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Prepared by: Dr Brad Hine, Dr Aaron Ingham, Dr Jen Smith and Dr Ian Colditz
CSIRO, Agriculture and Food

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Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

Associations between immune competence, health and performance of sheep in the Resource Flock

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Abstract

Genetic selection for production with little emphasis on health can lead to an increase in disease incidence. This trend is observed in many livestock species. Here we describe the development of methodology to assess general immune competence in sheep and demonstrate that immune competence traits are moderately heritable, suggesting reasonable genetic gains can be expected when selecting for immune competence in sheep.

The project explored associations between the resilience traits of immune competence, stress-responsiveness and temperament and important health and production traits in 2613 lambs and 945 adult ewes.

Immune competence was moderately unfavourably correlated with the temperament trait, flight time and moderately positively correlated with haptoglobin responses to the combined effects of vaccination and management induced stress. Favourable genetic correlations between immune competence, internal parasite resistance and several carcase characteristic traits including tenderness and intramuscular fat were observed in lambs. Although unfavourable genetic correlations were observed between immune competence and certain wool traits such as fibre diameter and yield, favourable correlations were observed for other wool traits such as staple strength.

We hypothesis that significant economic benefits can be achieved by selecting for immune competence in sheep, realised through reduced labour and therapeutic costs associated with monitoring and treating for disease, while having favourable impacts on many important production traits in sheep.

Executive Summary

Strategies aimed at reducing the incidence of disease in the Australian sheep industry have the potential to significantly reduce labour and therapeutic costs associated with disease monitoring and treatment. Development of such strategies is also expected to play a critical role in maintaining consumer confidence that the sheep industry is operating a a standard aligned with community expectation. Consumers are increasingly conscious of the health and welfare of food producing animals producing and increasingly concerned with the use of antibiotics to prevent and treat disease. With consumers demanding the highest possible standards of animal health and welfare through their purchasing choices, maintaining consumer confidence is critical to the future of the sheep industry.

Livestock face a variety of challenges from their production environment including exposure to infectious agents, climatic extremes, social stressors as a result of 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. Immune competence is an important component of an animal's resilience, reflecting it's ability to cope with infectious disease. The establishment of a protocol to assess immune competence in dairy cattle has enabled genetic selection strategies, aimed at breeding animals with enhanced 'general' disease resistance, to be developed and implemented in industry (Wilkie and Mallard 1999). This approach combines measures of both antibody-mediated immune responses (AMIR) and cell-mediated immune responses (CMIR) to assess 'general' immune competence. Pathogens differ in the way they infect the host animal. For instance, most bacteria live outside host cells while viruses replicate within host cells. Extra-ceullar pathogens are most effectively controlled by AMIR whereas intracellular pathogens are controlled by CMIR. Therefore individuals identified as having a balanced ability to mount both types of responses are expected to exhibit broad-based disease resistance against a wide range of pathogens. The success of this strategy has already been demonstrated in dairy cattle, beef cattle and pigs.

Genetic selection for production with little emphasis on health can lead to an increase in the incidence of disease. This trend has been observed in many livestock species. Therefore, this project aimed to explore the potential for genetic selection, aimed at improving 'general' immune competence to reduce the incidence of disease in the Australian sheep industry.

Specific objectives of the project were to:

1. Develop immunophenotyping tests to predict individuals at enhanced risk of disease or reduced productivity.
2. Develop immunophenotype indicator trait(s) suitable for implementation through genetics / genomics.
3. Develop initial estimates of genetic and phenotypic parameters for immune response traits in sheep.
4. Develop knowledge on stress responsiveness of lambs at weaning.

To explore associations between resilience traits and important health and production traits, 2613 lambs and 945 adult ewes were put through a testing protocol to assess the resilience traits, immune competence, stress-responsiveness and temperament. In addition, information on the following traits were obtained.

Traits measured by MLA on resource flock lambs which were used for analysis included:-

- Growth traits - birth weight (BW) and post-weaning weight (PW1, PW2).
- Body composition traits - eye muscle diameter (EMD) and fat cover (CFAT).
- Carcase traits – hot carcase weight (HCWT), carcase eye muscle area (CEMA), fat depth C (CCFAT), AUS-MEAT fat classification (CFATSCORE), fat depth GR (HGRFAT), intramuscular fat (IMF), fresh meat colour (CFL, Cfa, Cfb), post slaughter pH (PH24LL) and shear force (SHEARF5).

Traits measured by the Sheep CRC on follower ewes which were used for analysis included:-

- Growth and body condition traits - birth weight (BW), weaning weight (WW), yearling weight (YW), mature weight (MW) and body condition score (BCS1, BCS2).
- Body composition traits - eye muscle diameter (EMD) and fat cover (CFAT).
- Wool traits - greasy fleece weight (GFW), clean fleece weight (CFW), yield (YLD), fibre diameter (FD), standard deviation of fibre diameter (SDFD), co-efficient of variation of fibre diameter (CVD), fibre curvature (CURV), staple length (SL) and staple strength (SS) measured at hogget age.
- Health and other traits - post weaning dag score (pDAG), yearling dag score (yDAG) and post weaning worm egg count (pWEC).

Analysis was undertaken to estimate genetic parameters for immune competence traits to predict the genetic gains which might be expected when selecting for immune competence. Correlations, both phenotypic and genetic, between immune competence, other resilience traits and important health and production traits as described above were also determined. Key findings from the study include:-

- The heritability of the immune competence traits, AMIR, CMIRclo (CMIR to clostridial vaccine) and CMIRgud (CMIR to Gudair vaccine) were estimated to be moderate, suggesting a reasonable rate of genetic gain could be expected when selecting for immune competence in sheep.
- Moderate to strong positive genetic correlations between AMIR and CMIRclo and AMIR and CMIRgud were observed in ewes. However, AMIR and CMIR traits were not genetically correlated in lambs.
- Unfavourable moderate to strong genetic correlations between immune competence traits and the temperament trait, FT were observed in ewes and lambs; however, no significant differences in FT were observed between immume competence phenotype groups and the use of flight speed as a measure of temperament is not well established in sheep.
- Phenotypic and genetic correlations between immune competence traits and increases in serum haptoglobin induced by vaccination and weaning (lambs) or transportation (ewes) conducted on day 0 of testing were positive, suggesting that haptoglobin

responses induced by vaccination may provide a valuable indicator of the strength of the innate immune system in sheep.

- Immune competence traits were weakly unfavourably genetically correlated with WW in ewes but were not correlated with YW. Immune competence traits and growth traits were not genetically correlated in lambs.
- Weak to moderate positive genetic correlations were observed between immune competence traits and BCS in ewes which may have implications for reproductive performance.
- Moderately unfavourable genetic correlations were observed between immune competence traits and the wool traits, FD, YLD and SDFD. In contrast, genetic correlations between immune competence traits and SS were weakly favourable.
- Favourable weak to moderate genetic correlations between immune competence traits and the carcase traits SHEARF5, IMF and HGRFAT were observed in lambs. Results from carcase data were supported by live animal ultrasound scanning data which suggested that CFAT was also favourably genetically correlated with immune competence in lambs. These findings suggest selection for immune competence may also have benefits for carcase retail values and lamb eating quality.
- Favourable genetic correlations between immune competence traits and FAM in ewes and lambs and with WEC in lambs suggests that selection for immune competence will also improve resistance to internal parasites in sheep. Favourable correlations between immune competence traits and pDAG in ewes were also observed.

Based on results of the current study, we hypothesise that significant economic benefits can be achieved by selecting for immune competence in sheep, realised through reduced labour and therapeutic costs associated with monitoring and treating for disease, while having favourable impacts on many important production traits in sheep.

Australian biosecurity restrictions prevented the use of the immune competence testing procedure developed by Wilkie and Mallard in the current study. Therefore, a practical method was developed for immune competence testing suited to Australian conditions and compliant with Australian regulations that employed conventional commercial vaccines for immunphenotyping. In addition, by application of the test at a time when animals were exposed to management induced stress, weaning in the case of lambs and transport and handling in the case of ewes, the concept of immune competence phenotyping of animals developed by Wilkie and Mallard was extended in the current study to the concept of resilience testing. This represents a substantial conceptual advance on previous approaches to improving functional traits in livestock. We anticipate that immune phenotyping during this period of stress will provide a more rigourous test of the potential of sheep to be resilient and cope with the social and environmental challenges experienced during their productive life than is achieved using previous used methods.

Strategies aimed at reducing the incidence and impact of disease in Australian sheep flocks such as that described here have the potential to:

- Increase productivity
- Reduce disease treatment costs
- Improve animal health & welfare
- Reduce use of antibiotics in the food-chain

- Increase the productive life of breeding animals

Further development and validation of resilience phenotyping methods will be required to allow the development of a resilience selection index which could be used to rank sires presented for sale by seed-stock suppliers. Results from the current study will allow resilience phenotyping methods to be refined in future studies, improving the practicality of testing large numbers of sheep on farm. Central to this refinement is the need for development of field based tests to replace labour intensive laboratory tests, removing the need to transport samples.

Unfortunately detailed data on disease incidence (apart from WEC data) and health-related mortalities were not available (or numbers of records were insufficient) for animals enrolled in the current study. Therefore, future studies will be required to validate benefits of selecting for immune competence on disease incidence, mortality and disease treatment costs in sheep. The potential for additional resilience traits such as heat tolerance to be incorporated into resilience selection indexes also warrants further investigation. Therefore the goal of future projects will be to validate benefits of selecting for immune competence and to further refine testing procedures to assess resilience for integration into routine .

More specifically future projects should aim to:-

- Utilise detailed health and performance data collected (during feedlot finishing or lifetime production) in sheep to validate the ability of immune competence phenotyping measures to identify animals better able to cope with challenges posed by their production environment.
- Conduct detailed economic analysis to estimate the potential benefits of selecting for improved immune competence in sheep realised through reduced labour and therapeutic costs associated with monitoring and treating for disease and improved productivity.
- Further validate appropriate testing protocols to assess temperament in sheep.
- Refine testing protocols, minimising the number of farm visits required and time taken to conduct testing.
- Develop field based tests to replace laboratory assays, providing same day results during testing and removing the need to transport serum samples to the laboratory.
- Explore genetic markers for immune responsiveness traits
- Further develop a resilience index for sheep producers looking to improve the resilience of their flocks

In summary, the objectives of the current project were successfully achieved as evidenced by the new knowledge gained regarding associations between resilience traits and production traits and the potential benefits of selecting for immune competence to improve the health and welfare of sheep.

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1 Background

1.1 Project Background

*Taken from:-
Brad C. Hine, Bonnie A. Mallard, Aaron B. Ingham, Ian G. Colditz. (2014) Immune competence in livestock. In 'Breeding focus 2014 – Resilience'. (Eds. S. Hermesch and S. Dominik) pp. 49-64. (Animal Genetics and Breeding Unit, University of New England, Armidale, NSW, Australia) ISBN 978-1-921-597-65-7.

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 of our livestock species. Currently we are developing testing procedures to assess 'general immune competence' of beef cattle, dairy cattle and sheep on-farm. Immune competence traits will be combined with measures of temperament and ability to cope with management induced stress to estimate an animal's resilience. By exploring associations between resilience and important production traits we aim to develop breeding strategies which will identify animals highly suited to their production environment.

1.1.1 Introduction

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. 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. 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 livestock 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). Thus progeny of parents with high genetic potential for milk production have a higher incidence of mastitis than 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 livestock species. The animal production sector is becoming increasingly aware of this issue and is 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 antibiotics to control disease in livestock and the potential food contamination issues that arise from their misuse. However, it must also be acknowledged that selection for increased productivity remains a key profit driver for our livestock industries. Alternative strategies that address these consumer concerns while reducing the incidence of disease, and as a consequence, production losses and treatment costs associated with disease are therefore required. 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. In support of this concept, 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 livestock 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 tradeoff 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. T he 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).

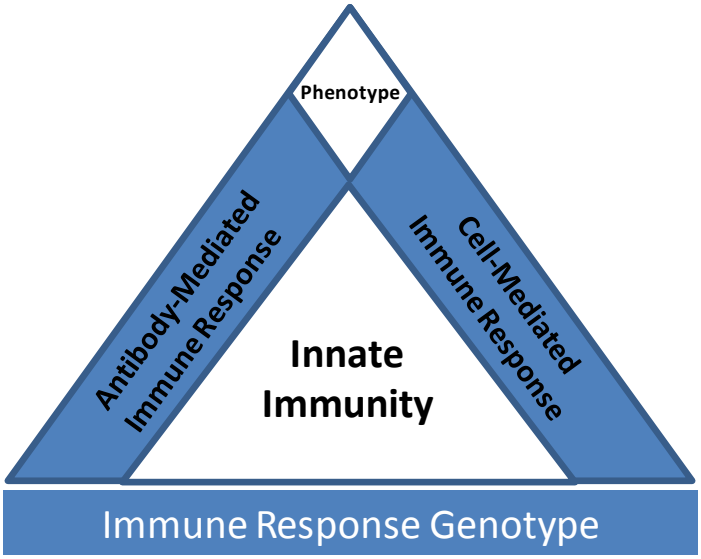

Figure 1. Genetic variation in the ability to resist disease is due to a large number of additive genetic effects which together regulate innate and adaptive immune responses (Source: adapted from Wilkie and Mallard 1999)

2 Project Objectives

2.1 Project Purpose (as outlined in original application)

Sheep respond to challenges created by infectious organisms and husbandry practices through immunological, physiological and behavioural defence reactions. The three modalities of host defence are highly integrated and their activation uses resources that would otherwise be directed towards production. Research over a number of years has highlighted that the level of activity of the immune system is associated with robustness of the animal in the face of environmental stressors and can be an indicator of future health and performance (Schmid-Hempel, 2003).

Immune responsiveness is moderately heritable in farm animals (Flori et al., 2011; Thompson-Crispi et al., 2012) and research in pigs (Magnusson et al., 1998; Wilkie and Mallard, 1999) and dairy cattle (Thompson-Crispi et al., 2012a; Wagter et al., 2000) has illustrated that selection for general immune responsiveness as a quantitative trait has resulted in enhanced productivity and decreased disease incidence.

The purpose of this project is to immunophenotype sheep in the Resource Flock and Follower Flock and examine associations with health and production traits recorded on these animals. The principle behind development of the assessment protocol is that animal's differ in the activity (strength) of their immune system and in the bias between the two arms of the adaptive immune response: cell mediated (Type 1) and antibody (Type 2) responses. The tests are designed to measure strength and balance of these components of the immune response. The tests use commercial vaccines to avoid issues with carcase contamination or use of

unregistered products. The panel measures a combination of innate and adaptive immune responses including haptoglobin, skin temperature, antibody, and delay type hypersensitivity in skin together with cortisol and worm egg count in faeces. Weaning creates a period of physiological and social stress in animals and immunphenotyping animals at this time provides an opportunity to assess immune responsiveness.

2.2 Project Objectives (as outlined in original submission)

1. Develop immunophenotyping tests to predict individuals at enhanced risk of disease or reduced productivity.
2. Develop immunophenotype indicator trait(s) suitable for implementation through genetics / genomics.
3. Develop initial estimates of genetic and phenotypic parameters for immune response traits in sheep.
4. Develop knowledge on stress responsiveness of lambs at weaning.

2.3 Project Plan (as outlined in original submission)

Immune phenotype measurements were made on progeny of the Resource Flock during weaning at Kirby in 2013 and 2014 and Katanning 2014 and 2015. Sheep to be phenotyped will be 2013 and 2014 lambs from terminal sires at Kirby and 2014 and 2015 lambs from terminal sires Katanning (about 600 lambs per site per year), and follower ewes at both sites while non-pregnant (about 600 ewes at each site).

The panel of tests used a commercial clostridial multivalent vaccine to test antibody and cell mediated immune responses, and commercial Gudair Johnes disease vaccine to measure cell mediated immune responses.

Antibody responses were measured via change in antibody titre following vaccination, and cell mediated immune responses will be measured by change in skin thickness on the medial aspect of the thigh to an intradermal antigen injection following prior vaccination with clostridial multivalent vaccine and Gudair Johnes disease vaccine.

The clostridial antigen methodology has been developed in MLA project B.STU.0244. Faecal samples will be collected on the day of weaning if monitor WECs indicate adequate burdens for phenotyping are present. Otherwise, the closest WEC taken as part of lamb phenotyping will be used.

Lambs were scored for FAMACHA eye colour and blood sample taken for haematocrit or haemoglobin determination. These latter, easy to measure traits provide additional indications of efficacy of immune responses in coping with internal parasitism.

The assessment protocol follows:

Day	Operation
Day 0	Wean Weight Temperament test (to be confirmed) Vaccinate with Ultravac 5 in 1 clostridial vaccine (Zoetis) Vaccinate with Gudair (Zoetis) Collect blood sample (measure antibody and haptoglobin) Collect faecal Sample
Day 3	Collect blood sample (to assess stress response indicators)
Day 9	Collect blood sample (to assess antibody production) Conduct skin test (tetanus toxoid and guidair)
Day 11	Collect blood sample (to assess antibody production) Measure response to skin test (skin thickness, skin temperature) Weigh

Method development is needed for the Gudair intradermal skin test. A trial will be conducted on around 40 sheep to establish the appropriate dose of antigen (diluted vaccine) for intradermal injection and to characterise nature of response.

While antagonism between cell mediated and antibody mediated arms of the immune response is a classical and well validated concept in immunology, a strength of the current assessment protocol is that both arms of the immune response are measured to the one antigen. A common confounding factor in assessment of bias between antibody mediated immune responses and cell mediated immune responses is the use of different antigens known to preferential stimulate antibody or cell mediated immune responses respectively to assess these two arms of the immune response. By using tetanus toxoid which stimulates both antibody and cell mediated immune responses to measure both arms together with Gudair to measure cell mediated immunity this will be able to distinguish between antigen specific and general (less specific) activity of the immune response.

Ewes will be immunophenotyped with the same panel of measures at a separate time of year.

3 Methodology

3.1 Study Animals

All animals enrolled in the study were either progeny from the Sheep genetics MLA resource flocks (tested as lambs) or the Sheep CRC follower ewe flock (tested as adults) run at both the Kirby Field Station (University of New England) located at Armidale, NSW and the Katanning Field Station (Department of Agriculture and Food, Western Australia) located at Katanning, WA. As part of the project a total of 2,613 lambs (mixed sex) and 945 adult sheep (ewes only) were immune competence phenotype tested. Details of animals enrolled in the study are presented in Table 1.

Table 1. Description of animals enrolled in the study

Location	Drop[#]	Sex[±]	Progeny of Program[†]	Immune Competence Tested
Kirby	2013	M	Resource flock	380
	2013	F	Resource flock	420
	2014	M	Resource flock	287
	2014	F	Resource flock	312
Katanning	2014	M	Resource flock	296
	2014	F	Resource flock	318
	2015	M	Resource flock	299
	2015	F	Resource flock	301
Kirby	2012	F	Follower flock	338
	2013	F	Follower flock	134
Katanning	2012	F	Follower flock	364
	2013	F	Follower flock	109
TOTAL				**3,558**

[#] Drop = Year born
[±] M=Male (castrated), F=Female
[†] Resource flock = Sheep genetics MLA resource flock, Follower flock = Sheep CRC follower ewe flock

3.2 Sheep Genetics MLA Resource Flock

The MLA Resource Flock began as the Sheep CRC's Information Nucleus Flock (INF) in 2007. Over five years, the INF gathered a database of biological and genetic traits by joining 5,000 ewes each year to 100 industry sires.

This has evolved into a reference flock for hard to measure traits such as intramuscular fat, meat quality, tenderness and fatty acid profile. Data is used for ongoing investigation of new traits. As the Resource Flock evolves there will be an increased focus on co-investment with industry groups and individual breeders, utilising data already collected.

Industry Outcomes:

- Breeding values for eating quality and meat yield developed
- Development of eating quality indexes
- Development of a single step for meat eating quality ASBVs
- Development of single step analysis for other traits is ongoing
- Improvement in accuracy of genomic testing
- Bio-bank of DNA - assessing the value of full genomic sequence analysis
- High-density and sequence data will provide clues to genes affecting traits
- Development of technology to measure IMF content
- Grading of yearling merinos
- Data included in MERINOSELECT and LAMBPLAN improving accuracies and providing linkage
- Valuable resource for other research projects

(source: http://www.sheepgenetics.org.au/Resources/MLA-Resource-Flock).

