELISA protocol and results obtained using each sample type compared. The correlation between results obtained using spun and decanted serum, spun serum and whole blood and decanted serum and whole blood are shown in Figures 18, 19 and 20, respectively. Assay results from spun serum, decanted serum and whole blood were highly correlated (R^2 ~ 0.9) suggesting that comparable results can be obtained using each of the sample types.

Figure 18. Correlation between results obtained using spun versus decanted serum

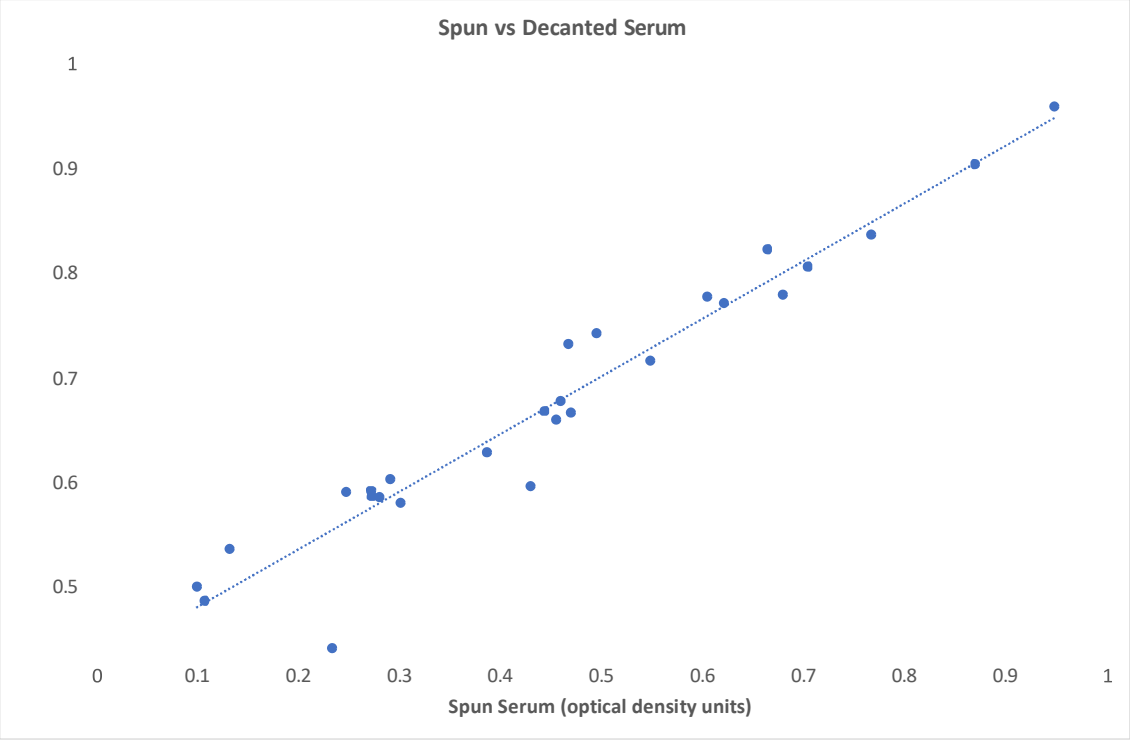


Figure 19. Correlation between results obtained using spun serum versus whole blood

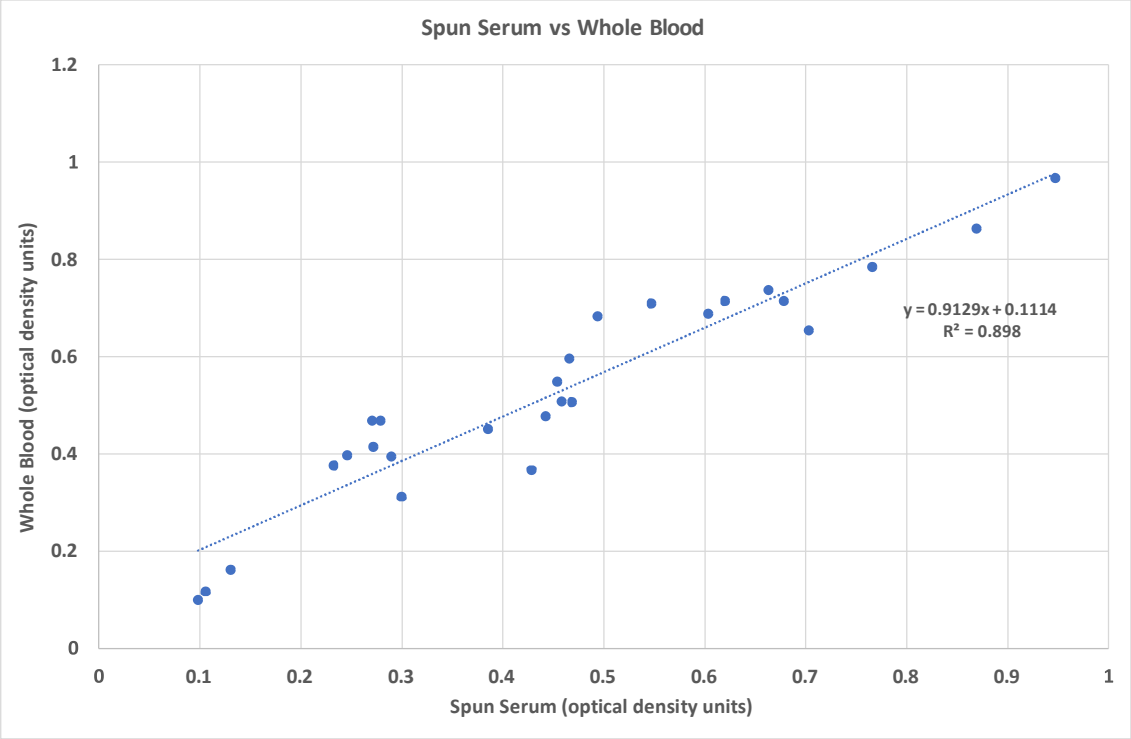
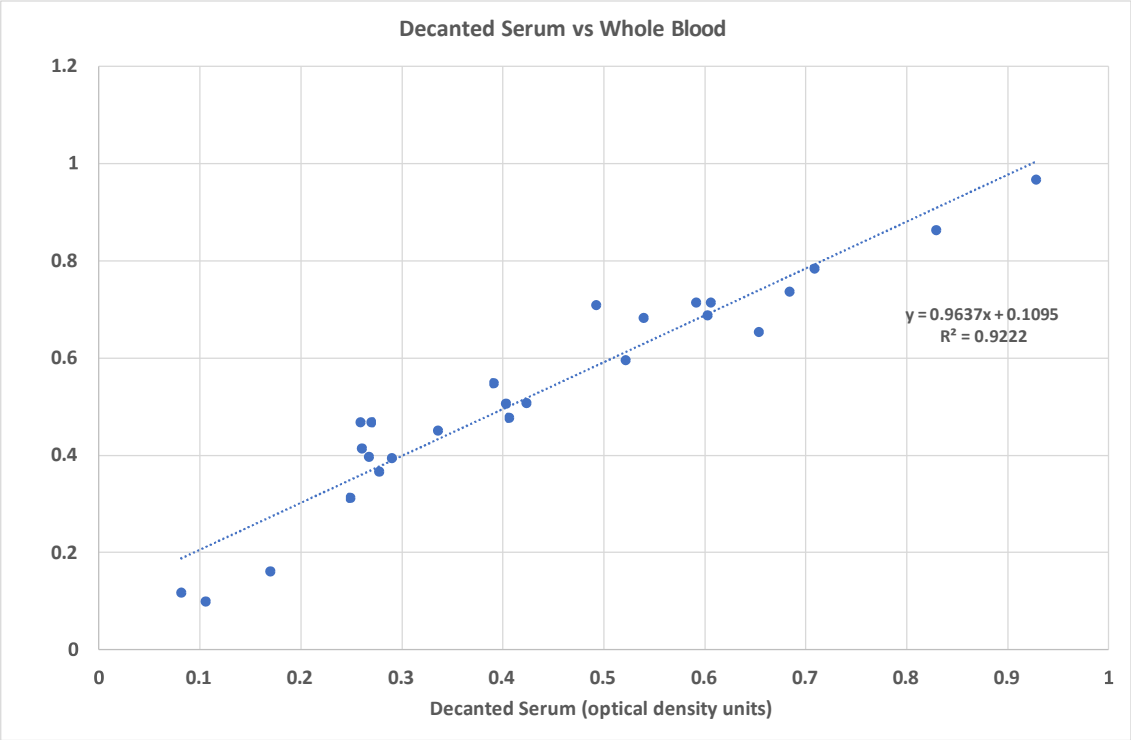


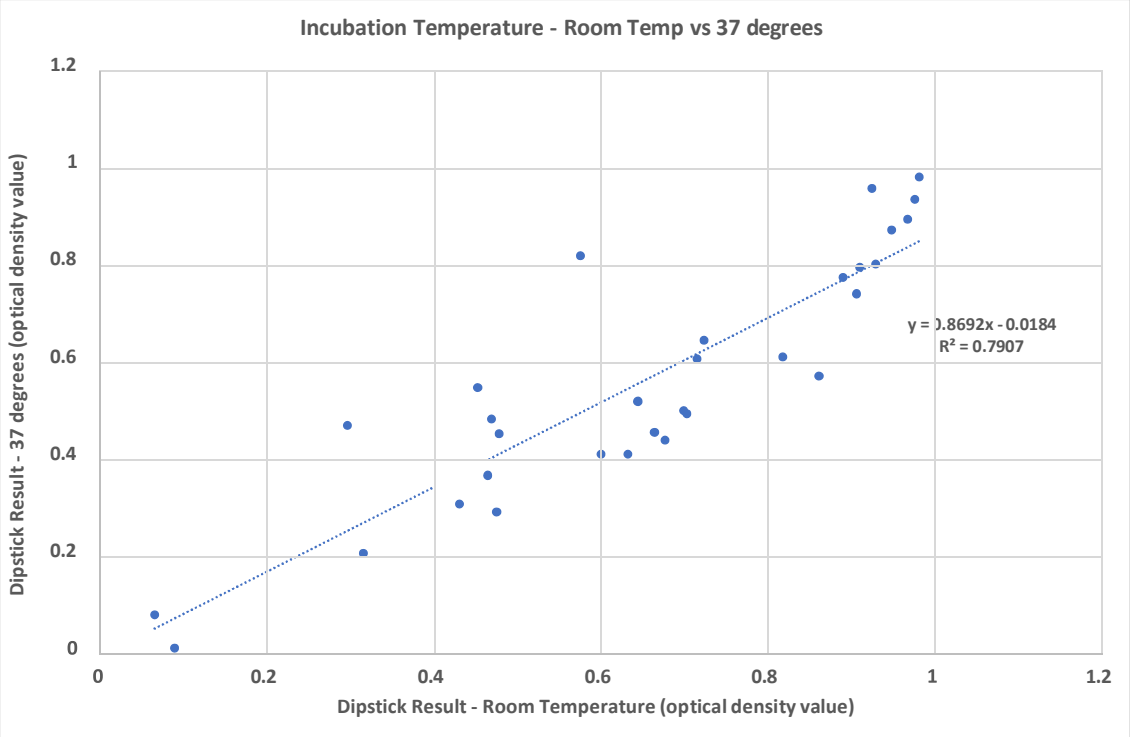
Figure 20. Correlation between results obtained using decanted serum versus decanted serum