3.3 Sheep CRC Follower Ewe Flock

The Sheep CRC Follower Ewe Flock has evolved from the Sheep CRC Information Nucleus Flock (INF).

The INF is a progeny testing scheme where progeny of selected industry sires are measured for a large range of traits, most of them not commonly measured on commercial studs. The INF program has three main objectives. The first objective is to obtain estimates of (quantitative) genetic parameters. These are heritabilities of new traits and correlations (genetic and phenotypic) of these traits with existing production traits as well as estimates of genotype by environment interactions. The second objective is to undertake genome association analysis, resulting in the ability to predict breeding values based on genotypic information. The third objective is to enhance estimates of breeding values of animals in commercial studs. This refers to increasing the accuracy of Australian Sheep Breeding Values (ASBVs) due to additional information measured on relatives. This increase in ASBV accuracy depends on the additive genetic relationship between INF progeny and commercial breeding animals as well as the extent to which certain traits are measured in commercial flocks.

(source: Van der Werf, J.H.J., B. P. Kinghorn and R. G. Banks. 2010. Design and role of an information nucleus in sheep breeding programs. Animal Production Science, 2010, 50, 1–6).

3.4 On-farm Testing

The immune competence, stress responsiveness and the temperament of a total of 2613 lambs and 945 ewes were assessed on-farm using the testing protocol timetable described in Table 2. In attempt to assess immune competence whilst under the influence of management induced stress, immune competence testing of lambs was aligned with the weaning period and immune competence testing of ewes with handling and road transport.

Table 2. Timetable for testing procedures conducted on farm

Day	Operation
Day 0	Wean (lambs only) Road transport (ewes only) Liveweight recording FAMACHA Flight Speed Testing Vaccinate with clostridial vaccine (Zoetis) and Gudair (Zoetis) Collect blood sample Collect faecal Sample
Day 3 or 4 **(standardised within flock cohort)**	Collect blood sample Liveweight recording (where possible)
Day 14 **(dependant on vaccination history, standardised within flock cohort)**	Collect blood sample Conduct skin test (DTH) Liveweight recording (where possible)
Day 16 **(dependant on vaccination history, standardised within flock cohort)**	Collect blood sample Measure response to skin test (DTH) Liveweight recording

NOTES:

- Timing of blood sample collection (to assess antibody-mediated immune responses, AMIR) and Delayed Type Hypersensitivity (DTH) testing (to assess cell-mediated immune responses, CMIR) post vaccination were adjusted based on the history of clostridial vaccination of animals from each flock. All lambs tested had received a single clostridial vaccination at marking prior to testing while ewes had received a prime and boost vaccination as lambs and an annual booster each year as adults prior to testing.

- Clostridial vaccines, used to induce immune responses during immune competence testing, all contained tetanus toxoid antigen. Antibody responses to tetanus toxoid were used to assess AMIR. All Kirby sheep tested, (ewes and lambs) were vaccinated with ultravac 5in1 (Zoetis) on day 0 of testing. All Katanning ewes and 2015 drop lambs were vaccinated with

Glanvac6 (Zoetis) and all Katanning 2014 drop lambs were vaccinated with Glanvac3S (Zoetis) on day 0 of testing.

- To the best of our knowledge, the vaccination history of animals within each year drop at each site (Kirby or Katanning) was consistent at the time of testing. The following timetable of events was used for lambs having received only a primary clostridial vaccination.

- Day 0 of testing coincided with the commencement of weaning for all lambs and handling and road transport for all ewes.

- All lambs tested were weighed a minimum of twice (start and end of testing period) and maximum of four times during the weaning period (depending on facility availability).

3.5 Detailed Methodology for Testing Procedures Conducted On-Farm

Faecal sampling – Faecal samples were collected by taking a grab sample directly from the rectum of animals while in the race using standard operating procedures.

Blood sample collection – Blood samples were collected using jugular venipuncture. A total of 2*10ml serum tubes are collected at any single blood collection. Serum was collected 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 procedures.

Vaccinations – Animals received a clostridial and gudair vaccination (as described in Table 2 notes) on day 0 of testing. All vaccinations were administered subcutaneously high on the neck as per manufacturer's instructions.

DTH skin test – To elicit DTH responses, a test or control sample was injected intradermally in the wool free groin area of the sheep using an insulin syringe with 30G needle. Prior to injection, injection sites were identified and skin thickness measurements taken with calipers to provide a baseline skin thickness. 48Hrs post-injection, changes in skin thickness at the site of injection were assessed again using calipers. All animals received a total of 3 intradermal injections as part of the testing procedure including an injection of clostridial vaccine (as described in Table 2 notes, test reaction 1) and saline (control reaction) on one side of the groin and an injection of Gudair vaccine (Zoetis) on the other side of the groin. Where test sites and control sites were located on the same side of the groin, injections sites were well separated and the control site located above the test site to avoid interference between reactions. Injection volumes were 100µL for clostridial vaccines and saline and 50µL for Gudair for DTH testing of all sheep tested with the exception of the 2013 drop Kirby lambs which received 100µL of clostridial vaccine, saline and Gudair. Following initial testing of this cohort of lambs, the volume of Gudair injections was reduced from 100 µL to 50µL to optimise the magnitude of the DTH reactions observed.

Flight Speed Testing– Sheep were released from a weigh crate and their flight time recorded using electronic equipment as per standard operating procedures (Burrow et al. 1988). Flight speed testing procedures were standardised for all animals tested in each flock cohort.

3.6 Assessing Antibody-Mediated Immune Responsiveness (AMIR)

Production of antibody, more specifically anti-tetanus toxoid serum IgG1, in response to vaccination was used to assess AMIR. Animals were vaccinated with a commercially available clostridial vaccine (as described in Table 2 notes) on day 0 of testing and antibody production to a component of the multi-valent vaccines, tetanus toxoid, was assessed between day 14 and 16 of testing in lambs and between day 12 and 14 of testing in ewes (depending on vaccination history). However, responses for all animals within a flock cohort were always assessed on the same day post-vaccination. For each flock cohort, testing was undertaken to determine which of the two blood samples collected post-vaccination would be analysed for antibody level. The post-vaccination blood sample collected on the day which represented the maximal response observed in that herd cohort of animals was then analysed to determine antibody levels.

All lambs immune competence tested had received a single clostridial vaccination at marking prior to testing while ewes had received a prime and boost vaccination as lambs and an annual booster each year as adults prior to testing. As animals enrolled in the study had already received a clostridial vaccination prior to testing at yard weaning, serum collected on Day 0 of testing was not assessed for baseline antibody levels. The rationale for this decision was based on the following factors:-

1) Circulating antibody produced in response to previous vaccinations was still detectable in serum at the start of testing and therefore adjusting post-testing antibody level values (assessed on day 14 or 16 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 vaccinations.
2) To the best of our knowledge, the clostridial vaccination history of ewes and lambs in each flock cohort was identical and therefore the response assessed during testing at weaning represents cumulative response to the vaccination given at day 0 and any previous vaccinations administered to animals.
3) As lambs were generally between 5 and 6 months of age at weaning (depending on flock) and the half-life of maternal antibody in ruminants expected to be approximately 21 days (Fulton *et al.,* 2004), any influence of maternal antibody on responses to vaccination during testing were expected to be minimal.

To assess AMIR, total serum IgG1 antibody against tetanus toxoid antigen (kindly provided by Zoetis, Australia) was determined using an in-house developed indirect ELISA method based on the methodology described by Mallard et al. (1997) with modifications. 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 total IgG1 was bound using mouse anti-sheep IgG1 (Clone MCM1; Beh, 1987) and detected using goat anti-mouse IgG (whole molecule) conjugated to alkaline phosphatase (Product No. A3562; Sigma Aldrich).

3.7 Assessing Cell-Mediated Immune Responsiveness (CMIR)

DTH responses to clostridial and Gudair vaccine components were used to assess CMIRclo and CMIRgud, respectively. The skin testing methodology used in testing procedures is described in detail in section 3.5. The magnitude of DTH responses were calculated as the log of (double skin fold thickness (DSFT) at test reaction site / DSFT at control reaction site) at 48 hours post-injection (T48). For analysis, the log of (DSFT at test reaction site / DSFT at control reaction site) at T0 was fitted as a covariate in statistical models (see section 3.12).

3.8 AMIR, CMIR and Combined Immune Response (CIR) Groupings

To identify High, Average and Low immune phenotypes for AMIR, CMIRclo and CMIRgud, sheep were ranked 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 3.12 and were standardised, by dividing each residual value by the standard deviation of all residual values for that trait. Sheep with a standardised residual value which was > 1.0 were considered High responders, sheep with a standardised residual value < -1.0 were considered Low responders and sheep with a standardised residual value ≤ 1.0 and ≥ -1.0 were considered average for that trait (Table 3).

A combined immune response (CIR) trait was also calculated by combining (with equal weighting) measures of AMIR and CMIR. CIR provides an indicator of an animals overall immune responsiveness and is the trait animals would be selected on if aiming to improve the immune competence and general disease resistance of a flock. Standardisation of residual values was undertaken to ensure equal weighting was given to both AMIR and CMIR traits and the values summed together to generate CIRclo (AMIR + CMIRclo), CIRgud (AMIR + CMIRgud) and CIRclo+gud (AMIR + CMIRclo+gud) values to investigate phenotypic and genetic correlations between CIR and other traits.

When categorising animals as High, Average or Low for immune competence to investigate phenotypic group differences in measured traits, sheep with standardised residuals for both AMIR andCMIRclo which were > 0.5 were considered High for CIRclo, sheep with standardised residual values for both traits which were < -0.5 were considered Low for CIRclo and all other animals considered average for CIRclo. A similar method was used to identify High, Average and Low responder animals for CIRgud using standardised residual values for AMIR and CMIRgud and for CIRclo+gud using standardised residual values for AMIR and CMIRclo+gud where CMIRclo+gud represents the average of CMIRclo and CMIRgud values (Table 3).

Table 3. Criteria used to define immune competence groupings. Numbers in table refer to standardised residual values for traits listed. Residual values were standardised by dividing each residual value by the standard deviation of all residual values for that trait. Therefore values in the table represent standard deviations from the mean. For example, for an animal to be classified as a high responder for AMIR, their AMIR residual value would need to be >1.0 standard deviation above the mean of all residual values for that trait.

Immune Competence Grouping	Trait Assessed	Low	Average	High
AMIR	AMIR	<-1.0	≥-1.0 to ≤1.0	>1.0
CMIRclo	CMIRclo	<-1.0	≥-1.0 to ≤1.0	>1.0
CMIRgud	CMIRgud	<-1.0	≥-1.0 to ≤1.0	>1.0
CIRclo	AMIR & CMIRclo	<-0.5	≥-0.5 to ≤0.5	>0.5
CIRgud	AMIR & CMIRgud	<-0.5	≥-0.5 to ≤0.5	>0.5
CIRclo+gud	AMIR & CMIRclo+gud	<-0.5	≥-0.5 to ≤0.5	>0.5

3.9 Assessing Stress-Responsiveness

Haptoglobin is an acute phase protein whose levels in serum increase in response to stress allowing it to be used as a stress response indicator. In the current study, increases in serum haptoglobin levels in response to weaning (lambs) or handling and transport (ewes) were used to assess responsiveness to management-induced stress. Serum collected on day 0 of testing was analysed to provide a baseline haptoglobin concentration for each animal and changes in serum haptoglobin detected on day 3 or 4 post weaning or handling and transport (standardised within flock cohort) was used to assess stress responsiveness to management procedures. Serum haptoglobin was analysed using the method described by Jones and Mould, D.L. (1984) with minor modifications. All standard, control and test samples were run in triplicate. Where triplicate sample values had a CV>15%, samples were repeated. Test sample values were calculated from a standard curve produced using ovine serum with known haptoglobin concentration. Control samples were run on all assay plates to monitor assay performance. The assay is based on the reaction of Haptoglobin with excess haemoglobin, to form a complex that initiates a peroxidase reaction which releases oxygen from introduced hydrogen peroxide oxidising colourless guaiacol to brown coloured tetraguaiacol. As endogenous peroxidase can affect results, serum blanks for each test sample were run in the assay and values subtracted from test values. Haemolysis of blood samples, which occasionally occurs during collection, releases haemoglobin which can interfere with the haptoglobin assay. Therefore, a separate haemoglobin assay was performed on all samples tested and serum haptoglobin concentration values adjusted for haemoglobin content, as described previously (Slocombe and Colditz, 2012). Both unadjusted (HaptoUncorr) and adjusted (HaptoCorr) haptoglobin values were analysed.

Average daily weight gain during the weaning period (WtGain) was also recorded in lambs as an indirect measure of responsiveness to management-induced stress. All lambs tested were weighed a minimum of twice (start and end of testing period) and maximum of four times during the weaning period (depending on facility availability). Timing of weighing and number of times lambs were weighed was consistent within each flock cohort. Weight gain was calculated as the mean of average daily gain recorded between each weighing event.

3.10 Assessing Temperament

Flight time (FT) was measured to assess temperament during testing. Details of methodology used to collect flight speed data are presented in section 3.5.

3.11 Traits Measured

Traits measured on animals in the current study during testing included the immune competence traits, AMIR and CMIR, the stress response trait haptoglobin (HaptoUncorr and HaptoCorr), the temperament trait FT measured on all animals and the growth traits weaning weight (WW) and weight gain during weaning (WtGain) measured in lambs and mature weight (MW) measured on ewes. Where worm burdens were sufficiently high, (mean worm egg count (WEC) > 1000 eggs per gram on a subset of 10 animals in the mob), WEC were conducted on lambs at day 0 of testing. Where counts were not sufficiently high to collect informative WEC data at the time of testing, lambs were sampled at a later date. When collecting faecal samples for WEC from lambs, samples were also assigned a faecal consistency score (FCS). For ewes, historical WEC data was obtained (where available) from the SheepCRC for analysis. All ewes and lambs (with the exception of the 2013 drop Kirby follower ewes which were drenched just prior to testing) were given a FAMACHA score (FAM) on day 0 of testing. FAM assessment involves scoring the colour of the ocular mucous membranes of individual animals and provides a measure of clinical anaemia. As such, FAM provides an indirect indicator of Haemonchosis (van Wyck et al. 1997).

Traits measured by MLA on resource flock lambs which were used for analysis included:-

- Growth traits - birth weight (BW) and post-weaning weight (PW1, PW2).
- Body composition traits - eye muscle diameter (EMD) and fat cover (CFAT).
- Carcase traits – hot carcase weight (HCWT), carcase eye muscle area (CEMA), fat depth C (CCFAT), AUS-MEAT fat classification (CFATSCORE), fat depth GR (HGRFAT), intramuscular fat (IMF), fresh meat colour (CFL, Cfa, Cfb), post slaughter pH (PH24LL) and shear force (SHEARF5).

Traits measured by the SheepCRC on follower ewes which were used for analysis included:-

- Growth and body condition traits - birth weight (BW), weaning weight (WW), yearling weight (YW), mature weight (MW) and body condition score (BCS1, BCS2).
- Body composition traits - eye muscle diameter (EMD) and fat cover (CFAT).
- Wool traits - greasy fleece weight (GFW), clean fleece weight (CFW), yield (YLD), fibre diameter (FD), standard deviation of fibre diameter (SDFD), co-efficient of variation of fibre diameter (CVD), fibre curvature (CURV), staple length (SL) and staple strength (SS) measured at hogget age.
- Health and other traits - post weaning dag score (pDAG), yearling dag score (yDAG) and post weaning worm egg count (pWEC).

A detailed description of each trait measured is presented in Table 4.

Table 4. Trait descriptions

	Description
Immune Competence Traits	
AMIR	Antibody-mediated immune response. Assessed by measuring production of anti-tetanus toxoid serum IgG1 antibody in response to vaccination.
CMIRclo	Cell-mediated immune response. Assessed by measuring delayed type hypersensitivity (DTH) response to clostridial vaccine components.
CMIRgud	Cell-mediated immune response. Assessed by measuring delayed type hypersensitivity (DTH) response to Gudair vaccine components.
CMIRclo+gud	Cell-mediated immune response. Assessed by measuring delayed type hypersensitivity (DTH) response to clostridial and Gudair vaccine components.
CIRclo	Combined immune response calculated by combining (with equal weighting) measures of AMIR and CMIRclo. An indicator of an animals overall immune responsiveness.
CIRgud	Combined immune response calculated by combining (with equal weighting) measures of AMIR and CMIRgud. An indicator of an animals overall immune responsiveness.
CIRclo+gud	Combined immune response calculated by combining (with equal weighting) measures of AMIR and CMIRclo+gud. An indicator of an animals overall immune responsiveness.
Stress Responsiveness Traits	
HaptoUncorr	Change in serum haptoglobin concentration in response to weaning (lambs) or handling and transport (ewes). Haptoglobin is an acute phase protein produced in response to stress.
HaptoCorr	Change in serum haptoglobin concentration (adjusted for haemoglobin content) in response to weaning (lambs) or handling and transport (ewes). Haptoglobin is an acute phase protein produced in response to stress.
WtGain	Average daily weight gain during the weaning period (lambs only).
Temperament Traits	
FT	Flight time.
Growth and Body Condition Traits	
BW	Birth weight.
WW	Weaning weight.
PW	Post-weaning weight (lambs only)
YW	Yearling weight (ewes only).
MW	Mature weight (ewes only).
BCS	Body condition score (score 1 (light condition) -5 (fat condition), ewes only)
Body Composition Traits	
EMD	Eye muscle depth (from ultrasound scanning of live animals, ewes only).
CFAT	Fat depth (from ultrasound scanning of live animals, ewes only).
Wool Traits	
GFW	Greasy fleece weight (ewes only).
CFW	Clean fleece weight (ewes only).
YLD	Scoured yield (ewes only).
FD	Fibre diameter (ewes only).
SDFD	Standard deviation of fibre diameter (ewes only).
CVD	Co-efficient of variation of fibre diameter (ewes only).
CURV	Fibre curvature (ewes only).
SL	Staple length (ewes only).
SS	Staple strength (ewes only).

Carcase Traits	
HCWT	Hot carcase weight measured immediately following slaughter (lambs only).
CEMA	Eye muscle area measured between 12th and 13th ribs (lambs only).
CCFAT	Fat depth measured at loin C site on cold carcase (lambs only).
CFATSCORE	AUS-MEAT fat classification (scale 1 (leanest) – 5 (fattest), lambs only).
HGRFAT	Fat measurement GR measured on hot carcase (lambs only).
IMF	Intramuscular fat measured on frozen sample – caudal end of the *M. longissimus thoracis et lumborum* (LL) (lambs only)
CFL	Fresh colour measured by Minolta Chroma meter on sample 30-40 minutes after cutting three components L* value, a*value, b* value (lambs only).
Cfa	Fresh colour measured by Minolta Chroma meter on sample 30-40 minutes after cutting three components La* value, aa*value, ba* value (lambs only).
Cfb	Fresh colour measured by Minolta Chroma meter on sample 30-40 minutes after cutting three components Lb* value, ab*value, bb* value (lambs only).
PH24LL	pH measured on LL 24 hours post-slaughter (lambs only).
SHEARF5	Shear force measured on loin muscle – Day5 (lambs only).
Health and other Traits	
FAM	FAMACHA score (scale 1 (optimal) -5 (anaemic)).
pDAG	Dag score (score 1 (no dag) -5 (heavy dag), measured post-weaning, ewes only)
yDAG	Dag score (score 1 (no dag) -5 (heavy dag), measured at yearling age, ewes only)
wWEC	Worm egg count (measured at weaning, lambs only).
pWEC	Worm egg count (measured post-weaning, ewes only).
wFCS	Faecal consistency score (score 1 (firm pellets) – 5 (liquid manure), measured at weaning, lambs only).

3.12 Statistical Analysis

Univariate animal models were run in ASReml (Gilmour et al. 2009) to estimate variance components and heritabilities for immune competence, stress responsiveness, temperament and production traits. Data for each trait was tested for normality by assessing skewness and kurtosis and transformed where required to improve normality. Fixed effects assessed in all models included contemporary group (CG, incorporating property of origin, year drop, flock management group and sampling date), sex (lamb data only), dam age (maiden or adult, ewe data only), breed (merino or maternal for ewe data, merino, maternal, terminal 1 or terminal 2 for lamb data) and birth type (BT, single or multiple). The dams of all lambs tested in the study were adult ewes and therefore dam age was not fitted as a fixed effect in models when analysing lamb data. For breed assignment, maternal was assigned to animals which were merino x border leicester (or similar), terminal 1 to animals which were merino x white suffolk (or similar) and terminal 2 to animals which were maternal x white suffolk (or similar). Covariates assessed in models (where relevant for the individual trait) included age at measurement, WW, YW (ewe data only), MW (ewe data only), DSFT at test site / DSFT at control site at T0 (for CMIR traits, CMIRclo and CMIRgud) and baseline haptoglobin measured on day 0 of testing (for Hapto traits, HaptoUncorr and HaptoCorr). Details of fixed effects and covariates assessed in models when analysing each lamb trait and ewe trait are detailed in Tables 5 and 6, respectively. The main effect of CG along with relevant covariates were retained in models regardless of their *P* values. However, models were reduced by removing other fixed effects which were non-significant ($P > 0.05$) terms.

Least square means were generated from the linear model for each of the production traits, fitting relevant fixed effects, and the significance of differences between immune competence grouping (low, average, high) based on CIRclo, CIRgud and CIRclo+gud analysed. The predict function was used in ASReml to test group differences. Contrasts were evaluated by Bonferroni *t* statistics for multiple comparisons.

Table 5. Description of transformations applied to traits for analysis and fixed effects / covariates assessed in models when analysing individual **lamb** traits. Fixed effects and covariates shown in bold were retained in models when analysing respective trait and those not in bold were tested but removed from final model as they were non-significant (p > 0.05).

Trait	Transformation	Fixed effects[#]	Covariates
CMIRclo	Log	**CG, breed, BT,** sex	**(DSFT at test site / DSFT at control site) at T0, age at measurement**
CMIRgud	Log	**CG, breed,** BT, sex	**(DSFT at test site / DSFT at control site) at T0,** age at measurement
AMIR	None	**CG, breed**, BT, sex	age at measurement
HaptoUncorr	Log	**CG, BT,** breed, sex	**baseline hapto measured on day 0 of testing,** age at measurement
HaptoCorr	Log	**CG, BT,** breed sex	**baseline hapto measured on day 0 of testing,** age at measurement
WtGain	None	**CG, BT,** breed, sex	**WW,** age at measurement
FT	Log	**CG, BT, sex,** breed	age at measurement
BW	None	**CG (of dam), BT,** breed, sex	age at measurement
WW	None	**CG, BT, sex,** breed	age at measurement
PW1	None	**CG, BT, breed, sex**	**age at measurement**
PW2	None	**CG, BT, breed, sex**	**age at measurement**
EMD	None	**CG, BT,** breed, sex	age at measurement
CFAT	None	**CG, BT, sex,** breed	**age at measurement**
HCWT	None	**CG, BT, breed, sex**	**age at measurement**
CEMA	None	**CG, BT, breed, sex**	age at measurement
CCFAT	None	**CG, breed, sex,** BT	age at measurement
CFATSCORE	None	**CG, breed, BT,** sex	age at measurement
HGRFAT	None	**CG, breed, BT, sex**	age at measurement
IMF	None	**CG,** breed, BT, sex	age at measurement
CFL	None	**CG, sex,** breed, BT	age at measurement
Cfa	None	**CG,** breed, BT, sex	age at measurement
Cfb	None	**CG, BT,** breed, sex	age at measurement
PH24LL	Log	**CG,** breed, BT, sex	age at measurement
SHEARF5	Log	**CG, BT, sex,** breed	age at measurement
FAM	None	**CG, BT,** breed, sex	age at measurement
wWEC	Log	**CG, BT, sex,** breed	age at measurement
wFCS	None	**CG,** breed, BT, sex	age at measurement

[#]CG=contemporary group incorporating property of origin, year drop, flock management group and sampling date effects.

[±]Non estimable due to either insufficient between animal variation in trait values or insufficient data available for some contemporary groups.

Table 6. Description of transformations applied to traits for analysis and fixed effects / covariates assessed in models when analysing individual **ewe** traits. Fixed effects and covariates shown in bold were retained in models when analysing respective trait and those not in bold were tested but removed from final model as they were non-significant ($p > 0.05$).

Trait	Transformation	Fixed effects[#]	Covariates
CMIRclo	Log	**CG,** breed, BT, dam age	**(DSFT at test site / DSFT at control site) at T0,** MW
CMIRgud	Log	**CG, breed**, BT, dam age	**(DSFT at test site / DSFT at control site) at T0, MW**
AMIR	None	**CG, breed**, BT, dam age	MW
HaptoUncorr	Log	**CG, dam age,** breed, BT	**baseline hapto measured on day 0 of testing, MW**
HaptoCorr	Log	**CG, dam age,** breed, BT	**baseline hapto measured on day 0 of testing, MW**
FT	Log	**CG,** breed, BT, dam age	MW
EMD	None	**CG, breed,** BT, dam age	**age at measurement, YW**
CFAT	None	**CG,** breed, BT, dam age	**age at measurement, YW**
BW	None	**BT, dam age,** breed	
WW	None	**CG, BT, dam age, breed**	**age at measurement**
YW	None	**CG, BT, dam age,** breed	**age at measurement**
BCS1	None	**CG, dam age**, breed, BT	
BCS2	None	**CG,** breed, BT, dam age	
GFW	None	**CG, BT, dam age,** breed	**YW,** age at measurement
CFW	None	**CG, BT, dam age,** breed	**YW,** age at measurement
YLD	None	**CG,** breed, BT, dam age	YW, age at measurement
FD	None	**BT, dam age,** CG, breed,	**YW,** age at measurement
SDFD	None	**CG, breed, BT,** dam age	**YW,** age at measurement
CVD	None	**CG, breed,** BT, dam age	**YW,** age at measurement
CURV	None	**CG, breed,** BT, dam age	YW, age at measurement
SL	None	**CG, BT,** breed, dam age	**YW,** age at measurement
SS	None	**CG, BT,** breed, dam age	YW, age at measurement
FAM	None	Non estimable[±]	Non estimable[±]
pDAG	None	**CG,** breed, BT, dam age	
yDAG	None	**CG, BT, dam age,** breed	
pWEC	Log	**CG,** breed, BT, dam age	**WW,** age at measurement

[#]CG=contemporary group incorporating property of origin, year drop, flock management group and sampling date effects.

[±]Non estimable due to either insufficient between animal variation in trait values or insufficient data available for some contemporary groups.

4 Results

4.1 Trait Parameters

For traits measured in ewes, a description of summary statistics are presented in Tables 7 and 8, respectively.

Table 7. Description of summary statistics for traits measured in **lambs**.

Trait	Units	n[#]	Mean	Min	Max	StdDev
Immune Competence Traits						
CMIRclo (T0)	log (increase in skin fold thickness (mm))	2572	0.06	-0.34	0.44	0.11
CMIRclo (T48)	log (increase in skin fold thickness (mm))	2571	1.29	-0.14	2.59	0.34
CMIRgud (T0)	log (increase in skin fold thickness (mm))	2572	0.09	-0.61	0.49	0.12
CMIRgud (T48)	log (increase in skin fold thickness (mm))	2571	2.17	0.00	3.34	0.41
AMIR	optical density units	2569	1.26	0.00	2.92	0.72
Stress Responsiveness Traits						
HaptoUncorr (baseline)	mg/mL serum	2580	0.10	0.00	14.54	0.68
HaptoUncorr (post weaning)	mg/mL serum	2580	1.29	0.00	12.68	1.47
HaptoCorr (baseline)	mg/mL serum	2580	-0.02	-0.86	14.29	0.65
HaptoCorr (post weaning)	mg/mL serum	2580	1.01	-1.84	12.32	1.42
WtGain (during weaning)	kg	2458	0.08	-1.48	1.21	0.21
Temperament Traits						
FT	time (seconds)	1743	1.05	0.32	7.01	0.59
Growth Traits						
BW	kg	2503	4.94	1.40	9.50	1.21
WW	kg	2479	27.37	8.80	54.20	7.31
PW1	kg	2504	30.22	9.00	59.20	8.56
PW2	kg	2425	35.33	13.60	67.20	8.53
Body Composition Traits						
EMD	mm	1622	26.27	14	39	3.96
CFAT	mm	1623	3.02	1.0	7.7	1.15
Carcase Traits						
HCWT	kg	2147	22.93	12.40	39.30	3.85
CEMA	cm^2	1974	14.83	5.76	26.00	2.64
CCFAT	mm	1976	4.42	0.3	17.0	2.38
CFATSCORE	score 1-5	1704	2.90	1	5	0.97
HGRFAT	mm	2176	13.37	1	44	5.94
IMF	%	1623	5.08	2.21	11.74	1.27
CFL		1863	36.07	28.62	45.19	2.57
Cfa		1864	19.80	12.79	25.63	1.73
Cfb		1862	2.86	-5.39	12.20	4.75
PH24LL	pH	2174	5.69	5.30	6.99	0.22
SHEARF5	newtons	1630	33.73	14.92	99.73	12.42
Health and Other Traits						
FAM	score 1-5	2576	1.87	1	4	0.66
wWEC	eggs / gram of faeces	2516	1289	0	22240	1597
wFCS	score 1-5	2518	2.07	1	5	1.00

[#] n=Number of observations recorded

Table 8. Description of summary statistics for traits measured in **ewes**.

Trait	Units	n[#]	Mean	Min	Max	StdDev
Immune Competence Traits						
CMIRclo (T0)	log (increase in skin fold thickness (mm))	943	0.08	-0.34	0.47	0.10
CMIRclo (T48)	log (increase in skin fold thickness (mm))	943	1.62	0.99	2.70	0.29
CMIRgud (T0)	log (increase in skin fold thickness (mm))	943	0.10	-0.41	0.46	0.11
CMIRgud (T48)	log (increase in skin fold thickness (mm))	943	2.32	1.58	3.20	0.30
AMIR	optical density units	943	1.18	0.00	2.75	0.52
Stress Responsiveness Traits						
HaptoUncorr (baseline)	mg/mL serum	943	0.12	0.00	9.59	0.58
HaptoUncorr (post weaning)	mg/mL serum	943	1.58	0.00	9.24	1.59
HaptoCorr (baseline)	mg/mL serum	943	-0.01	-0.40	9.37	0.53
HaptoCorr (post weaning)	mg/mL serum	943	1.29	-0.69	8.98	1.53
Temperament Traits						
FT	time (seconds)	918	0.91	0.38	8.17	0.49
Growth and Body Condition Traits						
BW	kg	926	4.51	1.80	7.80	0.89
WW	kg	928	25.39	9.60	50.60	5.48
YW	kg	939	41.39	23.00	69.00	8.86
MW	kg	942	57.59	34.00	96.40	8.74
BCS1	score 1-5	937	3.24	2	4.5	0.48
BCS2	score 1-5	918	2.90	2	5	0.42
Body Composition Traits						
EMD	mm	455	22.42	13	34	3.77
CFAT	mm	455	2.02	1	4	0.50
Wool Traits						
GFW	kg	818	3.48	1.20	6.40	0.97
CFW	kg	818	2.58	0.90	5.00	0.70
YLD	%	829	74.23	56.60	89.50	4.56
FD	µM	829	18.88	13.00	38.20	3.97
SDFD	µM	829	3.50	1.90	9.00	1.27
CVD	%	829	18.20	11.90	31.50	3.24
CURV	degrees / mm	829	64.47	32	115	11.65
SL	mm	829	103.03	49	127	13.69
SS	newtons / kilotex	829	38.53	6	69	12.61
Health and Other Traits						
FAM	score 1-5	809	1.74	1	3	0.66
pDAG	score 1-5	692	1.55	1	5	1.55
yDAG	score 1-5	793	1.82	1	5	0.80
pWEC	eggs / gram of faeces	535	1082	40	19800	1622

[#] n=Number of observations recorded

4.2 Genetic parameters for immune competence traits

Genetic parameters for the immune competence traits AMIR, CMIRclo, CMIRgud and CMIRclo+gud and genetic and phenotypic correlations between traits estimated for lambs and ewes are presented in Tables 9 and 10, respectively. The heritability of a trait describes the proportion of observed variance of a trait that is attributable to genetics. A correlation describes the relationship between two traits. A phenotypic correlation describes the combined influence of the genetic and environmental components, whereas genetic correlations only describe the inherent genetic component.

Table 9. Genetic parameters for immune competence traits measured in **lambs**. Heritabilities are shown in bold, phenotypic correlations above the diagonal and genetic correlations below the diagonal.

	AMIR	CMIRclo	CMIRgud
AMIR	**0.25 ± 0.05**	0.14 ± 0.02	0.08 ± 0.02
CMIRclo	-0.08 ± 0.17	**0.20 ± 0.05**	0.18 ± 0.02
CMIRgud	0.09 ± 0.16	0.17 ± 0.17	**0.22 ± 0.05**

Table 10. Genetic parameters for immune competence traits measured in **ewes**. Heritabilities are shown in bold, phenotypic correlations above the diagonal and genetic correlations below the diagonal.

	AMIR	CMIRclo	CMIRgud
AMIR	**0.47 ± 0.12**	0.16 ± 0.03	0.04 ± 0.03
CMIRclo	0.29 ± 0.19	**0.42 ± 0.12**	0.43 ± 0.03
CMIRgud	0.57 ± 0.31	0.74 ± 0.21	**0.14 ± 0.09**

4.3 Genetic parameters for stress responsiveness, production, health and other traits and correlations with immune competence traits

Genetic parameters for stress responsiveness, production, health and other traits and phenotypic and genetic correlations between these traits and immune competence traits in lambs and ewes are presented in Tables 11 and 12, respectively.

Table 11. Genetic parameters for production traits and phenotypic and genetic correlations between production traits and immune competence/stress responsiveness traits measured in **lambs**. Values describing phenotypic variance (Vp), heritability (h2), phenotypic and genetic correlations (rp and rg, respectively) are presented with standard errors for each estimate shown in brackets.

			AMIR		CMIRclo		CMIRgud		CIRclo		CIRgud		CIRclo+gud	
	V_p	h^2	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
Stress Responsiveness Traits														
HaptoUncorr*	0.05	0.31 (0.06)	0.31 (0.02)	0.37 (0.13)	0.13 (0.02)	0.29 (0.15)	0.12 (0.02)	-0.06 (0.15)	0.29 (0.02)	0.51 (0.14)	0.30 (0.02)	0.21 (0.14)	0.32 (0.02)	0.38 (0.14)
HaptoCorr*	0.05	0.28 (0.06)	0.30 (0.02)	0.36 (0.14)	0.12 (0.02)	0.28 (0.16)	0.11 (0.02)	-0.10 (0.16)	0.28 (0.02)	0.49 (0.15)	0.28 (0.02)	0.17 (0.15)	0.30 (0.02)	0.34 (0.15)
WtGain	0.03	0.21 (0.05)	-0.05 (0.02)	0.27 (0.17)	0.01 (0.02)	0.05 (0.18)	-0.05 (0.02)	-0.07 (0.18)	-0.03 (0.02)	0.24 (0.19)	-0.07 (0.02)	0.14 (0.17)	-0.05 (0.02)	0.18 (0.18)
Temperament Traits														
FT*	0.01	0.23 (0.07)	0.03 (0.02)	-0.19 (0.19)	0.01 (0.03)	-0.46 (0.26)	-0.01 (0.03)	0.18 (0.24)	0.03 (0.03)	-0.45 (0.27)	0.02 (0.02)	0.01 (0.23)	0.02 (0.03)	-0.22 (0.25)
Growth Traits														
BW	1.25	0.97 (0.06)	0.03 (0.02)	0.13 (0.11)	0.01 (0.02)	0.09 (0.12)	0.01 (0.02)	0.18 (0.11)	0.02 (0.02)	0.15 (0.12)	0.03 (0.02)	0.18 (0.11)	0.03 (0.02)	0.19 (0.12)
WW	19.07	0.25 (0.05)	0.01 (0.02)	-0.09 (0.12)	0.03 (0.02)	0.09 (0.13)	0.01 (0.02)	0.07 (0.12)	0.03 (0.02)	-0.01 (0.13)	0.01 (0.02)	-0.01 (0.12)	0.03 (0.02)	0.00 (0.13)
PW1	26.33	0.51 (0.07)	-0.01 (0.02)	-0.18 (0.13)	0.03 (0.02)	0.10 (0.14)	-0.01 (0.02)	0.15 (0.13)	0.01 (0.02)	-0.07 (0.15)	-0.01 (0.02)	-0.02 (0.13)	0.00 (0.02)	-0.02 (0.14)
PW2	31.23	0.49 (0.06)	-0.04 (0.02)	-0.08 (0.13)	0.02 (0.02)	0.05 (0.14)	-0.02 (0.02)	0.05 (0.13)	-0.01 (0.02)	-0.03 (0.15)	-0.04 (0.02)	-0.01 (0.13)	-0.02 (0.02)	-0.01 (0.14)
Body Composition Traits														
EMD	8.23	0.35 (0.08)	0.01 (0.03)	0.12 (0.17)	0.01 (0.02)	0.16 (0.18)	-0.01 (0.03)	0.27 (0.18)	0.01 (0.02)	0.20 (0.19)	0.00 (0.03)	0.26 (0.18)	0.00 (0.03)	0.27 (0.19)
CFAT	0.67	0.21 (0.07)	0.02 (0.03)	0.17 (0.21)	-0.04 (0.02)	-0.03 (0.23)	-0.05 (0.03)	0.35 (0.24)	-0.01 (0.02)	0.16 (0.25)	-0.02 (0.03)	0.40 (0.23)	-0.02 (0.02)	0.32 (0.24)
Carcase Traits														
HCWT	5.76	0.11 (0.06)	0.00 (0.02)	-0.16 (0.25)	-0.02 (0.02)	0.17 (0.27)	-0.01 (0.02)	-0.05 (0.26)	-0.01 (0.02)	0.01 (0.28)	-0.01 (0.02)	-0.14 (0.25)	-0.01 (0.02)	-0.06 (0.27)

CEMA	4.83	0.37 (0.07)	0.01 (0.02)	0.10 (0.15)	0.00 (0.02)	0.07 (01.6)	0.01 (0.02)	-0.10 (0.16)	0.01 (0.02)	0.13 (0.17)	0.02 (0.02)	0.00 (0.15)	0.01 (0.02)	0.05 (0.16)
CCFAT	3.58	0.12 (0.06)	0.02 (0.02)	-0.17 (0.23)	0.00 (0.02)	-0.25 (0.25)	-0.04 (0.02)	0.30 (0.26)	0.02 (0.02)	-0.29 (0.26)	-0.01 (0.02)	0.07 (0.24)	0.00 (0.02)	-0.09 (0.25)
CFATSCORE	0.20	0.12 (0.07)	-0.01 (0.02)	-0.19 (0.26)	-0.03 (0.02)	0.12 (0.28)	0.00 (0.02)	-0.20 (0.28)	-0.03 (0.03)	-0.05 (0.29)	0.00 (0.02)	-0.25 (0.27)	-0.02 (0.02)	-0.16 (0.28)
HGRFAT	14.90	0.09 (0.05)	0.01 (0.02)	0.17 (0.26)	0.02 (0.02)	0.05 (0.27)	-0.02 (0.02)	0.18 (0.27)	0.02 (0.02)	0.17 (0.29)	-0.01 (0.02)	0.23 (0.27)	0.00 (0.02)	0.23 (0.28)
IMF	1.15	0.46 (0.08)	0.00 (0.03)	0.18 (0.16)	0.02 (0.03)	0.15 (0.17)	0.00 (0.03)	0.12 (0.16)	0.02 (0.03)	0.25 (0.18)	0.00 (0.03)	0.21 (0.16)	0.01 (0.03)	0.26 (0.17)
CFL	3.80	0.20 (0.07)	-0.01 (0.02)	-0.09 (0.20)	-0.03 (0.02)	-0.19 (0.21)	0.02 (0.02)	-0.07 (0.20)	-0.02 (0.02)	-0.21 (0.22)	0.01 (0.02)	-0.10 (0.20)	-0.01 (0.02)	-0.18 (0.21)
Cfa	1.24	0.19 (0.07)	-0.02 (0.02)	0.04 (0.21)	0.00 (0.02)	0.02 (0.22)	0.02 (0.02)	0.14 (0.21)	-0.01 (0.02)	0.06 (0.23)	0.00 (0.02)	0.12 (0.21)	0.00 (0.02)	0.11 (0.22)
Cfb	1.66	0.13 (0.06)	-0.02 (0.02)	0.01 (0.24)	-0.03 (0.02)	0.20 (0.25)	0.01 (0.02)	0.21 (0.25)	-0.03 (0.02)	0.16 (0.27)	-0.01 (0.02)	0.14 (0.25)	-0.02 (0.02)	0.20 (0.26)
PH24LL*	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^
SHEARF5*	0.01	0.19 (0.07)	0.01 (0.03)	-0.11 (022)	-0.02 (0.02)	-0.38 (0.26)	0.02 (0.02)	-0.48 (0.26)	-0.01 (0.02)	-0.40 (0.29)	0.02 (0.03)	-0.42 (0.26)	0.01 (0.03)	-0.50 (0.28)
Other Traits														
FAM	0.31	0.16 (0.05)	-0.05 (0.02)	-0.17 (0.19)	-0.01 (0.02)	-0.46 (0.20)	0.01 (0.02)	-0.28 (0.19)	-0.04 (0.02)	-0.45 (0.21)	-0.03 (0.02)	-0.31 (0.18)	-0.04 (0.02)	-0.44 (0.19)
wWEC*	0.30	0.37 (0.06)	-0.03 (0.02)	-0.32 (0.14)	0.03 (0.02)	-0.16 (0.16)	-0.05 (0.02)	-0.28 (0.15)	0.00 (0.02)	-0.37 (0.17)	-0.06 (0.02)	-0.41 (0.14)	-0.03 (0.02)	-0.44 (0.16)
FCS	0.64	0.20 (0.05)	-0.02 (0.02)	-0.14 (0.17)	0.01 (0.02)	-0.08 (0.18)	-0.02 (0.02)	-0.02 (0.18)	-0.01 (0.02)	-0.15 (0.19)	-0.03 (0.02)	-0.11 (0.17)	-0.02 (0.02)	-0.14 (0.18)

*log transformed

^residual covariance constrained to zero.