4.6.5 Incubation Period

Randomly selected samples were assayed using either the standard dipstick-based ELISA protocol with incubation steps (post addition of block) conducted at room temperature for 1 hour or using a modified protocol in which incubation steps (post addition of block) were conducted at 37°C for 15 minutes and results obtained using each protocol compared. The correlation between results obtained when incubation steps were conducted at room temperature versus at 37°C are shown in Figure 21. Assay results using different incubation temperatures and time were reasonably well correlated (R^2 ~ 0.8) suggesting that comparable results were obtained using both protocols. However, further validation will be required to confirm that modifying the protocol to incorporate incubation steps at 37°C for shorter periods to reduce sample processing times yields comparable results to those obtained using the standard protocol.

Figure 21. Correlation between results obtained when incubation steps (post addition of block) were conducted at room temperature for 1 hour versus at 37°C for 15 minutes.

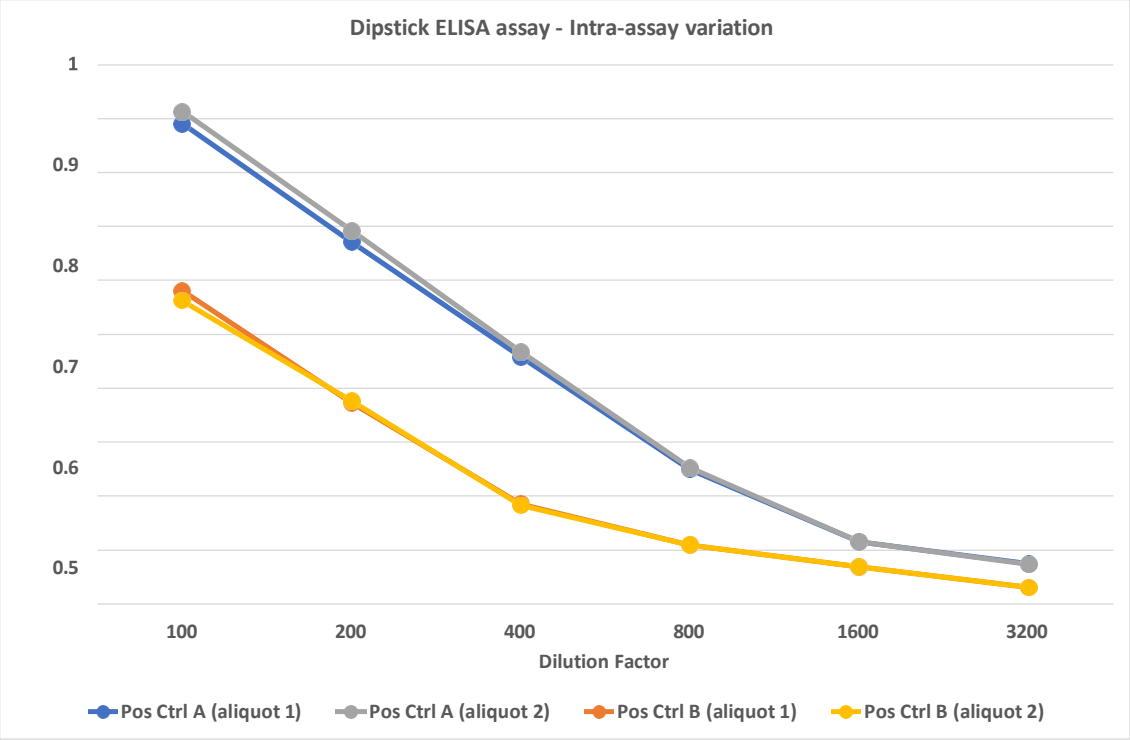


4.6.6 Intra-assay and Inter-assay Variation

Initial indications are that both the intra- and inter-assay variation observed using the dipstick- based ELISA protocol are well within acceptable ranges (Coefficient of Variation (CV) < 10%). However, to accurately assess the intra-assay and inter-assay variation, large numbers of samples will need to be analysed in replicates both in the same assay run and across assay runs, respectively. Results when two positive control

samples, Pos Ctrl A and Pos Ctrl B, were serially diluted and each diluted sample aliquoted into different dipsticks and tested in the same assay run are shown in Figure 22 demonstrating the high repeatability of assay results.