#correlation values suggesting traits are considered weakly correlated are highlighted in green, moderately correlated are highlighted in blue and strongly correlated highlighted in red. When interpreting results the size of the error associated with the estimate relative to the estimate itself should be considered.

Table 12. Genetic parameters for production traits and phenotypic and genetic correlations between production traits and immune competence/stress responsiveness traits measured in **ewes**. Values describing phenotypic variance (Vp), heritability (h2), phenotypic and genetic correlations (rp and rg, respectively) are presented with standard errors for each estimate shown in brackets.

			AMIR		CMIRclo		CMIRgud		ClRclo		ClRgud		ClRclo+gud	
	V_p	h^2	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
Stress Responsiveness Traits														
HaptoUncorr*	0.04	0.29 (0.12)	0.16 (0.03)	0.21 (0.23)	0.09 (0.03)	0.04 (0.25)	0.06 0.03)	0.79 (0.34)	0.17 (0.03)	0.17 (0.23)	0.15 (0.03)	0.50 (0.22)	0.17 (0.03)	0.35 (0.21)
HaptoCorr*	0.05	0.36 (0.12)	0.14 (0.03)	0.15 (0.22)	0.10 (0.03)	0.04 (0.23)	0.07 (0.03)	0.76 (0.30)	0.16 (0.03)	0.17 (0.23)	0.15 (0.03)	0.49 (0.21)	0.17 (0.03)	0.35 (0.21)
Temperament Traits														
FT*	0.01	0.13 (0.11)	-0.01 (0.03)	-0.58 (0.22)	-0.10 (0.03)	-0.36 (0.35)	n/a^	n/a^	-0.08 (0.03)	-0.62 (0.33)	-0.04 (0.03)	-0.83 (0.40)	-0.07 (0.03)	-0.75 (0.35)
Growth and Body Condition Traits														
BW	0.62	0.25 (0.11)	0.02 (0.03)	0.21 (0.25)	0.03 (0.04)	0.05 (0.26)	0.03 (0.04)	-0.31 (0.38)	0.04 (0.04)	0.16 (0.25)	0.04 (0.04)	-0.02 (0.26)	0.04 (0.04)	0.05 (0.25)
WW	14.8	0.03 (0.09)	-0.06 (0.04)	0.32 (0.18)	0.06 (0.12)	-0.15 (0.21)	0.03 (0.04)	-0.29 (0.84)	-0.01 (0.04)	-0.43 (0.64)	-0.02 (0.04)	-0.04 (0.60)	-0.01 (0.04)	-0.32 (0.58)
YW	23.3	0.55 (0.13)	-0.02 (0.04)	0.02 (0.19)	0.01 (0.12)	0.02 (0.18)	0.07 90.04)	0.19 (0.30)	-0.00 (0.04)	0.06 (0.19)	0.04 (0.04)	0.05 (0.20)	0.02 (0.04)	0.06 (0.19)
BCS1	0.08	0.37 (0.12)	0.05 (0.04)	0.20 (0.21)	-0.05 (0.04)	0.05 (0.23)	0.02 (0.03)	0.47 (0.34)	-0.01 (0.04)	0.18 (0.21)	0.04 90.04)	0.33 (0.21)	0.02 (0.04)	0.27 (0.02)
BCS2	0.13	0.32 (0.11)	0.04 (0.04)	0.18 (0.22)	0.00 (0.04)	0.45 (0.23)	0.03 (0.03)	0.41 (0.40)	0.03 (0.04)	0.40 (0.21)	0.04 (0.04)	0.20 (0.24)	0.04 (0.04)	0.32 (0.22)
Body Composition Traits														
EMD	6.0	0.41 (0.19)	0.03 (0.05)	0.11 (0.26)	n/a^	n/a^	0.05 (0.05)	0.24 (0.43)	-0.01 (0.05)	-0.00 (0.27)	0.05 (0.05)	0.21 (0.28)	0.02 (0.05)	0.09 (0.27)
CFAT	0.13	0.28 (0.18)	0.04 (0.05)	-0.14 (0.32)	n/a^	n/a^	-0.03 (0.05)	0.33 (0.48)	-0.01 (0.05)	0.01 (0.30)	0.02 (0.05)	0.09 (0.32)	-0.00 (0.05)	0.08 (0.30)
Wool Traits														
GFW	0.24	0.69 (0.14)	-0.02 (0.04)	0.09 (0.18)	0.06 (0.04)	-0.04 (0.19)	-0.03 (0.04)	0.10 (0.30)	0.02 (0.04)	0.01 (0.18)	-0.04 (0.04)	0.07 (0.19)	-0.01 (0.04)	0.02 (0.18)
CFW	0.16	0.65 (0.14)	-0.01 (0.04)	0.03 (0.19)	0.01 (0.04)	-0.24 (0.19)	-0.05 (0.04)	-0.19 (0.29)	-0.01 (0.04)	-0.15 (0.18)	-0.04 (0.04)	-0.07 (0.19)	-0.03 (0.04)	-0.14 (0.18)
YLD	17.9	0.52 (0.13)	0.02 (0.04)	-0.16 (0.20)	-0.12 (0.04)	-0.57 (0.18)	-0.06 (0.04)	-0.77 (0.32)	-0.07 (0.04)	-0.46 (0.18)	n/a^	n/a^	-0.06 (0.04)	-0.48 (0.18)

FD	3.0	0.56 (0.13)	0.06 (0.04)	0.28 (0.19)	0.04 (0.04)	0.20 (0.19)	0.05 (0.04)	0.65 (0.29)	0.05 (0.04)	0.31 (0.18)	0.07 (0.04)	0.48 (0.18)	0.07 (0.04)	0.40 (0.18)
SDFD	0.42	0.33 (0.12)	0.04 (0.04)	0.20 (0.23)	0.03 (0.04)	0.35 (0.23)	-0.02 (0.04)	0.49 (0.35)	0.05 (0.04)	0.35 (0.22)	0.02 (0.04)	0.37 (0.23)	0.03 (0.04)	0.39 (.22)
CVD	5.9	0.46 (0.14)	0.03 (0.04)	0.01 (0.21)	0.04 (0.04)	0.30 (0.21)	-0.05 (0.04)	0.13 (0.36)	0.04 (0.04)	0.20 (0.20)	-0.01 (0.04)	0.08 (0.22)	0.02 (0.04)	0.16 (0.21)
CURV	113.4	0.62 (0.14)	0.01 (0.04)	0.01 (0.19)	0.06 (0.04)	0.13 (0.20)	0.02 (0.04)	0.05 (0.31)	0.05 (0.04)	0.09 (0.18)	0.02 (0.04)	0.01 (0.20)	0.04 (0.04)	0.06 (0.18)
SL	130.3	0.67 (0.15)	-0.04 (0.04)	-0.03 (0.19)	-0.01 (0.04)	-0.15 (0.19)	0.04 (0.04)	0.43 (0.30)	-0.04 (0.04)	-0.13 (0.18)	-0.02 (0.04)	0.10 (0.20)	-0.02 (0.04)	-0.03 (0.18)
SS	83.8	0.32 (0.13)	0.02 (0.04)	0.20 (0.24)	-0.02 (0.04)	0.12 (0.25)	-0.04 (0.04)	0.14 (0.38)	0.00(0.04)	0.21 (0.24)	-0.01 (0.04)	0.21 (0.25)	-0.01 (0.04)	0.22 (0.24)
Other Traits														
FAM	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^	n/a^
pDAG	0.74	0.12 (0.11)	-0.05 (0.04)	0.12 (0.25)	-0.00 (0.04)	-0.29 (0.37)	-0.02 (0.04)	0.68 (0.72)	-0.04 (0.04)	-0.55 (0.36)	-0.05 (0.04)	-0.23 (0.38)	-0.05 (0.04)	-0.40 (0.36)
yDAG	0.54	0.15 (0.10)	-0.02 (0.04)	0.40 (0.33)	0.01 (0.04)	-0.63 (0.38)	-0.02 (0.04)	0.18 (0.48)	0.00 (0.04)	-0.08 (0.33)	0.00 90.04)	0.08 (0.33)	-0.01 (0.04)	0.09 (0.32)
pWEC*	0.11	0.27 (0.16)	0.01 (0.04)	-0.10 (0.30)	0.02 (0.04)	-0.07 (0.32)	0.02 (0.05)	0.13 (0.47)	0.01 (0.04)	-0.11 (0.34)	0.01 (0.04)	-0.03 (0.35)	0.01 (0.04)	-0.05 (0.33)

*log transformed

^ non estimable due to either insufficient between animal variation in trait values or insufficient data available for some contemporary groups..

#correlation values suggesting traits are considered weakly correlated are highlighted in green, moderately correlated are highlighted in blue and strongly correlated highlighted in red. When interpreting results the size of the error associated with the estimate relative to the estimate itself should be considered.

4.4 Influence of immune competence phenotype on stress responsiveness, production, health and other traits

Ewes and lambs were categorised as low (Lo), average (Avg) or high (Hi) responders for CIRclo, CIRgud and CIRclo+gud as described in section 3.8. Numbers of lambs and ewes in each immune competence phenotype grouping are shown in Tables 13 and 14, respectively. CIR provides an indicator of an animals overall immune responsiveness as it is generated from the combination of antibody and cellular response values, and is the trait animals would be selected on if aiming to improve the immune competence and general disease resistance of a herd. Therefore, most emphasis should be placed on the influence of CIR, rather than AMIR or CMIR when interpreting results. We hypothesise that when selecting animals based on immune competence that maximum benefit will be achieved by eliminating low responder CIR phenotype animals rather than selecting high responder CIR phenotype animals.

Table 13. Numbers of **lambs** classified as high (Hi), average (Avg) or low (Lo) responders for CIRclo, CIRgud and CIRclo+gud for analysis.

	Hi	Avg	Lo	Total
CIRclo	296	2002	275	**2573**
CIRgud	311	2018	244	**2573**
CIRclo+gud	310	1972	291	**2573**

Table 14. Numbers of **ewes** classified as high (Hi), average (Avg) or low (Lo) responders for CIRclo, CIRgud and CIRclo+gud for analysis.

	Hi	Avg	Lo	Total
CIRclo	94	747	102	**943**
CIRgud	94	753	96	**943**
CIRclo+gud	96	743	104	**943**

The influence of immune competence phenotype on stress responsiveness, production, health and other traits was assessed by comparing group least square means for each trait.

Stress responsiveness and temperament:

For lambs and ewes immune competence phenotype predicted group means for stress responsiveness traits are presented in Tables 15 and 16, respectively.

Table 15. Predicted group means for stress responsiveness and temperament traits in **lambs** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	logHaptoUncorr[#]	**logHaptoCorr**[#]	**logFT**[#]	**WtGain**[#]
CIRclo[±]	P < 0.001	P < 0.001	ns	ns
Hi	0.366 (0.012)[a]	0.304 (0.013)[a]	0.307 (0.007)	0.070 (0.010)
Avg	0.282 (0.004)[b]	0.216 (0.005)[b]	0.298 (0.002)	0.082 (0.004)
Lo	0.193 (0.013)[c]	0.128 (0.014)[c]	0.304 (0.007)	0.099 (0.010)
CIRgud[±]	P < 0.001	P < 0.001	ns	P < 0.05
Hi	0.383 (0.012)[a]	0.322 (0.013)[a]	0.297 (0.007)	0.055 (0.010)[a]
Avg	0.278 (0.004)[b]	0.212 (0.005)[b]	0.300 (0.002)	0.087 (0.004)[b]
Lo	0.186 (0.014)[c]	0.118 (0.014)[c]	0.298 (0.007)	0.085 (0.011)[b]
CIRclo+gud[±]	ns	ns	ns	ns
Hi	0.365 (0.012)	0.305 (0.013)	0.303 (0.007)	0.064 (0.010)
Avg	0.282 (0.004)	0.216 (0.005)	0.298 (0.002)	0.083 (0.004)
Lo	0.192 (0.012)	0.127 (0.013)	0.304 (0.007)	0.096 (0.010)

[#] Where the group effect was significant least square means with different superscripts are significantly different
[±]Significance of group effect, ns=non-significant

Table 16. Predicted group means for stress responsiveness and temperament traits in **ewes** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	logHaptoUncorr[#]	**logHaptoCorr**[#]	**logFT**[#]
CIRclo[±]	P < 0.001	P < 0.001	ns
Hi	0.410 (0.022)[a]	0.350 (0.023)[a]	0.274 (0.008)
Avg	0.333 (0.007)[b]	0.271 (0.007)[b]	0.270 (0.003)
Lo	0.287 (0.021)[b]	0.219 (0.022)[b]	0.272 (0.008)
CIRgud[±]	ns	ns	ns
Hi	0.388 (0.022)	0.323 (0.023)	0.265 (0.008)
Avg	0.338 (0.007)	0.277 (0.007)	0.272 (0.003)
Lo	0.266 (0.022)	0.196 (0.023)	0.267 (0.008)
CIRclo+gud[±]	ns	ns	ns
Hi	0.418 (0.021)	0.357 (0.023)	0.263 (0.008)
Avg	0.333 (0.007)	0.271 (0.007)	0.272 (0.003)
Lo	0.278 (0.021)	0.209 (0.022)	0.269 (0.008)

[#] Where the group effect was significant least square means with different superscripts are significantly different
[±]Significance of group effect, ns=non-significant

Growth and body condition:

For lambs and ewes immune competence phenotype predicted group means for growth and body condition traits are presented in Tables 17 and 18, respectively.

Table 17. Predicted group means for growth traits in **lambs** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	BW[#]	WW[#]	PW1[#]	PW2[#]
CIRclo[±]	ns	ns	ns	ns
Hi	4.49 (0.05)	27.84 (0.23)	30.48 (0.29)	35.68 (0.34)
Avg	4.47 (0.01)	27.63 (0.07)	30.51 (0.09)	36.14 (0.15)
Lo	4.49 (0.05)	27.31 (0.24)	29.86 (0.30)	35.60 (0.35)
CIRgud[±]	ns	ns	ns	ns
Hi	4.57 (0.05)	27.65 (0.23)	30.44 (0.28)	35.54 (0.33)
Avg	4.47 (0.01)	27.62 (0.07)	30.45 (0.09)	36.08 (0.15)
Lo	4.41 (0.05)	27.63 (0.26)	30.36 (0.32)	36.21 (0.37)
CIRclo+gud[±]	ns	ns	ns	ns
Hi	4.56 (0.05)	27.83 (0.23)	30.59 (0.28)	35.87 (0.33)
Avg	4.46 (0.01)	27.63 (0.07)	30.45 (0.09)	36.04 (0.15)
Lo	4.46 (0.05)	27.36 (0.23)	30.18 (0.29)	36.08 (0.34)

[#] Where the group effect was significant least square means with different superscripts are significantly different

[±]Significance of group effect, ns=non-significant

Table 18. Predicted group means for growth and condition traits in **ewes** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	BW[#]	WW[#]	YW[#]	BCS1	BCS2
CIRclo[±]	ns	ns	ns	ns	ns
Hi	4.52 (0.08)	25.48 (0.41)	41.03 (0.47)	3.22 (0.03)	2.88 (0.04)
Avg	4.52 (0.03)	25.48 (0.15)	41.53 (0.13)	3.26 (0.01)	2.85 (0.01)
Lo	4.47 (0.08)	25.49 (0.40)	41.71 (0.47)	3.27 (0.03)	2.83 (0.04)
CIRgud[±]	ns	ns	ns	ns	ns
Hi	4.50 (0.08)	25.13 (0.41)	41.43 (0.48)	3.28 (0.03)	2.90 (0.04)
Avg	4.53 (0.03)	25.49 (0.14)	41.55 (0.13)	3.26 (0.01)	2.85 (0.01)
Lo	4.42 (0.08)	25.82 (0.41)	41.11 (0.48)	3.25 (0.03)	2.78 (0.04)
CIRclo+gud[±]	ns	ns	ns	ns	ns
Hi	4.58 (0.08)	25.44 (0.40)	41.39 (0.47)	3.24 (0.03)	2.88 (0.04)
Avg	4.52 (0.03)	25.51 (0.15)	41.54 (0.14)	3.26 (0.01)	2.85 (0.01)
Lo	4.38 (0.08)	25.33 (0.40)	41.27 (0.46)	3.25 (0.03)	2.80 (0.04)

[#] Where the group effect was significant least square means with different superscripts are significantly different

[±]Significance of group effect, ns=non-significant

Carcase and wool:

For lambs and ewes immune competence phenotype predicted group means for carcase traits in lambs and wool traits in ewes are presented in Tables 19 and 20, respectively.

Table 19. Predicted group means for carcase traits in **lambs** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	HCWT[#]	**CEMA**[#]	**CCFAT**[#]	**CFATSCORE**[#]	**HGRFAT**[#]	**IMF**[#]
CIRclo[±]	ns	ns	ns	ns	ns	ns
Hi	22.83 (0.16)	14.69 (0.15)	4.41 (0.13)	2.86 (0.03)	13.39 (0.25)	5.09 (0.07)
Avg	22.89 (0.06)	14.86 (0.05)	4.41 (0.05)	2.92 (0.01)	13.36 (0.09)	5.08 (0.02)
Lo	22.87 (0.16)	14.59 (0.15)	4.45 (0.13)	2.89 (0.03)	12.95 (0.26)	5.09 (0.08)
CIRgud[±]	ns	ns	ns	ns	ns	ns
Hi	22.74 (0.15)	14.87 (0.14)	4.29 (0.12)	2.89 (0.03)	12.95 (0.24)	5.01 (0.07)
Avg	22.91 (0.06)	14.84 (0.05)	4.42 (0.05)	2.92 (0.01)	13.37 (0.09)	5.10 (0.02)
Lo	22.79 (0.17)	14.49 (0.16)	4.44 (0.14)	2.86 (0.04)	13.40 (0.27)	5.09 (0.08)
CIRclo+gud[±]	ns	ns	ns	ns	ns	ns
Hi	22.79 (0.15)	14.82 (0.14)	4.26 (0.13)	2.86 (0.03)	12.99 (0.24)	5.07 (0.07)
Avg	22.90 (0.06)	14.86 (0.05)	4.43 (0.05)	2.92 (0.01)	13.38 (0.09)	5.08 (0.02)
Lo	22.80 (0.16)	14.45 (0.15)	4.43 (0.13)	2.90 (0.03)	13.25 (0.25)	5.12 (0.07)
	CFL[#]	**Cfa**[#]	**Cfb**[#]	**logPH24LL**[#]	**logSHEARF5**[#]	
CIRclo[±]	ns	ns	ns	ns	ns	
Hi	36.06 (0.13)	19.75 (0.07)	2.68 (0.09)	0.82 (0.00)	1.52 (0.01)	
Avg	36.04 (0.05)	19.79 (0.03)	2.74 (0.03)	0.83 (0.00)	1.52 (0.00)	
Lo	36.29 (0.14)	19.87 (0.08)	2.94 (0.09)	0.82 (0.00)	1.52 (0.01)	
CIRgud[±]	ns	ns	ns	n/a	ns	
Hi	36.08 (0.13)	19.85 (0.07)	2.72 (0.08)	-	1.52 (0.01)	
Avg	36.07 (0.05)	19.79 (0.03)	2.75 (0.03)	-	1.52 (0.00)	
Lo	36.05 (0.15)	19.81 (0.08)	2.81 (0.10)	-	1.53 (0.01)	
CIRclo+gud[±]	ns	ns	ns	ns	ns	
Hi	36.18 (0.13)	19.82 (0.07)	2.76 (0.08)	0.83 (0.00)	1.53 (0.01)	
Avg	36.03 (0.05)	19.78 (0.03)	2.73 (0.03)	0.83 (0.00)	1.52 (0.00)	
Lo	36.19 (0.14)	19.88 (0.08)	2.90 (0.09)	0.82 (0.00)	1.52 (0.01)	

[#] Where the group effect was significant least square means with different superscripts are significantly different. n/a = Non estimable due to either insufficient between animal variation in trait values or insufficient data available for some contemporary groups.
[±]Significance of group effect, ns=non-significant

Table 20. Predicted group means for wool traits in **ewes** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	GFW[#]	CFW[#]	YLD[#]	FD[#]	SDFD[#]
CIRclo[±]	ns	ns	ns	ns	ns
Hi	3.60 (0.05)	2.65 (0.04)	73.73 (0.45)	19.12 (0.18)	3.58 (0.07)
Avg	3.47 (0.01)	2.57 (0.01)	74.28 (0.13)	18.95 (0.05)	3.52 (0.02)
Lo	3.54 (0.05)	2.62 (0.04)	74.12 (0.43)	18.93 (0.18)	3.48 (0.07)
CIRgud[±]	ns	ns	ns	ns	ns
Hi	3.54 (0.05)	2.64 (0.04)	74.68 (0.46)	19.19 (0.19)	3.59 (0.07)
Avg	3.48 (0.01)	2.57 (0.01)	74.15 (0.12)	18.96 (0.05)	3.52 (0.02)
Lo	3.60 (0.05)	2.66 (0.04)	74.24 (0.45)	18.76 (0.18)	3.50 (0.07)
CIRclo+gud[±]	ns	ns	ns	ns	ns
Hi	3.56 (0.05)	2.64 (0.04)	74.08 (0.44)	19.11 (0.18)	3.54 (0.07)
Avg	3.48 (0.01)	2.58 (0.01)	74.20 (0.13)	18.94 (0.05)	3.52 (0.02)
Lo	3.54 (0.05)	2.63 (0.04)	74.34 (0.43)	18.96 (0.17)	3.53 (0.07)

	CVD[#]	CURV[#]	SL[#]	SS[#]
CIRclo[±]	P < 0.05	ns	ns	ns
Hi	18.41 (0.26)[a]	64.73 (1.11)	103.72 (1.18)	38.55 (1.00)
Avg	18.22 (0.08)[a]	64.36 (0.30)	102.84 (0.31)	38.61 (0.31)
Lo	17.94 (0.25)[a]	63.63 (1.08)	104.38 (1.14)	38.31 (0.96)
CIRgud[±]	ns	ns	ns	ns
Hi	18.42 (0.27)	63.27 (1.14)	103.47 (1.21)	38.17 (1.02)
Avg	18.18 (0.08)	64.55 (0.29)	102.92 (0.30)	38.67 (0.31)
Lo	18.29 (0.26)	63.49 (1.13)	104.19 (1.19)	38.08 (1.01)
CIRclo+gud[±]	ns	ns	ns	ns
Hi	18.21 (0.26)	64.16 (1.10)	103.93 (1.16)	38.45 (0.99)
Avg	18.22 (0.08)	64.46 (0.30)	102.71 (0.30)	38.65 (0.31)
Lo	18.16 (0.25)	63.40 (1.07)	105.13 (1.13)	38.08 (0.96)

[#] Where the group effect was significant least square means with different superscripts are significantly different

[±]Significance of group effect, ns=non-significant

Health traits:

For lambs and ewes immune competence phenotype predicted group means for health traits are presented in Tables 21 and 22, respectively.

Table 21. Predicted group means for health traits in **lambs** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	FAM[#]	logwWEC[#]	wFCS[#]
CIRclo[±]	ns	ns	ns
Hi	1.87 (0.03)	2.75 (0.03)	2.05 (0.05)
Avg	1.87 (0.01)	2.76 (0.01)	2.07 (0.02)
Lo	1.95 (0.03)	2.76 (0.03)	2.10 (0.05)
CIRgud[±]	ns	ns	ns
Hi	1.87 (0.03)	2.70 (0.03)	2.05 (0.05)
Avg	1.88 (0.01)	2.77 (0.01)	2.07 (0.02)
Lo	1.86 (0.04)	2.80 (0.04)	2.17 (0.05)
CIRclo+gud[±]	ns	ns	ns
Hi	1.87 (0.03)	2.72 (0.03)	2.06 (0.05)
Avg	1.88 (0.01)	2.77 (0.01)	2.07 (0.02)
Lo	1.87 (0.03)	2.77 (0.03)	2.11 (0.05)

[#] Where the group effect was significant least square means with different superscripts are significantly different

[±]Significance of group effect, ns=non-significant

Table 22. Predicted group means for health traits in **ewes** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	FAM[#]	pDAG[#]	yDAG[#]	logpWEC[#]
CIRclo[±]	n/a	P < 0.05	ns	ns
Hi	-	1.56 (0.10)[a]	1.85 (0.08)	2.84 (0.04)
Avg	-	1.51 (0.04)[a]	1.82 (0.03)	2.83 (0.01)
Lo	-	1.85 (0.10)[b]	1.89 (0.08)	2.83 (0.04)
CIRgud[±]	n/a	ns	ns	ns
Hi	-	1.55 (0.11)	1.76 (0.09)	2.81 (0.04)
Avg	-	1.53 (0.03)	1.83 (0.03)	2.83 (0.01)
Lo	-	1.81 (0.11)	1.88 (0.08)	2.89 (0.05)
CIRclo+gud[±]	n/a	ns	ns	ns
Hi	-	1.60 (0.10)	1.85 (0.08)	2.82 (0.04)
Avg	-	1.51 (0.04)	1.82 (0.03)	2.84 (0.01)
Lo	-	1.82 (0.10)	1.88 (0.08)	2.85 (0.04)

[#] Where the group effect was significant least square means with different superscripts are significantly different. n/a = Non estimable due to either insufficient between animal variation in trait values or insufficient data available for some contemporary groups.
[±]Significance of group effect, ns=non-significant

Body composition and confirmation traits:

For lambs and ewes immune competence phenotype predicted group means for body confirmation and composition traits in lambs and body confirmation and composition traits in ewes are presented in Tables 23 and 24, respectively.

Table 23. Predicted group means for body confirmation traits in **lambs** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	EMD[#]	CFAT[#]
CIRclo[±]	ns	ns
Hi	26.33 (0.19)	3.09 (0.06)
Avg	26.38 (0.07)	3.12 (0.03)
Lo	26.22 (0.21)	3.18 (0.06)
CIRgud[±]	ns	ns
Hi	26.16 (0.19)	3.04 (0.06)
Avg	26.41 (0.07)	3.13 (0.02)
Lo	26.17 (0.23)	3.16 (0.07)
CIRclo+gud[±]	ns	ns
Hi	26.31 (0.19)	3.08 (0.06)
Avg	26.40 (0.07)	3.12 (0.03)
Lo	26.13 (0.20)	3.17 (0.06)

Table 24. Predicted group means for body composition and confirmation traits in **ewes** classified as high, average or low responders for CIRclo, CIRgud and CIRclo+gud.

	EMD[#]	CFAT[#]
CIRclo[±]	ns	ns
Hi	21.76 (0.33)	1.91 (0.05)
Avg	21.47 (0.13)	1.92 (0.02)
Lo	21.91 (0.33)	1.86 (0.05)
CIRgud[±]	ns	ns
Hi	21.90 (0.35)	1.93 (0.05)
Avg	21.53 (0.12)	1.91 (0.02)
Lo	21.45 (0.37)	1.89 (0.06)
CIRclo+gud[±]	ns	ns
Hi	21.90 (0.33)	1.90 (0.05)
Avg	21.47 (0.13)	1.92 (0.02)
Lo	21.77 (0.34)	1.85 (0.05)

5 Discussion

5.1 Genetic parameters for immune competence traits

The heritability of the immune competence traits AMIR, CMIRclo and CMIRgud were estimated at 0.25 (± 0.05), 0.20 (± 0.05) and 0.22 (± 0.05) in lambs and 0.47 (± 0.12), 0.42 (± 0.12) and 0.14 (± 0.09) in ewes, respectively. These heritability estimates are considered moderate, suggesting a reasonable rate of genetic gain can be expected when selecting for immune competence in sheep. As described above, CMIR was assessed by measuring DTH responses to both 5in1 clostridial vaccine (CMIRclo) and Gudair vaccine (CMIRgud) in all animals tested. Results indicated that DTH responses to both vaccines were moderate to strongly positively correlated in ewes with phenotypic and genetic correlations between the two traits estimated to be 0.43 (± 0.03) and 0.74 (± 0.21), respectively, but only weakly positively correlated in lambs with phenotypic and genetic correlations between the two traits estimated to be 0.18 (± 0.02) and 0.17 (± 0.17), respectively.

In the current study, weak positive phenotypic correlations (0.16 ± 0.03 & 0.04 ± 0.03) and moderate to strong positive genetic correlations (0.29 ± 0.19 & 0.57 ± 0.31) were observed in ewes between AMIR and CMIRclo and AMIR and CMIRgud, respectively. No significant correlations (either phenotypic and genetic) were observed between AMIR and CMIR traits in lambs. Supporting the results observed in ewes in the current study, Hine et al. (2016) reported strong positive genetic correlations between AMIR and CMIR traits in Australian beef cattle. These results are in contrast to findings in North American dairy cattle where Thompson et al. (2012) reported 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). While 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 study suggest that based on the positive genetic correlations observed in ewes and the absence of significant correlations in lambs, selecting for AMIR in sheep wil l also improve (or at least not reduce) the ability of animals to mount CMIR and vice versa. On this basis it is tempting to suggest that at least in ewes, measuring just CMIR or AMIR (but not both) is all that is required to improve the general disease resistance of your flock.

However, it is important to consider that even when AMIR and CMIR traits 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. 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 livestock including sheep.

5.2 Relationships between immune competence, stress-responsiveness and temperament traits

Correlations between immune competence and temperament were investigated in the current study. Results suggested that immune competence traits in both ewes and lambs were generally negatively (unfavourably) correlated with the temperament trait, FT. This was an unexpected result as immune competence and FT have been shown to be strongly positively (favourably) genetically correlated in beef cattle (Hine *et al.*, 2016). Previous studies in cattle have demonstrated that calm animals (high FT) perform better in the feedlot environment as evidenced by their higher average daily weight gains and lower mortality as compared to their nervous (low FT) counterparts (Fell *et al.*, 1999). Results from the current study suggest that selection for immune competence may have a negative impact on temperament; however, the use of flight speed as a measure of temperament is not as well established in sheep as it is in cattle and no significant effects of CIR phenotype group (CIRclo, CIRgud or CIRclo+gud) on FT were observed in either ewes or lambs. Both FT and agitation scoring has been used previously to assess temperament in sheep with results suggesting that the genetic correlation between FT and agitation score is favourable, but small and generally not significant (Brown *et al.,* 2015). It is also important to recognise, that genetic progress can be made simultaneously in traits even when those traits are unfavourably genetically correlated. Further studies are required to identify the most appropriate means of assessing the temperament of sheep and to better understand the relationship between temperament test outcomes and inherent behavioural characteristics of individual animals.

Correlations between immune competence and the stress-responsiveness traits hapto and WtGain were also investigated. Results indicated that AMIR and WtGain were weakly positively genetically correlated in lambs (0.27 ± 0.17) and CMIR and WtGain were not correlated (0.05 ± 0.17 (CMIRclo), -0.07 ± 0.18 (CMIRgud)). Similar genetic correlations between immune competence and weight gain over the weaning period have been reported in beef calves (Hine et al., 2016). However, the large errors associated with the correlation estimates in both studies mean the results are difficult to interpret and a trend in group weight gain differences in lambs was observed over the weaning period with WtGain in high immune competence lambs generally being lower than in their low immune competence counterparts. Although group WtGain differences were not significant for CIRclo and CIRclo+gud groupings, low and average CIRgud animals had significantly higher WtGain (0.085 ± 0.011 & 0.087 ± 0.004 kg/day, respectively) over the weaning period than did high CIRgud animals (0.055 ± 0.004 kg/day). Weight gain during the weaning period was monitored as an indirect measure of an animal's ability to cope with the management-induced stress. The relationship between immune competence and stress coping ability in sheep is difficult to interpret based on the results of the current study, therefore further studies will be required to evaluate associations between immune competence and temperament in sheep.

Haptoglobin is an acute phase protein whose expression is up-regulated during periods of heightened stress. Therefore increases in serum haptoglobin concentration can be used as a stress response indicator. Results of the current study suggest that the immune competence traits, AMIR and CMIR are generally phenotypically and genetically positively correlated with increases in serum haptoglobin associated with yard weaning (lambs) or transport and handling (ewes). Furthermore, significant group differences in serum haptoglobin responses to transportation or yard weaning were observed in ewes and lambs, respectively, with higher serum haptoglobin increases observed in high versus average or low CIRclo, CIRgud and

CIRclo+gud immune competence animals in response to management induced stress. This was an unexpected result as we hypothesised that immune competence would be favourably correlated with stress-coping ability in sheep. However, when interpreting these results it is important to consider that acute phase proteins such as haptoglobin play an important role in innate immunity and as such their levels are expected to increase in response to immune challenges such as vaccination as well as management induced stress. As ewes and lambs were vaccinated at the commencement of testing in the current study, to allow the immune competence of animals when under stress to be assessed, increases in serum haptoglobin detected post-weaning (lambs) and post handling and transportation (ewes) are likely to be due to the combined effects of the stressor and stimulation of the immune system through vaccination at the commencement of testing. This effect was confirmed in a pilot trial conducted in beef cattle prior to this study in which calves were vaccinated with 7in1 (test) or saline (control) at the commencement of yard weaning and the influence of vaccination on haptoglobin responses assessed 3 days post weaning. Unfortunately the confounding influence of vaccination on haptoglobin responses induced by management induced stress could not be avoided in the current study if we wanted to assess the immune competence of animals when under stress. The use of alternative indicators of stress responsiveness such as serum cortisol or serum amyloid A were considered when designing the current project; however, serum cortisol responses are rapid and very dynamic, requiring very strict timing of sampling which was not practical when testing large numbers of animals on commercial farms and measurement of serum amyloid A at the scale required here was cost prohibitive. The influence of vaccination on these alternative indicators would also require investigation. On this basis it was decided to proceed with measurement of haptoglobin as a stress response indicator in the current project. In future studies we plan to investigate the use of faecal cortisol as a stress response indicator. Measuring cortisol in faeces is expected to allow more accurate baseline cortisol levels to be determined at the commencement of weaning and provide more flexibility in sample timing as compared to measuring serum cortisol.

The immune competence testing procedure described here assesses the strength of an animals adaptive immune system. Although it is well acknowledged that strong adaptive immune responses are underpinned by strong innate immune responses, and therefore animals exhibiting strong adaptive responses are expected to also exhibit strong innate responses, potential benefits could be gained from direct assessment of both innate and adaptive when assessing immune competence in livestock. The strong positive associations between immune competence and haptoglobin responses observed in the current study suggest that haptoglobin responses induced by vaccination may provide a valuable indicator of the strength of the innate immune system in sheep and warrants further investigation.

5.3 Relationships between immune competence and production traits

It has long been considered that resistance to disease in livestock may incur a production cost as a consequence of nutrients being redirected from production to support immune function. 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 animals with inferior immune competence. In contrast, short term effective activation of the immune system to clear infection in animals with superior immune competence allows them to quickly return to pre-infection production levels. In the current study we investigated correlations between immune

competence traits and various production traits describing growth and body condition and wool and carcase characteristics.

Growth and Body Condition Traits

Results suggested that genetic and phenotypic correlations between immune competence and growth traits were generally weak and not significant in both ewes and lambs. This result was supported by the finding that no significant differences in growth traits were observed between immune competence phenotype groups in either ewes or lambs. A weak negative (unfavourable) genetic correlation was observed between CIR and WW in ewes; however, errors associated with the correlation estimates were large and no genetic correlation between CIR and YW were observed in ewes. Genetic correlations between CIR and WW in lambs were negligible. 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*., 1998a). 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). Common to these studies was the intensification of the production systems investigated whether animals were housed or on pasture and the increased disease challenges that come with intensification. In the current study, ewes and lambs were managed in extensive pasture based production systems. We hypothesise that the production benefits gained by selecting for enhanced immune competence will be increased when animals are exposed to a more challenging environment.

Favourable associations between immune competence and reproductive traits in dairy cattle have been reported (Thompson-Crispi *et al*., 2012b). In a study across 42 herds in Canada, favourable associations were observed between immune competence and number of artificial services, and time from first service to conception. In the current study, results suggested that immune competence traits are weak to moderately positively genetically correlated with BCS in ewes, suggesting that selection for immune competence may also select for increased body condition. Positive genetic correlations between immune competence and fat cover have also been reported in beef cattle (Hine *et. al*, 2016). It is well established that maintaining body condition score in breeding females is a critical factor in achieving reproductive success and therefore we speculate that immune competence and fertility traits may be favourably genetically correlated. Although the reproductive performance of ewes assessed for immune competence in the current study was not investigated, we are planning to validate links between immune competence and reproductive performance in future studies.

Wool Traits

Unfavourable weak to moderate genetic correlations were generally observed between immune competence traits and YLD, FD and SDFD. In contrast, weak favourable genetic correlations were observed between immune competence traits and SS. Regardless, no significant differences between immune competence phenotype groups were observed for any of the wool traits measured with the exception of CVD, which tended to be lower in Low CIRclo

ewes (17.94 ± 0.25) as compared to Average (18.22 ± 0.08) and High (18.41 ± 0.26) CIRclo ewes. When interpreting these results it is important to recognise that genetic progress can be made simultaneously in traits even when those traits are unfavourably genetically correlated as evidenced by the success of the sheep industry in reducing fibre diameter while simultaneously increasing fleece weight (Taylor and Atkins, 1997). Therefore, genetic progress to simultaneously improve both immune competence and wool traits (YLD, FD and SDFD) is still possible. Furthermore, the favourable genetic correlations between immune competence and SS along with the absence of any significant genetic correlations between immune competence and GFW observed in the current study may suggest that breeding for enhanced immune competence is not expected to result in nutrients being redirected from wool production to support immune function in high responder animals.

Carcase Traits

The relationships between immune competence and various carcase traits in lambs were investigated in the current study. Moderate negative (favourable) genetic correlations between immune competence and SHEARF5 were observed. It is well recognised that tenderness has a significant impact on meat eating quality, therefore this result suggests that benefits in lamb eating quality may be achieved as a consequence of selecting for improved immune competence. Results also demonstrated that immune competence was favourably genetically correlated with IMF and HGRFAT, albeit weakly, suggesting that selection for immune competence may also lead to improvements in carcase retail value. Findings from carcase data were supported by live animal ultrasound scanning data which suggested that CFAT was also favourably genetically correlated with immune competence in lambs.