Figure 22. Comparison of results from duplicate positive control samples A & B (Pos Ctrl A & Pos Ctrl B) when serially diluted.



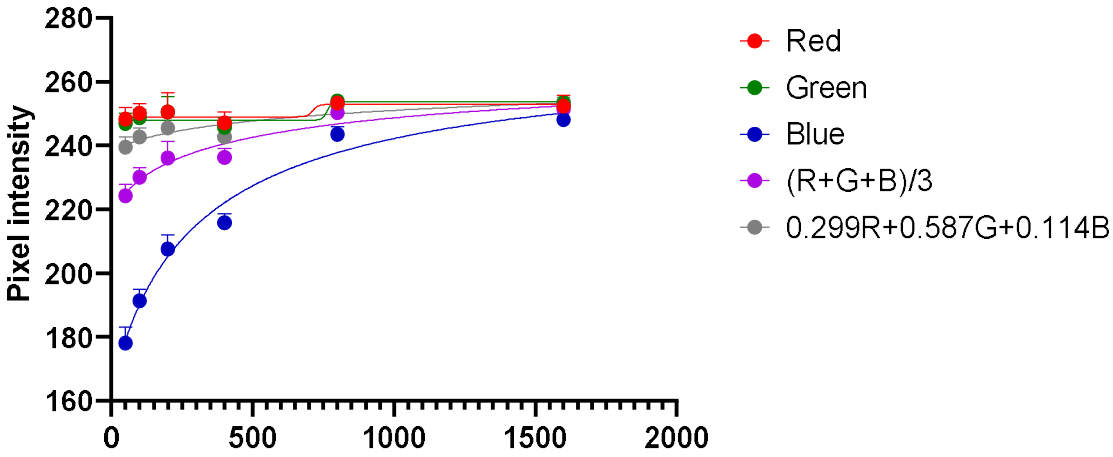
4.6.7 Quantification of Results

To assess the performance of the newly developed dipstick-based ELISA, solutions were transferred from dipstick tubes to low-binding plates following colour development and quantified using a plate reader. For the dipstick-based ELISA test to be practical to apply pen-side on farm it is important that specialised equipment is not required. Therefore, we propose that colour development in the dipstick-based ELISA be either assessed visually (in dipstick tubes) against a colour gradient chart (semi-quantitative) or by transferring solutions from dipstick tubes to a low-binding plate and quantifying colour development in images collected with the phone App (ProCam8). Transferring solutions from individual dipstick tubes to a low binding plate will allow for multiple assay results to be captured in a single image.

A positive control sample was serially diluted and tested using the dipstick-based ELISA protocol. Colour development was captured using the ProCam8 phone App and pixel intensity quantified in various colour channels was evaluated using ImageJ software (Figure 23). Results showed that quantification of pixel intensity in the blue channel had the greatest dynamic range and that the model curve fitted the data well (R^2 = 0.96, data not shown). These results suggest that capturing images using the ProCam8 app and subsequent analysis of images in ImageJ by quantification of pixel intensity in the blue channel may provide a practical means of quantifying results from the dipstick- based ELISA when conducted pen-side. However, further

validation to determine the correlation between results generated using ImageJ and a plate reader will be required.

Figure 23. Pixel intensity of dipstick solutions following the assaying of a serially diluted positive control sample.



5. Conclusion

Methodology to assess the immune competence of beef cattle, which is both practical to apply to farm and does not restrict future sale of tested animals, was developed as part of a previous MLA co-funded project (B.STU.0244). Results from that initial study suggested that immune competence is moderately heritable and favourably correlated with stress-responsiveness and temperament. Results also suggested that animals with enhanced immune competence had significantly reduced disease incidence, significantly fewer mortalities and incurred substantially lower health-related costs during feedlot finishing. This project further validated the potential benefits of selecting for improved immune competence, in terms of reduced disease incidence and mortalities during feedlot finishing, in Australian feedlots.

5.1 Key findings

- A significant favourable association between immune competence phenotype and mortalities during feedlot finishing was observed, with steers below average for immune competence being three times more likely to die during feedlot finishing than steers above average for immune competence.
- Enhanced immune competence was associated with a reduced “pull rate” during feedlot finishing; however, the effect was not significant. The average immune competence rank of steers with no health issues recorded at the feedlot was 659 versus 690 for steers with ≥1 health issue recorded.
- Immune competence phenotype was not significantly associated with number of offal defects or lung lesion scores observed at processing. However, when the number of offal defects observed was treated as a binary trait (zero or ≥1 defect detected) a trend suggesting that immune competence was unfavourably

associated with offal defects being detected at processing. It is noteworthy that this trend was no longer evident when offal defects were treated as an ordinal trait or when the offal defect category 'kidney other' for steers from a single processing plant was excluded from analysis on the basis that 69% of all steers processed were recorded to have this defect which was considered very unlikely to be accurate.

- There was strong evidence to support the notion that there was a favourable association between immune competence phenotype and weight change over weaning: however, the observed relationship was not strong
- Immune competence phenotype was not associated with growth traits, suggesting selection for immune competence will not compromise productivity.
- Immune competence phenotype was not associated with carcase traits assessed in the current study, suggesting selection for immune competence will not compromise carcase characteristics or eating quality.
- A 'pen-side' assay which can be used to assess antibody-mediated immune responses as part of immune competence phenotype testing was developed. This development will allow immune competence phenotype testing to be conducted in 'real time' into the future.