Genetic correlations between immune competence and HCWT and CEMA in lambs were negligible, suggesting that breeding for enhanced immune competence is not expected to result in nutrients being redirected from meat production to support immune function in high responder animals as was hypothesised to be the case for wool production. Combined, results of the current study suggest significant benefits in carcase eating quality and retail value could be achieved by selecting for immune competence without impacting on carcase weights. Although the biological basis of associations between immune competence and carcase characteristic traits observed in the current study were not investigated, we hypothesise that these associations are the result of interactions between the endocrine and immune systems of individual animals which are known to be highly integrated (Colditz and Hine, 2015).

5.4 Relationships between immune competence and health traits

Worm egg counts were conducted on immune competence tested ewes and lambs (where possible) to investigate associations between immune competence and resistance to internal parasites. Multi-drug resistance of common internal parasites is a major issue for the Australian sheep industry. Results suggested that immune competence is favourably genetically correlated with wWEC in lambs. Favourable genetic correlations were also observed between immune competence and FAM in lambs. Although genetic correlations were favourable, no significant differences in wWEC or FAM were observed between CIR phenotype groups in lambs. In contrast, genetic correlations between immune competence and pWEC in ewes were negligible. Differing results observed in ewes as compared to lambs may be a consequence of the use of historical WEC data for ewes and WEC data collected at (or close to) the time of immune competence testing for lambs. It is also plausible that the

relationship between immune competence and internal parasite resistance varies with age in sheep. Favourable genetic correlations were also observed between immune competence and FCS in lambs and pDAG in ewes. Furthermore, pDAG scores were lower in High and Average CIRclo (1.56 ± 0.08) which may have potential economic benefits for sheep producers through reduced incidence of breech strike, reduced crutching costs and reduced wool contamination at shearing.

The immune competence testing protocol developed here utilises commercial vaccines to induce measureable antibody and cell-mediated immune responses. Since the initial development of the immune competence testing protocol the Barbers Pole worm vaccine 'BarberVax' has been commercially released and BarberVax vaccination programs have been introduced on many farms in high Barbers Pole worm risk environments. As part of future studies we aim to investigate the potential of incorporating antibody responses to the BarberVax vaccine as one of a series of immune parameters measured as part of our current immune competence testing protocol. The inclusion of antibody responses to BarberVax (both peak antibody response and/or longevity of antibody responses) in immune competence tests is expected to further improve our ability to identify animals with enhanced general disease resistance.

Unfortunately, detailed data on disease incidence (apart from WEC data) and health-related mortalities were not available (or numbers of records were insufficient) for animals enrolled in the current study to allow detailed analysis of the influence of immune competence phenotype on disease incidence, mortality and disease treatment costs. In a recently completed MLA project investigating associations between immune competence and health and performance of beef cattle in the feedlot, significant reductions in health associated costs were incurred during feedlot finishing for high immune competence steers ($3.53) as compared to low immune competence steers ($103.36) demonstrating the substantial improvements in animals health and welfare which can be achieved through selection for immune competence in livestock (Hine *et al.,* 2016). Future studies will aim to utilise detailed health and performance data collected (during feedlot finishing or lifetime production) in sheep to validate the ability of immune competence phenotyping measures to identify animals better able to cope with challenges posed by their production environment. Detailed economic analyses will also be undertaken to estimate the potential economic benefits of selecting for improved immune competence in sheep realised through reduced labour and therapeutic costs associated with monitoring and treating for disease and improved productivity.

6 Conclusions/Recommendations

6.1 Conclusion

Based on results from the current study, we hypothesise that significant economic benefits can be achieved by selecting for immune competence in sheep, realised through reduced labour and therapeutic costs associated with monitoring and treating for disease, while having favourable impacts on many important production traits in sheep.

Strategies aimed at reducing the incidence and impact of disease in the Australian sheep industry such as that described here have the potential to:

- Increase productivity
- Reduce disease treatment costs
- Improve animal health & welfare
- Reduce use of antibiotics in the food-chain
- Improve consumer confidence in the Australian sheep industry

6.2 Objectives achieved

Specific indicative objectives were to:

- Develop immunophenotyping tests to predict individuals at enhanced risk of disease or reduced productivity. **Achieved**
- Develop immunophenotype indicator trait(s) suitable for implementation through genetics / genomics. **Achieved**
- Develop initial estimates of genetic and phenotypic parameters for immune response traits in sheep. **Achieved**
- Develop knowledge on stress responsiveness of lambs at weaning. **Achieved**

6.3 Future projects

Future projects will aim to:-

- Utilise detailed health and performance data collected (during feedlot finishing or lifetime production) in sheep to validate the ability of immune competence phenotyping measures to identify animals better able to cope with challenges posed by their production environment.
- Conduct detailed economic analysis to estimate the potential benefits of selecting for improved immune competence in sheep realised through reduced labour and therapeutic costs associated with monitoring and treating for disease and improved productivity.
- Further validate appropriate testing protocols to assess temperament in sheep.
- Refine testing protocols, minimising the number of farm visits required and time taken to conduct testing.
- Develop field based tests to replace laboratory assays, providing same day results during testing and removing the need to transport serum samples to the laboratory.
- Explore genetic markers for immune responsiveness traits
- Further develop a resilience index for sheep producers looking to improve the resilience of their flocks

6.4 Publications and conference proceedings (see appendix for articles without a link)

Hine BC, Mallard BA, Ingham AB, Colditz IG. (2014) Immune competence in livestock. In 'Breeding focus 2014 – Resilience'. (Eds. S. Hermesch and S. Dominik) pp. 49-64. (Animal Genetics and Breeding Unit, University of New England, Armidale, NSW, Australia) ISBN 978-1-921-597-65-7.

Colditz IG, Hine BC. (2015) A consideration of biological responses related to resilience in farm animals. *Animal Production Science*. http://dx.doi.org/10.1071/AN15297

Hine B, Wiiffels G, Ingham A, Colditz I. Potential benefits of selecting for improved resilience in Northern beef cattle. (2016) *"In Proceedings of the Northern Beef Research Update Conference"*, Rockhampton, QLD, Australia 15th – 18th August, 2016. Improving animal health and welfare for productivity in Northern Australia section pp 70-76.

Dominik S, Hine, B. Selection for immune competence in beef breeding programs modelled on potential reductions in the incidence of bovine respiratory disease. (2016) In 'Breeding focus 2016 – Animal Welfare'. (Eds. S. Hermesch and S. Dominik) (Animal Genetics and Breeding Unit, University of New England, Armidale, NSW, Australia).

7 Key Messages

7.1 Key project findings

- The heritability of the immune competence traits, AMIR, CMIRclo and CMIRgud were estimated to be moderate, suggesting a reasonable rate of genetic gain could be expected when selecting for immune competence in sheep.
- Moderate to strong positive genetic correlations between AMIR and CMIRclo and AMIR and CMIRgud were observed in ewes. However, AMIR and CMIR traits were not genetically correlated in lambs.
- Unfavourable moderate to strong genetic correlations between immune competence traits and the temperament trait, FT were observed in ewes and lambs; however, no significant differences in FT were observed between immume competence phenotype groups and the use of flight speed as a measure of temperament is not well established in sheep.
- Phenotypic and genetic correlations between immune competence traits and increases in serum haptoglobin induced by vaccination and weaning (lambs) or transportation (ewes) conducted on day 0 of testing were positive, suggesting that haptoglobin responses induced by vaccination may provide a valuable indicator of the strength of the innate immune system in sheep.
- Immune competence traits were weakly unfavourably genetically correlated with WW in ewes but were not correlated with YW. Immune competence traits and growth traits were not genetically correlated in lambs.
- Weak to moderate positive genetic correlations were observed between immune competence traits and BCS in ewes which may have implications for reproductive performance.
- Moderately unfavourable genetic correlations were observed between immune competence traits and the wool traits, FD, YLD and SDFD. In contrast, genetic correlations between immune competence traits and SS were weakly favourable.

- Favourable weak to moderate genetic correlations between immune competence traits and the carcase traits SHEARF5, IMF and HGRFAT were observed in lambs. Results from carcase data were supported by live animal ultrasound scanning data which suggested that CFAT was also favourably genetically correlated with immune competence in lambs. These findings suggest selection for immune competence may also have benefits for carcase retail values and lamb eating quality.
- Favourable genetic correlations between immune competence traits and FAM in ewes and lambs and with WEC in lambs suggests that selection for immune competence will also improve resistance to internal parasites in sheep. Favourable correlations between immune competence traits and pDAG in ewes were also observed.
- Based on results of the current study we hypothesise that significant economic benefits can be achieved by selecting for immune competence in sheep, realised through reduced labour and therapeutic costs associated with monitoring and treating for disease, while having favourable impacts on many important production traits in sheep.

7.2 Acknowledgements

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9 Appendix

9.1 Appendix A

Immune competence in livestock

Brad C. Hine[1], Bonnie A. Mallard[2], Aaron B. Ingham[3], Ian G. Colditz[1]

[1]CSIRO Agriculture Flagship, Chiswick, New England Highway, Armidale, NSW, 2350, Australia.

[2]Department of Pathobiology, Ontario Veterinary College, and Centre of Genetic Improvement of Livestock, University of Guelph, 50 Stone Road, Guelph, ON, N1G2W1.

[3]CSIRO Agriculture Flagship, Queensland Bioscience Precinct, 306 Carmody Road, St. Lucia, QLD 4067, Australia.

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 of our livestock species. Currently we are developing testing procedures to assess 'general immune competence' of beef cattle, dairy cattle and sheep on-farm. Immune competence traits will be combined with measures of temperament and ability to cope with management induced stress to estimate an animal's resilience. By exploring associations between resilience and important production traits we aim to develop breeding strategies which will identify animals highly suited to their production environment.

Introduction

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 bodily 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 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 paper 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 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 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. The contribution of immune competence to general resilience will be discussed in further detail later in the chapter.

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 target cells. Collectively, the actions of such 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. Equally responses must be of a magnitude that effectively eliminates pathogens without causing self harm.

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 livestock industries. 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 cattle has been estimated at between 0.15 to 0.37 (Lyons *et al*., 1991; Uribe *et al*., 1995; Van Dorp *et al*., 1998). Thus progeny of parents with high genetic potential for milk production have a higher incidence of mastitis than 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 livestock species. The animal production sector is becoming increasingly aware of this issue and is 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 antibiotics to control disease in livestock and the potential food contamination issues that arise from their misuse. However, it must also be acknowledged that selection for increased productivity remains a key profit driver for our livestock industries. Alternative strategies that address these consumer concerns while reducing the incidence of disease, and as a consequence, production losses and treatment costs associated with disease are therefore required. 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).

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. In support of this concept, it has been reported that cell-mediated and antibody mediated immune responses are negatively genetically correlated in dairy cattle even though they work in coordination to protect the host (Hernandez *et al*., 2006; Thompson-Crispi *et al*., 2012b). An inverse relationship between antibody production and macrophage function, an important component of cell-mediated immunity, was first reported in Biozzi mice selected for high and low antibody production (Hale and Howard, 1981). A similar relationship has since been reported in cattle selected for resistance or susceptibility to *Brucella abortus* (Price *et al*., 1990). Furthermore, a recent study in dairy cattle has demonstrated that cattle which test positive for tuberculosis, which is largely controlled by cell-mediated immunity, have a lower incidence of mastitis, largely controlled by antibody-mediated immunity (Edwards, 2014). In contrast to these findings, monocyte function was found to be similar in

pigs selected for high and low overall immune responsiveness (Groves *et al*., 1993). Although such findings suggest 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, 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.

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 livestock 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 tradeoff 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 (Fig 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).

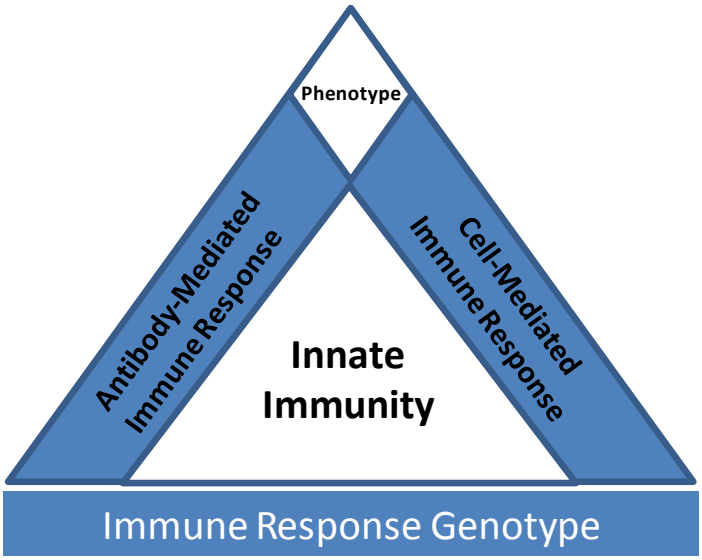

Figure 1. Genetic variation in the ability to resist disease is due to a large number of additive genetic effects which together regulate innate and adaptive immune responses (Source: adapted from Wilkie and Mallard 1999)

Heritability of Immune Competence Traits

The practicality and efficiency of the immune response testing protocol, developed by Mallard and colleagues for use in dairy cattle, has permitted the testing of large numbers of commercial dairy cows across diverse geographical locations in North America in order to estimate the heritability of immune responsiveness traits (Thompson-Crispi *et al*., 2012b). The heritability of a trait refers to the proportion of the observed variation between animals which can be directly attributed to differences in genetics. Genetic gains can be made quickly in highly heritable traits, whereas genetic progress in traits with low heritability, while still achievable, is expected to be proportionally slower. The heritability of antibody and cell mediated immune responsiveness in commercial dairy cattle has been estimated at 0.16-0.41 (with a standard error (SE) of 0.09-0.11, depending on time of sampling and antibody isotype measured) and 0.19 (SE = 0.10), respectively (Thompson-Crispi *et al*., 2012b). These estimates are in line with those reported in pigs selected for general immune responsiveness for eight generations, where the heritability of antibody and cell-mediated immune responsiveness was estimated at 0.27 and 0.16, respectively (Wilkie and Mallard, 1999). Heritability estimates of these traits in the initial cohort of Canadian Holstein sires owned by the Semex Alliance (http://www.semexusa.com/) are in the range of 0.3 to 0.48 (B.A. Mallard, *pers. comm*.). These heritability estimates are considered

moderate and they are comparable with the heritability of many highly selected production traits in livestock species (Safari and Fogarty, 2003). Therefore, reasonable genetic gains in general immune responsiveness traits can be expected when the traits are incorporated into livestock breeding programs.

Selection for Immune Competence – Associations with Disease Incidence, Reproduction and Productivity

Knowledge of associations between enhanced general immune responsiveness and incidence of disease, rates of reproduction and productivity in commercial livestock operations is critical to the success of selection strategies aimed at breeding high-producing animals with enhanced general immune responsiveness. In an early study conducted on both research and commercial dairy farms, it was reported that cows classified as high for antibody-mediated immune responsiveness had a lower incidence of mastitis when compared with average or low responders using data pooled across herds. High antibody responder cows also responded better to the commercial *Esherichia coli* J5 mastitis preventative vaccine (Wagter *et al*., 2000). It should be noted however, that in the same study, cows classified as high antibody responders had the highest incidence of mastitis in one of the three herds tested, with all mastitis cases in these cows recorded in first-parity cows rather than multiparous cows. This finding was limited to the research herd tested and was not observed in the two commercial herds tested. Disease incidence records carefully and systematically collected on commercial farms provide valuable data to quantify the success of selecting for improved general disease resistance (Guy *et al*., 2012). A more recent study reported incidence rates of clinical mastitis in 41 herds across Canada in dairy cattle classified as high, average or low for general immune responsiveness (Thompson-Crispi *et al*., 2013). Results from this study revealed that the average cases of mastitis reported per 100 cow years in high, average and low IR cows were 17.1, 27.9 and 30.7, respectively and that severity of mastitis cases was greatest in low IR cows. Associations between disease incidence and general immune responsiveness have also been investigated in a large commercial dairy herd in Florida (Thompson-Crispi *et al*., 2012c). Results showed that the incidence of mastitis was higher in average IR cows compared to high IR cows. Mastitis incidence tended to be higher in low IR as compared to high IR cows; however, the difference was not statistically significant. Although observed differences in the incidence of metritis and ketosis between IR phenotypes were not significant, displaced abomasums and retained foetal membranes were observed more frequently in low IR cows. The considerable research effort aimed at developing a strategy to assess general immune responsiveness and evaluating the success of that strategy to reduce the incidence of disease in commercial dairy herds has culminated in the licensing of the High Immune Response technology to the Semex Alliance. The Semex Alliance has been marketing semen from dairy sires with EBVs for enhanced general immune responsiveness in North America since January 2013 and is currently marketing this semen globally. Recent data collected from large commercial dairy farms in the United States demonstrated that daughters of Immunity+ sires have lower incidence of mastitis (8.8% versus 15.8%) and pneumonia (6.8% versus 9.1%) than do daughters from non-Immunity+ bulls in the same herd (Data courtesy of Jay Shannon, Sire Analyst, Semex Alliance).

It has long been considered that resistance to disease in livestock may incur a production cost as a consequence of nutrients being redirected from production to support immune function. 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. Enhanced immune responsiveness is expected to avoid the penalty to production that accompanies chronic immune activation and therefore may lead to improved productivity. In support of this concept, high IR pigs were found to have higher growth rates relative to their intermediate IR and low IR counterparts, significantly reducing the time taken to reach market weight (Mallard *et al*., 1998a). The relationship between antibody-mediated immune responsiveness and milk production has also been investigated in dairy cows. Among multiparous cows, high IR animals were found to have significantly higher milk production compared with low IR animals; however, in first-parity cows, milk production was higher in low IR animals than in average of high IR cows (Wagter *et al*., 2003). Favourable associations between general immune responsiveness and reproductive traits in dairy cattle have also been reported (Thompson-Crispi *et al*., 2012b). In a study across 42 herds in Canada, favourable associations were observed between general immune responsiveness and number of artificial services, and time from first service to conception. Clearly more research is required to determine associations between general immune responsiveness and important reproduction and production traits in livestock species. It is important to recognise however, that regardless of the outcome of these studies, genetic progress can be made simultaneously in traits even when those traits are unfavourably correlated. An example of this comes from the sheep industry where genetic progress in reducing fibre diameter while simultaneously increasing fleece weight, traits which are unfavourably correlated, has been successful (Taylor and Atkins, 1997).

Phenotype to Genotype

General immune responsiveness is a complex trait under polygenic control, having many genes each contributing to the variation observed in the trait (Wilkie and Mallard, 1999). Therefore it will be difficult to identify individual genes which have a major effect on general immune responsiveness which can be selected for in commercial populations of livestock. The use of EBVs or genomic based estimated breeding values (GEBVs) may help to overcome this issue by simultaneously selecting for genes contributing to the general immune responsiveness trait without the need to identify individual contributing genes (Thompson-Crispi *et al*., 2014). Estimation of GEBVs for traits is based on genetic markers across the genome that have a statistical association with those traits. Genome-wide association studies (GWAS) can be undertaken to explore associations between genetic markers and traits of interest. Various GWAS have been conducted in livestock to evaluate genetic differences in production, reproduction and health traits (Cole *et al*., 2011; Do *et al*., 2014). Recently, a GWAS was conducted to evaluate general immune responsiveness in Canadian Holstein cattle (Thompson-Crispi *et al*., 2014). This study identified several significant genetic markers, candidate genes and pathways associated with antibody and cell-mediated immune responsiveness in dairy cattle. Based on these findings it may be possible to calculate GEBVs for general immune responsiveness traits which could be incorporated into selection indices. However, studies based on larger reference populations are required to validate this approach. Associations between genetic markers and traits can differ between breeds and even between lines within breeds and therefore validation across multiple populations will be required.

Immune Competence as a Component of Resilience

Resilience can be described as the ability of an animal to maintain productivity in the face of diverse environmental challenges. Livestock respond to challenges from infectious agents and other environmental stressors through immunological, physiological and behavioural defence reactions. These three modalities of host defence are highly integrated and their activation uses resources that would otherwise be directed towards production (Colditz *et al*., 2002). Research over a number of years has highlighted that the level of activity of the immune system is associated with an animal's ability to thrive in the face of environmental stressors and can be an indicator of future health and performance (Schmid-Hempel *et al*., 2003). Such findings highlight the important contribution of immune competence to resilience.