5.2 Benefits to Industry

The Australian feedlot sector is actively seeking strategies which will allow them to reduce their reliance on antibiotics to treat disease while maintaining their existing high standards of animal welfare. This is becoming increasingly important as genetic selection for productivity alone, with no emphasis on health and fitness traits, is reducing the ability of animals to cope with disease challenges and ever-changing climatic conditions experienced in production environments are seeing animals exposed to disease challenges not previously encountered.

Results from the current study further validate the benefits of selecting beef cattle for immune competence, realised through reduced health associated disease and mortalities, in Australian commercial feedlot environments. Genetic strategies aimed at improving the inherent ability of animals to cope with disease challenges experienced in their production environment, used in conjunction with effective vaccination strategies and targeted management practices to reduce exposure to pathogens, have the potential to significantly reduce disease incidence, and subsequent reliance on antibiotics to treat disease, in Australian feedlots. Reducing reliance on antibiotics in feedlots, while maintaining optimal animal welfare standards, will be key to maintaining consumer confidence in Australian beef products moving forward and ensuring the industries continued social licence to operate.

6. Future research and recommendations

Future research should aim to:

- Further validate the benefits of selecting for immune competence in both commercial grass-fed and grain-fed production systems.
- Continue to refine immune competence testing procedures to reduce testing costs and improve practicality.
- Continue to investigate additional measures of immune competence which could be incorporated into testing procedures to improve the ability of the phenotype to predict favourable health outcomes.
- Genotype animals enrolled in the current study to enable immune competence phenotype data to be collected should contribute to the identification of genetic markers associated with improved immune competence.
- The animals enrolled in the study be included in reference populations to improve the accuracy of genomic predictions for immune competence in beef cattle.

7. Acknowledgements

The authors would like to gratefully acknowledge MLA for co-funding this project, co-operator herd owners and staff for providing access to animals and allowing us to conduct our resilience testing on their farms, co-operating feedlot owners and staff for monitoring health and performance data and co-operating processing plant owners and staff for allowing access to plants and providing offal defect data. Special thanks to Dr Tony Batterham (Bovine Dynamics) for facilitating access to feedlots and for providing health and performance data. We would also like to gratefully acknowledge the enormous effort made by numerous CSIRO technical staff Belinda Niemeyer, Graham Acton, Duncan Elks and Dan Driscoll to collect, collate and enter data for this project. Without your valued contribution this project would not have been possible.

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9. Appendix

Figure: Phenotypic correlation matrix for all traits assessed in the current study

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	CellIR	AbIR	IDEX	Wt1	rF81	rF82	rCruch8C	rADG_W	WtInd	rF8Ind	rADG_I	rADG_F	rCWT	rP8fat	rRibfat	rEMA	rausmar	rmsamar	rmeatoo	rfatoo	rph	rIoIntemp	rhump	ross
CellIR	1.00000 1659	0.16285 <.0001 1659	0.76591 <.0001 1659	0.00000 1.0000 1659	0.04918 0.0479 1619	-0.05616 0.0235 1626	-0.00513 0.8345 1659	0.15302 <.0001 1659	0.02098 0.4461 1321	-0.01996 0.5156 1063	0.00381 0.8900 1321	-0.06813 0.0286 1032	-0.06895 0.0343 943	-0.05457 0.0940 943	-0.08120 0.1273 354	0.04145 0.3446 522	0.00090 0.9830 599	0.01168 0.7900 522	0.03345 0.4300 599	0.01357 0.7489 599	0.03443 0.4324 522	0.07779 0.0964 458	0.01983 0.6513 522	0.04506 0.3042 522
AbIR	0.16285 <.0001 1659	1.00000 1659	0.71954 <.0001 1659	-0.00000 1.0000 1659	0.01199 0.6298 1619	-0.00536 0.8282 1626	-0.00155 0.9496 1659	-0.08485 0.0005 1659	-0.02413 0.3809 1321	-0.03059 0.3190 1063	-0.07493 0.0064 1321	0.00236 0.9397 1032	-0.01672 0.6081 943	-0.02067 0.5261 943	-0.04760 0.3719 354	0.02454 0.5759 522	0.03244 0.4440 599	0.02851 0.5157 522	0.02136 0.6143 599	0.00482 0.9095 599	-0.02977 0.4973 522	0.01034 0.8254 458	-0.00390 0.9292 522	0.01363 0.7560 522
IDEX	0.76591 <.0001 1659	0.71954 <.0001 1659	1.00000 1659	-0.00358 0.8842 1659	0.03799 0.1265 1619	-0.03450 0.1644 1626	-0.00440 0.8579 1659	0.04867 0.0475 1659	-0.00640 0.8163 1321	-0.02951 0.3365 1063	-0.04015 0.1447 1321	-0.03978 0.2017 1032	-0.04825 0.1388 943	-0.05444 0.0948 943	-0.09184 0.0841 354	0.04745 0.2792 522	0.00275 0.9483 599	0.00701 0.8730 522	0.04828 0.2545 599	0.00419 0.9213 599	0.00759 0.8626 522	0.05848 0.2116 458	0.03041 0.4881 522	0.04825 0.2711 522
Wt1	0.00000 1.0000 1659	-0.00000 1.0000 1659	-0.00358 0.8842 1659	1.00000 1659	0.00000 1.0000 1619	0.00000 1.0000 1626	0.00000 1.0000 1659	0.00000 1.0000 1659	0.17887 <.0001 1321	-0.00710 0.8172 1063	0.00000 1.0000 1321	0.04678 0.1331 1032	0.11114 0.0006 943	0.07191 0.0272 943	0.15042 0.0046 354	0.01950 0.6567 522	-0.05895 0.1640 599	-0.07509 0.0865 522	0.06140 0.1471 599	-0.03374 0.4260 599	-0.02868 0.5132 522	0.03246 0.4883 458	0.06924 0.1141 522	0.03971 0.3652 522
rF81	0.04818 0.0479 1619	0.01199 0.6298 1619	0.03799 0.1265 1619	0.00000 1.0000 1619	1.00000 1619	0.54189 <.0001 1599	-0.18270 <.0001 1619	-0.00332 0.8937 1619	0.03812 0.1705 1294	0.32445 <.0001 1042	0.05337 0.0550 1294	-0.11616 0.0002 1014	-0.08048 0.0144 925	0.00306 0.9259 925	0.00123 0.9818 346	-0.09212 0.0376 510	0.08086 0.0588 547	0.04722 0.2872 510	0.01061 0.8044 547	-0.01323 0.7576 547	-0.01217 0.7839 510	-0.01992 0.6745 447	-0.07256 0.1016 510	-0.15639 0.0004 510
rF82	-0.05616 0.0235 1626	-0.00539 0.8282 1626	-0.03450 0.1644 1626	0.00000 1.0000 1626	0.54189 <.0001 1599	1.00000 1626	-0.15828 <.0001 1626	-0.07543 0.0023 1626	0.05774 0.0372 1303	0.35063 <.0001 1048	0.09622 0.0168 1303	-0.01852 0.5548 1019	0.01476 0.6530 930	0.04178 0.2031 930	-0.00878 0.8704 348	-0.05127 0.2459 514	-0.02908 0.4961 550	-0.04686 0.2889 514	0.06650 0.1193 550	0.01391 0.7449 550	0.03592 0.4164 514	0.01380 0.7704 450	-0.01962 0.6572 514	-0.07829 0.0762 514
rCruch8C	-0.00513 0.8345 1659	-0.00155 0.9496 1659	-0.00440 0.8579 1659	0.00000 1.0000 1659	-0.18270 <.0001 1619	-0.15828 <.0001 1626	1.00000 1659	-0.01462 0.5517 1659	-0.02399 0.3837 1321	-0.17791 <.0001 1063	-0.03708 0.1780 1321	0.06404 0.0397 1032	0.04318 0.1852 943	-0.01459 0.6544 943	0.08041 0.1310 354	-0.01099 0.8022 522	-0.04063 0.3376 599	-0.04578 0.2965 522	-0.13784 0.0011 599	0.01628 0.7009 599	-0.09802 0.1856 522	0.05021 0.2836 458	0.04969 0.2571 522	0.07870 0.0724 522
rADG_W	0.15302 <.0001 1659	-0.08485 0.0005 1659	0.04867 0.0475 1659	0.00000 1.0000 1659	-0.00332 0.8937 1619	-0.07543 0.0023 1626	-0.01462 0.5517 1659	1.00000 1659	0.08038 0.0035 1321	-0.02807 0.3606 1063	0.12101 <.0001 1321	-0.01173 0.7067 1032	0.03479 0.2858 943	-0.00548 0.8665 943	-0.06357 0.2329 354	0.02885 0.5107 522	0.02057 0.6274 599	0.06116 0.1630 522	0.00297 0.9441 599	0.00775 0.8549 599	0.09642 0.0276 522	0.03511 0.4535 458	-0.00209 0.9620 522	0.00284 0.9483 522