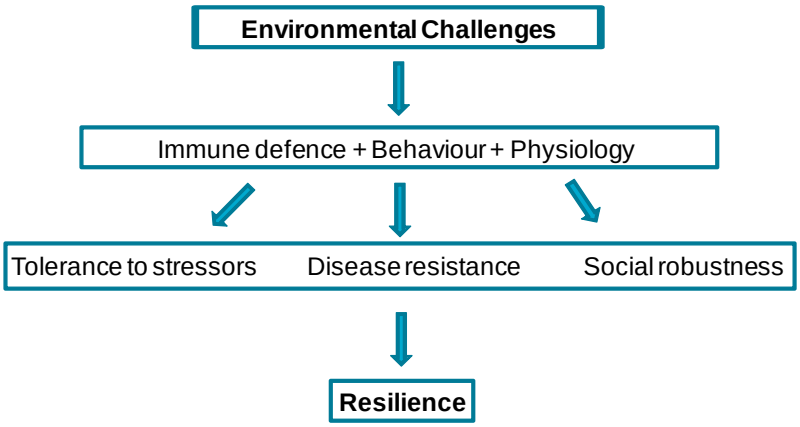

Figure 2. Resilience can be considered as the ability of an animal to maintain productivity in the face of diverse environmental challenges. Measures of disease resistance, tolerance to stressors and social robustness can be used in combination to predict an animal's resilience

The resilience of individual animals can be predicted by combining measures of their general immune competence, stress responsiveness and behaviour or temperament (Fig. 2). Livestock management practices, such as weaning, social mixing and animal handling, provide opportunities to simultaneously assess the various components of host defence contributing to resilience. For example, yard weaning of beef calves provides an opportunity in which to simultaneously assess the ability of calves to cope with the stress induced by the weaning process, the ability of calves to respond to immunological challenges whilst under stress and also assess the temperament of calves. It is well recognised that stress, both physiological and metabolic, negatively impacts on immune function. For example, the incidence of disease in dairy cows is highest during the periparturient period when cows are under physical and metabolic stress (Mallard *et al*., 1998b). Incidence rates of bovine respiratory disease in feedlot cattle are highest in the first few weeks after entering the feedlot when cattle are under stress as a consequence of adjusting to a new environment (Schnieder *et al*., 2009) and the stress of late pregnancy and early lactation induces a relaxation in immunity to gastrointestinal parasites in sheep during the periparturient period is well documented (Salisbury *et al*., 1970). Such findings suggest that assessing immune competence in animals when under stress may improve our ability to identify animals able to resist disease challenges during subsequent periods of heightened exposure to environmental stressors. When combined with measures of stress responsiveness and temperament, general immune responsiveness when under stress is expected to be a good predictor of resilience in livestock. Development of protocols to assess resilience phenotypes in livestock species will allow

selection of animals better adapted to the environmental challenges associated with their respective production environments.

Summary

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 livestock industries. A possible genetic solution to this problem is to develop breeding strategies aimed at enhancing general disease resistance of the animal while simultaneously making genetic gains in important production traits. Although immune responsiveness is a complex trait under polygenic control, general immune responsiveness can be assessed by inducing immune responses and objectively measuring such responses in livestock, allowing EBVs, and likely in the future, GEBVs to be calculated for individual animals. Selection for resistance to specific diseases carries the potential risk of inadvertently increasing susceptibility to other diseases. Selection of livestock for general immune responsiveness as an alternative to, or in conjunction with, selection for resistance to specific diseases reduces this risk and is expected to improve broad-based disease resistance. Extensive research in dairy cattle has demonstrated that animals with enhanced general immune responsiveness have a reduced incidence of disease in commercial herds. Furthermore, favourable associations between general immune responsiveness, production and reproduction traits have also been reported.

The ability to resist disease forms an important component of resilience, described as the ability to maintain productivity in the face of diverse environmental challenges. The resilience of livestock is becoming increasingly important as 1) selection pressure to increase productivity from livestock continues, 2) consumer awareness regarding the health and welfare of the animals producing their food increases and 3) consumer concern regarding the use of antibiotics in food-producing animals intensifies. The resilience of individual animals can be predicted using a combination of measures of general immune competence, stress responsiveness and temperament. Development of protocols to assess resilience phenotypes in livestock species will allow selection of animals better adapted to their production environment and help ensure the long-term future of livestock industries.

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9.2 Appendix B

Potential benefits of selecting for improved resilience in Northern beef cattle

B. Hine[A,B], G. Wijffels[A], A. Ingham[A] and I. Colditz[A]

[A] CSIRO Agriculture and Food, Armidale NSW and Brisbane Qld

[B] Corresponding Author (brad.hine@csiro.au)

Introduction

Livestock face a variety of challenges from their production environment including exposure to infectious agents, abiotic extremes, social stressors as a result of herd hierarchy and mixing with unfamiliar animals and management induced stressors imposed by standard husbandry procedures and practices. Challenges vary between environments. For instance, in Northern Australia, beef cattle experience seasonal challenges from ticks and buffalo flies, extreme heat and humidity, variable feed quality and long transport distances to market. Following pasture backgrounding, many Northern Australian cattle are then finished through feedlots or are destined for live export exposing them to a new set of challenges. Identifying animals better able to cope with these unique challenges could 1) improve animal health and welfare 2) reduce reliance on the use of antibiotics and anti-parasitic drugs thus slowing the emergence of multi-drug resistance and 3) improve productivity. It is also important to consider the significant influence consumers can have on an industry.

Consumers are increasingly conscious of the health and welfare of the animals producing their food and are demanding the highest possible standards of animal welfare through purchasing choices. Consumers are also increasingly concerned with the use of drugs in food-producing animals and the potential residue issues they pose. Therefore, breeding strategies aimed at improving the health and welfare of animals and reducing reliance on drugs to treat disease are expected to improve consumer confidence, help maintain the social licence to operate and, improve industry profitability.

We define resilience as the ability of an animal to maintain productivity in the face of diverse environmental challenges. Livestock respond to challenges from infectious agents and other environmental stressors through immunological, physiological and behavioural defence reactions. These three modalities of host defence are highly integrated, acting together to minimise the impact of challenges on the host (Colditz *et al*., 2002). The resilience of individual animals can be predicted by combining measures of their general immune competence, stress responsiveness, ability to tolerate climatic extremes and behaviour or temperament (Fig. 2). Livestock management practices, such as weaning, social mixing and animal handling, provide opportunities to simultaneously assess the various components of host defence contributing to resilience. For example, yard weaning of beef calves provides an opportunity to simultaneously assess the ability of calves to cope with weaning stress, the ability of calves to respond to immunological challenges whilst under stress and assess their temperament.

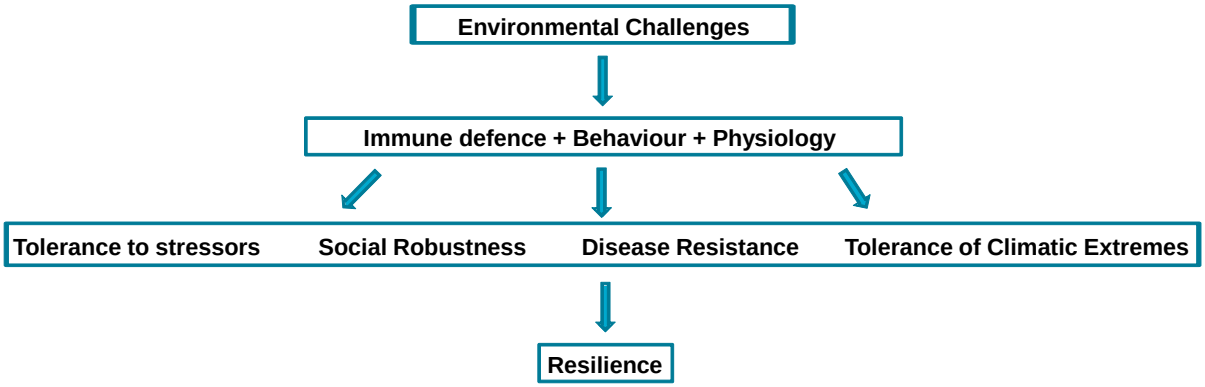

Figure 2. Resilience can be considered as the ability of an animal to maintain productivity in the face of diverse environmental challenges. Measures of disease resistance, tolerance to stressors, heat tolerance and social robustness can be used in combination to predict an animal's resilience

When assessing the resilience of livestock, the component measures used to define the resilience phenotype need to be tailored to the specific production environment. Here we propose a series of measures which could be used in conjunction to define resilience phenotypes specifically tailored for beef cattle grazing in various regions of Northern Australia.

Heat tolerance

The trend toward increased hot conditions in the cattle production regions of Australia is clear. Howden and Turnpenny, (1997) reported that for the Gayndah region (South East Queensland), the last 40 years has seen a 60% increase in days that cause heat stress in taurine cattle. With an intermediate warming scenario of an average temperature increase of 2.8°C by 2100, the number of heat stress days are estimated to increase to 139 days p.a. (as compared to the 58 heat stress days in the late 1990's). Furthermore, this region will face 92 days p.a. with high risk of heat related fatalities.

While the numbers and costs of cattle mortalities due to a discrete heat event can be calculated, total production losses over summers and on a national basis are difficult assessments. Sackett *et al.* (2006) estimated that Australian feedlots lose $16.5 million p.a. due to reductions in animal performance over summer.

The most obvious contribution to productivity loss in cattle from heat stress is decreased feed intake and subsequent slower weight gain. In beef cattle, there is a 0.4 kg/day average daily gain (ADG) depression for every 1°C increase in internal body temperature (Finch, 1986). A less obvious impact is the lower reproductive performance (Wheelock *et al.* 2010). All stages of bovine reproduction are affected by heat load.

Any stressor will redirect endocrine and metabolic processes toward maintenance of homeostasis and away from growth. The overt characteristics of heat stress: reduction of feed intake, reduced appetite and lassitude are the accumulation of the interactions of systemic endocrine, metabolic and inflammatory changes. The reduced feed intake most commonly experienced during heat stress has clouded much of the research and interpretation of the endocrine and metabolic effects that can be solely attributed to heat stress. However, the metabolic changes in heat stress cannot be explained by reduced feed intake alone. Heat-stressed ruminants fail to enlist the glucose saving mechanisms used by underfed animals; i.e. do not consume their fat stores and become slightly insulin insensitive

(Baumgard *et al.* 2011; Wheelock *et al.* 2010). It is likely, that to supply the glucose required for maintenance, protein in muscle is being catabolised to fuel gluconeogenesis in the liver.

There is now some evidence that the gut barrier function is disrupted in heat stress. The role of ruminal and intestinal dysfunction during heat stress in cattle was first proposed by Cronjé (2005). The disruption to gut function and integrity is a consequence of reduced blood flow to the viscera during heat stress, as the blood is directed to the skin and the mucosa of the respiratory tract for cooling. The lack of oxygen in the gut and liver, due to the reduced blood flow, compounds the situation thus setting off more inflammatory responses.

There has been research into different management tactics and tools with some adoption by producers and producer organisations (e.g. MLA, 2006). Based on research and their own experience, beef cattle nutritionists have manipulated buffering capacity, electrolyte balance and roughage: grain ratios of summer rations. These adjustments have met with success in some instances and not others, but this inconsistency is not understood.

Many researchers point to genetic selection as a means to equip the industry with heat tolerant breeds (Gaughan *et al*., 2010; Howden and Turnpenny, 1997). It is generally accepted that *Bos indicus* genotypes have greater heat tolerance than *Bos taurus* genotypes. There are exceptions. The Tuli, closely related to *Bos taurus* but tropically evolved, appears to have a high degree of heat tolerance (Hammond *et al*., 1998). This paper reported also that the rectal temperatures of Brahman cattle and Angus cattle (40.0 and 40.9°C respectively) were higher than the rectal temperature of Senepol cattle (39.6°C) under the same conditions.

Selective breeding for heat tolerance is a long and imprecise process but needs to be part of the answer. However, tools for detecting economically competitive heat tolerant phenotypes are limited because it is not understood which physiological parameters are most appropriate. Furthermore, the technology to measure these parameters in large numbers of animals in production environments is still under development or not yet in the pipeline.

Our current focus is on feedlot cattle where we are investigating inflammatory and metabolic responses to high heat load in growing steers in collaboration with Dr John Gaughan and team (University of Queensland, Gatton) (MLA B.FLT 0157). While the end-goal is to develop new nutritional and/or management approaches for alleviating heat stress in the feedlot, we are hopeful of discovering new parameters to define the heat-tolerant phenotype in *Bos taurus* cattle. This will provide tools for selective breeding and for assessing the suitability of animals for feedlot entry.

Tick resistance

Cattle tick (*Rhipecephalus microplus*) and tick borne disease (*Anaplasma marginale, Babesia bigemina, Babesia bovis*) have the highest economic impact of all diseases experienced in cattle in the north of Australia. A recent review commissioned by Meat and Livestock Australia estimated annual costs in excess of $160 million and attributed this to a combination of lost productivity and treatments (B.AHE.0010). Typical strategies used to control the incidence and severity of tick and tick borne disease are genetic improvement, chemical control, vaccination and management practices. A search of the patent literature over the last 10 years largely confirms the focus on these control strategies but identifies the occasional unconventional candidate. A breakdown of the results revealed a total of 68 patents of which 55 patents describing potential novel acaracides, 6 for vaccine antigens, 3 genetic

loci that could be significant for breeding approaches, and one each for probiotics, novel detection method, dsRNA (a form of chemical control) and freeze spraying (Derwent Innovation Index). Chemical control approaches have been highly successful when susceptible populations of ticks are targeted but increasingly ticks are showing high levels of resistance to acaricides. This issue has driven the ongoing search for new actives as identified in the patent search described above. Further complicating matters for producers are withholding times that must be applied following chemical application (limiting sale and movement of animals) and community concerns with the potential for residue contamination of foods and the environment.

Genetic control strategies are focussed on selective breeding programs that seek to include cattle that are tick resistant and / or eliminate those that are highly susceptible. This is largely achieved in industry by an indirect method through use of pure Indicine or crossbred Taurine and Indicine animals, as the Indicine breeds are reported to carry 5-10 times less ticks than taurine breeds (Jonsson 2014). Variation of resistance level within breeds does occur but it is difficult to take advantage of this fact as ranking animals for this trait in high numbers is not logistically or economically feasible. The main limitation being the intensive nature of recording tick levels on cattle, which is achieved via visual assessment of the animal. The tick burden is quantified as a score or as specific numbers of parasitising engorged adult ticks. Measurement of larvae is even more difficult given their near microscopic size and preference for difficult to access areas of the animal, that can place observers in harms way. The heritability of these traits is variable, ranging from 0.13 to 0.64 (Jonsson 2014), and this is most likely because the response mounted is complex, involves multiple functional pathways each of which may contribute at variable levels dependent on the different environmental or tick challenge methodology used.

The nature of host resistance to parasites is complex and involves many pathways (Campino 2006). The culmination of these pathways is reduced numbers of ticks, reduced viability or production of tick eggs. Resistance achieved via immunity is composed of both innate and acquired responses (Piper 2009, Kemp 1976). Antibody has been shown to be important in some studies but recent focus has been on the significance of the cellular response (Piper 2009). Genetic association studies have reinforced the importance of these pathways by identifying genes that are known to function in development of immune responses or wound repair, such as RIPK2 (Porto Neto 2012). Behavioural responses such as grooming, which is mediated by licking are important (Verissimo 2016). Other structural features of significance for enhancing cattle resistance to ticks include colour, hair density, and skin thickness (Shyma 2015).

We suggest that recent advances in technology should facilitate development of automated approaches for quantifying tick loads on animals and that this could be a productive area for future research. It may also be possible to measure resistance indirectly through an associated trait. In this respect, blood based immune parameters provide a further option. We have recently reported the use of blood based parameters for identification of worm resistant sheep allowing animals to be ranked following a single blood test (Andronicos 2014). Confidence in the value of such tests is enhanced by the observation that test results correlate well with conventional methods of counting parasite load (WEC in the case of worms). Significantly these phenotypes are amenable to pooling studies which greatly reduce the cost of genotyping studies and the method has been devised in a manner that allows both genotype and phenotype to be collected from a single sample. Given the importance of

cellular responses to tick resistance in cattle we believe that application of a similar approach in cattle may have great value in defining a new phenotype that can be routinely measured.

Temperament

It is easy to recognize that cattle differ in their behavioural reactions, for instance, to humans and to isolation from a group. When a behavioural response is expressed consistently on multiple occasions and in different situations it likely reflects the temperament of the animal. Cattle were domesticated from a wild progenitor, the auroch, which was hunted for food by humans. For these animals, fear of humans would have improved their chance of survival. During the process of domestication cattle were unintentionally selected for docility (Larson & Fuller, 2014); however, it was not until the 1970s that attempts were made to quantify the temperament of cattle and objectively breed for temperament traits. A number of methods for measuring temperament were explored including escape attempts of an animal isolated in a yard, flight distance when approached, and restlessness when held in a crush (Fordyce *et al.* 1982). The advantages of a standardised and automated method for measuring temperament led to the development of flight time, which is the time in seconds it takes an animal to travel a distance of approximately 2 metres when released from a crush (Burrow *et al.* 1988). The trait is moderately heritable and EBVs for flight time are available through Breedplan for Brahman and Santa Gertrudis sires while EBVs for docility, measured as restlessness in the crush or when held individually in a yard, are available for Limousins.

The behavioural responses we recognise as reflecting the temperament of the animal are accompanied by physiological responses such as release of the stress hormones cortisol and adrenalin. These hormones influence energy metabolism. It is therefore not surprising that favourable correlations exist between docile temperament (eg slow flight time), faster growth rate in the feedlot, more tender meat, and lower incidence of dark cutters (Kadel *et al.* 2006). Favourable temperament is also associated with a reduced occurrence of disease during feedlot finishing (Fell *et al*. 1999) but is not associated with resistance to internal or external parasites. In one study conducted during an AI program, more cows with a docile temperament were identified as in oestrus than cows with a poor temperament (reviewed by Haskell *et al.* 2014).

A second change in behaviour that is thought to have occurred early in the process of domestication was an increased capacity of cattle to habituate to the presence of humans and being handled (Wilkins *et al.* 2014). Whereas temperament is recognised by the consistency of a behavioural response over time, habituation is the change in response as the animal becomes accustomed to handling and to a new environment. A capacity to habituate underpins the training procedures used at weaning to teach young cattle to lead and move as a mob (Tyler *et al*. 2012). It has been proposed that genetic variation between animals in their capacity to habituate could be a valuable trait for selection (Wechsler & Lea, 2007); however to date, standardised tests for quantifying the capacity to habituate have not been developed. Further exploration of the genetics of habituation and its association with resilience of animals to environmental challenges is warranted.

Immune competence

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 cattle has been estimated at between 0.15 to 0.37 (Lyons *et al*.

1991; Uribe *et al*. 1995; Van Dorp *et al*. 1998) and selection focussed on high productivity in pigs has led to an increase in susceptibility to stress and disease (Prunier *et al*. 2010). Such findings suggest that selection for production traits with little or no emphasis on health and fitness traits has the potential to increase the incidence of disease in livestock production systems.

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 an 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 to protect the host. 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'.

Animal health can be improved through both targeted management practices and the implementation of genetic selection strategies aimed at breeding animals with improved immune competence. In combination, these approaches have the potential to dramatically improve animal health. Health and welfare are intimately linked and therefore improving animal health is expected to result in improved welfare outcomes for livestock. The concept of breeding for 'general' disease resistance was first proposed by Wilkie and Mallard (1999) and has been used successfully to reduce the incidence of disease in pigs and dairy cattle (Mallard and Wilkie 2007, Mallard *et al*. 2014). This approach combines measures of both antibody-mediated immune responses (AMIR) and cell-mediated immune responses (CMIR) to assess 'general' immune competence (Figure 2). Extra- and intra-cellular pathogens are most effectively controlled by AMIR and CMIR, respectively, therefore individuals identified as having a balanced ability to mount both types of responses are expected to exhibit broad-based disease resistance. Based on this concept, Mallard *et al*. established a protocol to assess immune competence in dairy cattle which has enabled genetic selection strategies, aimed at breeding animals with enhanced 'general' disease resistance, to be developed and implemented in industry. We are currently developing a similar testing protocol, based on a different set of antigens to those used by Mallard, to assess 'general' immune competence in *Bos Taurus* beef calves in Southern Australia during yard weaning as part of a joint Meat & Livestock Australia and CSIRO funded project. As part of the project we are investigating the potential for genetic selection, aimed at improving 'general' immune competence, to reduce the incidence of disease in Australian beef cattle with a particular focus on reducing bovine respiratory disease (BRD) incidence in the feedlot environment.