WtInd	0.02098 0.4461 1321	-0.02413 0.3809 1321	-0.00640 0.8163 1321	0.17887 <.0001 1321	0.03812 0.1705 1294	0.05774 0.0372 1303	-0.02399 0.3837 1321	0.08038 0.0035 1321	1.00000	0.00000 1.0000 1063	0.59837 <.0001 1321	0.00000 1.0000 1032	0.28641 <.0001 943	0.08826 0.0067 943	0.10595 0.0464 354	0.07722 0.0780 522	-0.01395 0.7421 599	-0.01307 0.7658 522	0.08306 0.0497 599	0.04792 0.2580 599	0.02483 0.5714 522	0.10700 0.0220 458	0.15220 0.0005 522	0.03501 0.4247 522
rF8Ind	-0.01996 0.5156 1063	-0.03059 0.3190 1063	-0.02951 0.3365 1063	-0.00710 0.8172 1063	0.32445 <.0001 1042	0.35063 <.0001 1048	-0.17791 <.0001 1063	-0.02807 0.3606 1063	0.00000 1.0000 1063	1.00000 1063	0.00169 0.9560 1063	-0.00370 0.9178 781	0.01426 0.7081 692	-0.01742 0.6473 692	0.04330 0.4174 353	-0.09749 0.0673 353	-0.00367 0.9425 390	-0.00612 0.9089 353	0.07560 0.1362 390	-0.11175 0.0273 390	-0.01749 0.7434 353	0.01824 0.7327 353	-0.02256 0.6727 353	-0.08245 0.1220 353
rADG_I	0.00381 0.8900 1321	-0.07493 0.0064 1321	-0.04015 0.1447 1321	0.00000 1.0000 1321	0.05337 0.0550 1294	0.09622 0.0168 1303	-0.03708 0.1780 1321	0.12101 <.0001 1321	0.59837 <.0001 1321	0.00169 0.9560 1063	1.00000 1321	-0.04998 0.1085 1032	0.34834 <.0001 943	0.06899 0.0342 943	0.05290 0.3210 354	0.07469 0.0882 522	0.01033 0.8075 599	0.01995 0.6493 522	0.08327 0.0491 599	0.07764 0.0966 599	0.05060 0.2485 522	0.11815 0.0114 458	0.14297 0.0011 522	0.01716 0.6957 522
rADG_F	-0.06813 0.0286 1032	0.00236 0.9397 1032	-0.03978 0.2017 1032	0.04678 0.1331 1032	-0.11616 0.0002 1014	-0.01852 0.5548 1019	0.06404 0.0397 1032	-0.01173 0.7067 1032	0.00000 1.0000 1032	-0.00370 0.9178 781	-0.04998 0.1085 1032	1.00000 1032	0.88483 <.0001 943	0.16055 <.0001 943	0.07337 0.1684 354	0.28263 <.0001 522	0.06295 0.1372 599	0.05149 0.2402 522	-0.11178 0.0082 599	-0.01934 0.6481 599	0.02961 0.4996 522	0.08464 0.0704 458	0.26022 <.0001 522	0.19197 <.0001 522
rCWT	-0.06895 0.0343 943	-0.01672 0.6081 943	-0.04825 0.1388 943	0.11114 0.0006 943	-0.08048 0.0144 925	0.01476 0.6530 930	0.04318 0.1852 943	0.03479 0.2858 943	0.28641 <.0001 943	0.01426 0.7081 692	0.34834 <.0001 943	0.88483 <.0001 943	1.00000 943	0.21234 <.0001 943	0.12518 0.0185 354	0.28153 <.0001 522	0.04690 0.2683 599	0.03127 0.4759 522	-0.08081 0.0562 599	-0.00471 0.9115 599	0.03721 0.3963 522	0.14152 0.0024 458	0.32820 <.0001 522	0.19656 <.0001 522
rP8fat	-0.05457 0.0940 943	-0.02067 0.5261 943	-0.05444 0.0948 943	0.07191 0.0272 943	0.00306 0.9259 925	0.04178 0.2031 930	-0.01459 0.6544 943	-0.00548 0.8665 943	0.08826 0.0067 943	-0.01742 0.6473 692	0.06899 0.0342 943	0.16055 <.0001 943	0.21234 <.0001 943	1.00000 943	0.41681 <.0001 354	0.04862 0.2675 522	-0.00428 0.9196 599	-0.00344 0.9375 522	-0.01806 0.6701 599	0.07313 0.0841 599	-0.00169 0.9693 522	0.22262 <.0001 458	0.11758 0.0072 522	0.05243 0.2317 522
rRibfat	-0.08120 0.1273 354	-0.04760 0.3719 354	-0.09194 0.0841 354	0.15042 0.0046 354	0.00123 0.9818 346	-0.00878 0.8704 348	0.08041 0.1310 354	-0.06357 0.2329 354	0.10595 0.0464 354	0.04330 0.4174 353	0.05290 0.3210 354	0.07337 0.1684 354	0.12518 0.0185 354	0.41681 <.0001 354	1.00000 354	-0.13343 0.0120 354	-0.05657 0.2885 354	-0.05388 0.3121 354	0.07900 0.1379 354	-0.00698 0.8959 354	0.08433 0.1132 354	0.16407 0.0002 354	-0.01635 0.7592 354	0.07157 0.1791 354
rEMA	0.04145 0.3446 522	0.02454 0.5759 522	0.04745 0.2792 522	0.01950 0.6567 522	-0.09212 0.0376 510	-0.05127 0.2459 514	-0.01099 0.8022 522	0.02885 0.5107 522	0.07722 0.0780 522	-0.09749 0.0673 353	0.07469 0.0882 522	0.28263 <.0001 522	0.28153 <.0001 522	0.04862 0.2675 522	-0.13343 0.0120 354	1.00000 522	0.17640 <.0001 522	0.17640 <.0001 522	-0.02295 0.6009 522	0.03869 0.4028 522	-0.06936 0.1135 522	0.11351 0.0151 458	0.16501 0.0002 522	0.24520 <.0001 522
rausmar	0.00090 0.9830 599	0.03244 0.4440 599	0.00275 0.9483 599	-0.05895 0.1640 599	0.08086 0.0588 547	-0.02908 0.4961 550	-0.04063 0.3376 559	0.02057 0.6274 559	-0.01395 0.7421 559	-0.00367 0.9425 390	0.01033 0.8075 559	0.06295 0.1372 559	0.04690 0.2683 559	-0.00428 0.9196 559	-0.05657 0.2885 354	0.17640 <.0001 522	1.00000 599	0.97173 <.0001 522	-0.13121 0.0019 599	-0.07655 0.0705 599	-0.14524 0.0009 522	-0.12883 0.0058 458	-0.04701 0.2837 522	0.09861 0.0243 522
rmsamar	0.01168 0.7900 522	0.02851 0.5157 522	0.00701 0.8730 522	-0.07509 0.0865 522	0.04722 0.2872 510	-0.04686 0.2889 514	-0.04578 0.2965 522	0.06116 0.1630 522	-0.01307 0.7658 522	-0.00612 0.9089 353	0.01995 0.6493 522	0.05149 0.2402 522	0.03127 0.4759 522	-0.00344 0.9376 522	-0.05388 0.3121 354	0.17640 <.0001 522	0.97173 <.0001 522	1.00000 522	-0.12805 0.0034 522	-0.08180 0.0618 522	-0.13245 0.0024 522	-0.10772 0.0211 458	-0.03401 0.4381 522	0.11011 0.0118 522
rmeatoo	0.03345 0.4300 599	0.02136 0.6143 599	0.04828 0.2545 599	0.06140 0.1471 599	0.01061 0.8044 547	0.06650 0.1193 550	-0.13784 0.0011 559	0.00297 0.9441 559	0.07560 0.1362 390	0.08327 0.0491 559	-0.11178 0.0082 559	-0.08081 0.0562 559	-0.01806 0.6701 559	0.07900 0.1379 354	-0.02295 0.6009 522	-0.13121 0.0019 599	-0.12805 0.0034 522	1.00000 599	0.01782 0.6741 559	0.08095 0.0646 522	0.05652 0.2273 458	0.05093 0.2454 522	-0.03727 0.3954 522
rfatoo	0.01357 0.7489 599	0.00482 0.9095 599	0.00419 0.9213 599	-0.03374 0.4260 599	-0.01323 0.7576 547	0.01391 0.7449 550	0.01628 0.7009 559	0.00775 0.8548 559	0.04792 0.2580 559	-0.11175 0.0273 390	0.07764 0.0966 559	-0.01934 0.6481 559	0.07313 0.0841 559	-0.00698 0.8959 354	0.03869 0.4028 522	-0.07655 0.0705 599	-0.08180 0.0618 522	0.01782 0.6741 559	1.00000 559	-0.08803 0.0444 522	0.14273 0.0022 458	0.03478 0.4278 522	0.06424 0.1428 522
rph	0.03443 0.4324 522	-0.02977 0.4973 522	0.00759 0.8626 522	-0.02868 0.5132 522	-0.01217 0.7839 510	0.03592 0.4164 514	-0.05802 0.1856 522	0.09642 0.0276 522	0.02483 0.5714 522	-0.01749 0.7434 353	0.05060 0.2485 522	0.02961 0.4996 522	0.03721 0.3963 522	-0.00169 0.9693 522	0.08433 0.1132 354	-0.06936 0.1135 522	-0.14524 0.0009 522	-0.13245 0.0024 522	0.08095 0.0646 522	-0.08803 0.0444 522	1.00000 522	0.09195 0.0492 458	0.03580 0.4143 522	0.00724 0.8689 522
rIoIntemp	0.07779 0.0964 458	0.01034 0.8254 458	0.05848 0.2116 458	0.03246 0.4883 458	-0.01992 0.6745 447	0.01380 0.7704 450	0.05021 0.2836 458	0.03511 0.4536 458	0.10700 0.0220 458	0.01824 0.7327 353	0.11815 0.0114 458	0.08464 0.0704 458	0.14152 0.0024 458	0.22262 <.0001 458	0.19407 0.0002 354	0.11351 0.0151 458	-0.12883 0.0058 458	-0.10772 0.0211 458	0.05652 0.2273 458	0.14273 0.0022 458	0.09195 0.0492 458	1.00000 458	0.03580 0.4143 522	0.07969 0.0885 458
rhump	0.01983 0.6513 522	-0.00390 0.9292 522	0.03041 0.4881 522	0.06924 0.1141 522	-0.07256 0.1016 510	-0.01962 0.6572 514	0.04969 0.2571 522	-0.00209 0.9620 522	0.15220 0.0005 522	-0.02256 0.6727 353	0.14297 0.0011 522	0.26022 <.0001 522	0.32820 <.0001 522	0.11758 0.0072 522	-0.01635 0.7592 354	0.16501 0.0002 522	-0.04701 0.2837 522	-0.03401 0.4381 522	0.05093 0.2454 522	0.03478 0.4278 522	0.03580 0.4143 522	0.06689 0.1530 458	1.00000 522	0.17398 <.0001 522
ross	0.04506 0.3042 522	0.01363 0.7560 522	0.04825 0.2711 522	0.03971 0.3652 522	-0.15639 0.0004 510	-0.07829 0.0762 514	0.07870 0.0724 522	0.00284 0.9483 522	0.03501 0.4247 522	-0.08245 0.1220 353	0.01716 0.6957 522	0.19197 <.0001 522	0.19656 <.0001 522	0.05243 0.2317 522	0.07157 0.1791 354	0.24520 <.0001 522	0.09861 0.0243 522	0.11011 0.0118 522	-0.03727 0.3954 522	0.06424 0.1428 522	0.00724 0.8689 522	0.07969 0.0885 458	0.17398 <.0001 522	1.00000 522