Following extensive research to validate the benefits of breeding for improved 'general' disease resistance in dairy cattle, the global breeding company Semex Pty. Ltd. are now marketing semen from sires with estimated breeding values for immune competence (Mallard *et al*. 2014). Such advances are allowing dairy producers to place direct selection emphasis on traits aimed at improving the health and welfare of animals in their herds. We propose that the development of immune competence testing protocols specific for beef cattle in Northern Australia will allow beef producers to select

animals with improved general disease resistance, improving the health and welfare of cattle in their herds.

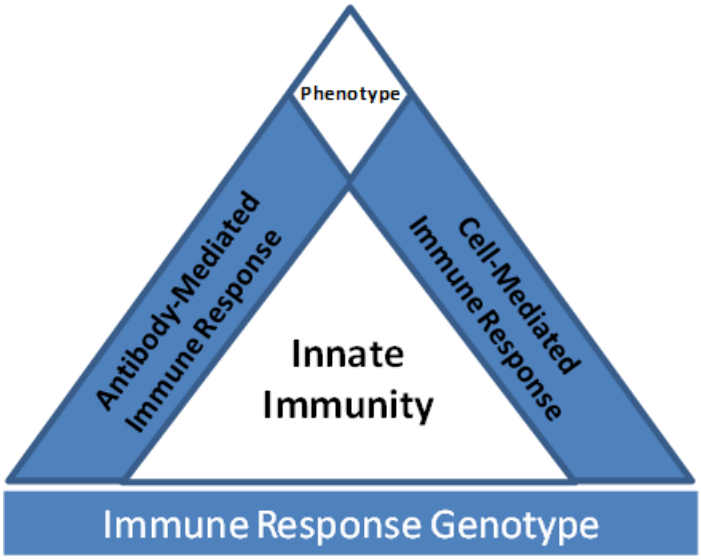

Figure 2. Genetic variation in the ability to resist disease is due to a large number of additive genetic effects which together regulate innate and adaptive immune responses (Source: adapted from Wilkie and Mallard 1999)

Summary

Future development of a resilience selection index specific to Northern Australia beef cattle will allow Northern cattle producers who are aiming to improve the resilience of their herds to make genetic gains in resilience traits. If improved resilience is correlated with an improved ability to cope with the challenges imposed by the feedlot and live export environments, feeder and live export cattle which are the progeny of high resilience indexing sires are expected to attract a premium for cattle producers.

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9.3 Appendix C

Selection for immune competence in beef breeding programs modelled on potential reductions in the incidence of bovine respiratory disease

Sonja Dominik and Brad C. Hine

CSIRO Agriculture and Food, Locked Bag 1, Armidale, 2350 NSW

Abstract

Livestock industries are expected to intensify as land resources for agricultural production decline and global demand for animal protein increases. As a consequence, strategies aimed at sustainably improving the health and welfare of livestock will be critical to the future of our livestock industries. This study has made a first attempt at modelling the potential benefits of incorporating measures of immune competence in beef cattle breeding programs with the aim of improving general disease resistance, and as a consequence animal welfare. This study explores a variety of selection strategies and estimates their potential economic benefits based on data stemming from the dairy industry. Results demonstrated that the estimated heritability and predicted relationship between immune competence and growth traits strongly affect the potential gains which can be expected in immune competence and also overall response to selection. The economic values used in this study were conservative, suggesting that higher selection genetic responses and dollar returns are possible. For more accurate predictions, it will be crucial to obtain genetic and phenotype parameters for immune competence and correlations with other traits specifically for beef cattle. Research is currently underway to determine such parameters for beef cattle. The study also emphasises the need for robust economic values for traits, such as immune competence, where potential economic benefits of the traits are not just purely driven by the cost versus profit of the product, but also strongly influenced through public perception of the industry.

Introduction

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 estimated at up to $20 per head (MLA Project AHW.087). Bovine respiratory disease 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 contributing to the BRD disease complex, however providing protection against the full complement of potential BRD causing agents and achieving protective responses in all vaccinated animals is difficult to achieve. Strategies, aimed at reducing the incidence of BRD in Australian feedlots, are required to complement BRD vaccination programs.

The establishment of a protocol to assess immune competence in dairy cattle has enabled genetic selection strategies, aimed at breeding animals with enhanced 'general' disease resistance, to be

developed and implemented in industry (Wilkie and Mallard 1999). This approach combines measures of both antibody-mediated immune responses (AMIR) and cell-mediated immune responses (CMIR) to assess 'general' immune competence. Extra- and intra-cellular pathogens are most effectively controlled by AMIR and CMIR, respectively, therefore individuals identified as having a balanced ability to mount both types of responses are expected to exhibit broad-based disease resistance. A similar testing protocol, based on differing antigens, to assess 'general' immune competence in beef calves during yard weaning is being developed as part of a joint Meat & Livestock Australia and CSIRO funded project (Hine *et al.* 2014). Currently, the potential for genetic selection, aimed at improving 'general' immune competence to reduce the incidence of disease in Australian beef cattle with a particular focus on reducing BRD incidence in the feedlot environment, is being investigated.

The beef industry is actively working towards improving the health and welfare of animals in their production systems. Including immune competence in beef breeding objectives is expected to promote improved health and welfare through improving general disease resistance. It is hypothesized that combining selection for important production and reproduction traits with selection for health and fitness traits, such as enhanced 'general' immune competence in a selection index will enable beef cattle producers to breed highly productive animals with an enhanced ability to resist disease challenges encountered in their production environment. Such strategies are expected to result in significant long-term economic gains for producers through reduced disease treatment costs, reduced reliance on the use of antibiotics to treat disease, decreased production losses, reduced processing penalties, improved health and welfare outcomes for animals, lower mortality in the herd and improved consumer confidence in products of the beef industry.

In an effort to predict the potential benefits of incorporating selection for 'general' immune competence in breeding programs, hypothetical selection index scenarios have been modelled drawing on available information from the dairy sector.

Material and Methods

Breeding objective traits and selection criteria

A selection index can be used to investigate the effect of including novel traits in breeding programs. It consists of two main components. The first component is called "breeding objective", includes traits that drive profit and targeted to be improved through genetic selection. The second component is called the selection criteria and includes traits that can be routinely measured ("selection criteria") to inform the breeding objective traits. In some cases if the breeding objective trait is easy and cheap to measure, it also acts as the selection criteria for that trait. An example of such a trait is live weight. However, for breeding objective traits that are difficult to measure, correlated traits can be used as selection criteria to inform the breeding objective trait. For example, marble score can only be obtained at slaughter. To inform marbling score as a breeding objective trait, intra muscular fat content as assessed at scanning of live animals is used as a correlated selection criteria trait.

For this study a simplified breeding objective for a beef cattle stud operation that is selling bulls to commercial producers of feeder cattle (seed-stock producer) was defined based on three breeding objective traits which characterise growth, reproduction and carcase quality. Growth is represented by sale weight (SW), which relates to live sale-weight at 17 months of age. Reproduction is represented

by cow weaning rate (CWR), which relates to percent of cows that wean a calf from the total number of cows mated. This is calculated as annual percent pregnant x (1 – reproductive waste) (Fordyce et al. 2014). Carcase quality is represented by marbling score (MS). Marbling score is a visual beef quality grading system, scored from 0 (low marbling) to 9 (highly marbled), referring to the visible fat between muscle fibres in the rib eye muscle (AUS-MEAT Limited 2010). Immune competence (Immuno) was included in the breeding objective and represents a combined measure of an animal's ability to mount both antibody-mediated and cell-mediated immune responses (Hine *et al*. 2011).

Immuno was also measured as selection criteria. Other selection criteria traits included live weight measured 200 and 400 days of age (WT200 and WT400) and intra muscular fat (IMF) assessed on live animals using ultrasound scanning of the rib-eye between the 12th and 13th rib. Traits and their phenotypic and genetic parameters, including economic values for the breeding objective traits are summarised in Tables 1 and 2. Weight at 200 days and WT400, IMF and Immuno were recorded on the selection candidates themselves, their sires, their dams and their half-sibs. Number of records from the different information sources are shown in Table 1. It was assumed that each sire was mated to 50 females and 40 of these half-sibs would be measured for selection criteria. Marbling score is not recorded, but informed through IMF.

Table 1. Breeding objective traits (BO) and selection criteria (SC), their abbreviations and units, and the number of records collected on the selection candidate, its dam, sire and half sibs

Trait	Abbreviation	Unit	BO	SC	Information sources*			
					Own	Dam	Sire	Half sibs
Sale-weight	SW	Kg	Yes	No	0	0	0	0
Cow weaning rate	CWR	%	Yes	No	0	0	0	0
Marbling score	MS	Score	Yes	No	0	0	0	0
200 day weight	WT200	Kg	No	Yes	1	1	1	40
400 day weight	WT400	Kg	No	Yes	1	1	1	40
Intramuscular fat	IMF	%	No	Yes	1	1	1	40
Immune competence	Immuno	stddev	Yes/No	Yes/No	1	1	1	40

*Information sources are in relation to the selection candidate

The economic values for the breeding objective traits, heritabilities and genetic and phenotypic parameters for SW, CWR, MS, WT 200 and 400 and IMF were adopted from Archer *et al.* (2004). Parameters for Immuno and its relationships with other production traits were estimated based on studies in dairy cattle which have estimated genetic parameters of general immune competence (Thompson-Crispi 2012 and Thompson-Crispi *et al*. 2012). Some of the traits used in this study were not represented amongst the published information, and therefore assumptions had to be made. For example, genetic correlations between general immune competence and four reported reproductive

traits (gestation length in heifers and cows, calf survival and calf size) were low and positive (ranging between 0.12 and 0.17) with one value low and negative correlation at -0.13 (Thompson-Crispi 2012). Consequently, it was assumed that Immuno and CWR have a low and positive correlation as was reflected in the majority of the dairy cattle estimates. Similar assumptions were made for other traits where published information was not available. The economic value for Immuno was based on information from the Canadian Dairy industry, where estimated breeding values for general immune competence are available for sires whose semen is marketed by Semex Pty. Ltd. (Mallard et al. 2014). It has been demonstrated that progeny from high immunity sires (being one standard deviation or more above the mean for antibody and cell-mediated immune responsiveness) had 25% fewer incidences of calf pneumonia (Mallard *et al*. 2014).

For the purposes of this project it has been assumed that a similar reduction in BRD could be achieved in beef cattle in the feedlot environment which are progeny of high immunity beef sires. The economic value for Immuno used is this study is flexible and can be tailored to different feed lot systems based on their annual turn-over of occupancy to account for the increased incidence of BRD expected to be associated with increased turn-over.

Economic value ($/per year) = Cost of BRD per head x % reduction in BRD incidence expected in high immune competence animals x annual turn-over (1)

The annual cost associated with BRD has been estimated to be $20 per head (MLA Project AHW.087) and a 25% reduction in BRD incidence was assumed as outlined above. In this study, the economic value was derived for a feed lot operation with an annual turn-over of three times capacity. Based on these assumptions a 25% improvement per phenotypic standard deviation would be valued at $5 per feedlot occupancy. This results in an economic value of $15 per year for a feedlot system where occupancy is turned over 3 times per annum. The economic value of $15 served as the most realistic estimate for immune competence in the selection index scenarios outlined below. However, because of the uncertainty of what the real economic values is, a sensitivity analysis explored economic values that were 25% higher ($18.75) and lower ($11.25) than what was assumed to be the most realistic value.

Selection index scenarios

Six different scenarios were modelled to explore the effect of including immune competence in beef breeding programs on selection response. The selection index scenarios and the abbreviations used to describe them throughout the text are detailed in Table 3. All indexes include the three major breeding objective traits SW, CWR and MS. For the first selection index, immune competence was included as a breeding objective trait, but not measured as a selection criteria, with Immuno informed by other correlated trait responses (Index1). The second index included Immuno as a breeding objective trait as well as a selection criterion (Index2). The inclusion of Immuno as a selection criterion adds another source of information, which increases index accuracy, and as Immuno in the breeding objective and selection criteria are genetically highly correlated is expected to increase the opportunity to drive genetic gains in this trait.

Different variations of Index1 and Index2 used a range of genetic parameters and economic values to explore various scenarios which either favour progress in immune competence or provide little opportunity to progress this trait. The sensitivity of selection responses were tested for Indexes 1 and

2. Index scenarios with genetic parameters that do not favour progress in Immuno used a low heritability of h^2 = 0.1 for Immuno and unfavourable genetic correlations between Immuno and liveweight traits (SW, 200WT and 400WT). These scenarios are labelled with a "↓" to depict unfavourable parameters. Scenarios that use a heritability of h^2=0.3 for Immuno and favourable genetic correlations between Immuno with liveweight traits are labelled with a "↑" to indicate favourable parameters. To test the sensitivity of responses to the economic value for Immuno, it was varied between \$11.25 (\$), \$15 (\$\$) and \$18.75 (\$\$\$) and labelled with the dollar signs as shown.

Table 2. Genetic standard deviation (σ_G), economic values for breeding objective traits (EV in \$) heritability (h^2 in bold) and genetic (above the diagonal) and phenotypic correlations (below the diagonal) for breeding objective traits and selection criteria

Trait	σ_G	EV ($)	EV*σ_G ($)	SW	CWR	MS	WT200	WT400	IMF	Immuno
SW	19.29	0.81	15.60	**0.31**	--	--	--	--	--	--
CWR	7.27	0.93	6.76	0	**0.05**	--	--	--	--	--
MS	0.44	0.01	0.00	0	0	**0.38**	--	--	--	--
WT200	9.49	--	--	0.68	0	0	**0.18**	0.75	-0.60	-0.20, +0.20
WT400	15.45	--	--	0.90	0	0	0.75	**0.25**	0	-0.20, +0.20
IMF	0.34	--	--	-0.02	0.09	0.72	0	-0.01	**0.12**	0.12
Immuno h^2=0.1	0.32	11.25, 15, 18.75	3.60, 4.80, 6.00	-0.20, +0.20	-0.12	0.12	-0.20, +0.20	-0.20, +0.20	0.12	**0.10**
Immuno h^2=0.3	0.55	11.25, 15, 18.75	6.19, 8.25, 10.31	-0.20, +0.20	-0.12	0.12	-0.20, +0.20	-0.20, +0.20	0.12	**0.30**

Abbreviations: SW: Sale weight, CWR: Cow weaning rate, MS: Marble score, WT200: 200-day weight, WT400: 400-day weight, IMF: Intramuscular fat, Immuno: Immune competence.

Table 3. Description of selection index scenarios

	Immuno Index1		Immuno Index2			
Abbreviation	\$\$↓	\$\$↑	\$↑	\$↓	\$\$↑	\$\$\$↑
Immuno included in*	BO	BO	BO/SC	BO/SC	BO/SC	BO/SC
Heritability						
h^2=0.1 (↓)	✓			✓		

h^2=0.3 (↑)		✓		✓		✓	✓
Correlations (WT/Immuno)						
negative (↓)	✓			✓		
positive (↑)		✓		✓		✓	✓
Economic value						
$11.25 ($)				✓	✓		
$15 ($$)	✓	✓			✓		
$18.75 ($$$)							✓

*BO=Breeding objective trait, SC=Selection criteria

Two variations of Index1 were modelled, both assuming an economic value of $15 for a unit of improvement in Immuno. The first variation assumed favourable genetic parameters for progress in Immuno (Index 1 $$↑) i.e. positive correlations with weight traits and moderate heritability. The second variation of the index assumed unfavourable parameters for progress in Immuno (Index 1 $$↓) with negative correlations with weight traits and low heritability.

Four variations of Index 2 were modelled. The correlations between Immuno and liveweight traits were either positive or negative and economic values varied between low, medium and high. The variations included Index 2 $↑, Index 2 $↓, Index 2 $$↑ and Index 2 $$$↑ (Table 3).

Herd parameters

For the purpose of this study a hypothetical Angus stud herd with 450 breeding cows (n_s) was used. The male and female generation interval (L_m and L_f), which is the age of sires and dams at birth of their selected progeny was 2 years of age. Each bull is mated each year to 50 cows, which determines the number of half-sibs that are available for measurement. The calving and survival rates were estimated at 90%. Each year 23 males and 90 females were used as replacements giving a selection intensity (i) for males of 1.69 (i_m) and for females of 0.88 (i_f). Seventy two bulls are sold commercially and used by those purchasers for three years with each bull producing 150 progeny. Therefore, each year bulls produced from this stud have an estimated total number of 10,800 commercial progeny (n_c).

Response to selection

The response to selection, per head per round of selection, for the multiple trait selection index was calculated for each of the selection index scenarios. Results reported include the standard deviation of the breeding objective (SD_{BO}), the genetic gain as trait and dollar responses per round of selection, the standard deviation of the index (SD_{Index}) which describes the total dollar response per head per round of selection, as well as the index accuracy (Acc) which is the ratio of SD_{index} and SD_{BO} and illustrates how well the breeding objective traits are described by the selection criteria. To calculate the genetic gain per year (R), the response per round of selection was multiplied by the selection

intensities for males and females and divided by the generation interval. The genetic gain per year per head was used in further calculations for discounted profit.

Discounted profit and net profit value

The discounted profit and net profit values were calculated to describe the long term value of the genetic gains made at the commercial herd level. The annual returns in year y were based on the genetic gain in dollars per year, starting in year 2 when commercial progeny of a sire are being born. Annual costs included health treatments at $30 per head and $10 per head to measure immune competence where applicable. It was assumed that for immune competence testing all animals in the herd are measured once. A discount rate of 7% per year was applied to returns and cost to calculate the discounted return in year y. The annual discounted profit per year was calculated by subtracting discounted annual cost from discounted returns per year. The annual discounted profit for each of the selection index scenarios was summed over an 11 year period to obtain the net profit value (NPV), providing a measure of profitability.

Discounted returns$_y$ = [(R_y + R_{y-1})*n_c]/(1+discount rate)^y-1, with R_y= genetic gain in year y, n_c = number of commercial progeny (2)

Discounted cost$_y$ = ((health cost + measurement cost)* n_s)/ (1+disount rate)$^{y-1}$, with n_s = head of cattle in stud herd, y = year (3)

Annual discounted profit$_y$= discounted returns$_y$– discounted cost$_y$ (4)

Net profit value (NPV) = $\sum_{y=0}^{11} discounted\ profit_y$ (5)

Results

The results from calculations using the different selection index scenarios described above are summarised in Table 4. The standard deviation of the selection index (SD_{index}, representing the total dollar response per head per round of selection) was generally higher for variations of Index 2 compared to Index 1, as a result of including Immuno as a selection criterion in addition to being a breeding objective trait. The standard deviation of the selection index increased with increasing economic values for Immuno. As expected, overall responses for Immuno were higher when favourable relationships with liveweight and higher heritability values were modelled. The lowest total dollar response, was found for Index1 $$↓ with the maximum difference to the most profitable scenario (Index2 $$$↑) being $5.08 per head per round of selection. Increases in total dollar response were realised when the additional selection response in Immuno was higher than losses in the other breeding objective traits, i.e. sale weight and cow weaning rate.

For Index 1 with favourable relationships between Immuno and liveweight (Index1 $$↑) a positive response for Immuno could still be achieved, despite the fact that Immuno was not included as a selection criterion. This was a result of correlated responses, which was a consequence of the responses achieved in in live weight traits. Consequently, if the relationships with live weight traits were unfavourable (Index1 $$↓) response in Immuno was unfavourable.

Table 4. Standard deviation of the breeding objective (SD_{BO}), of the index (SD_{Index}), Index Accuracy (Acc) and trait responses per round of selection (in $) for the breeding objective traits sale weight (SW), cow weaning rate (CWR), marbling score (MS) and immune competence (Immuno) used in selection index scenarios

	SD_{BO}	SD_{Index}	Acc	SW	CWR	MS	Immuno
Index1 $$↓	16.64	6.24	0.37	6.82	0.00	0.00	-0.58
Index1 $$↑	19.94	7.93	0.40	6.80	0.00	0.00	1.12
Index2 $↑	18.94	9.18	0.48	6.54	-0.30	0.00	2.95
Index2 $↓	16.63	6.40	0.39	6.77	-0.02	0.00	-0.38
Index2 $$↑	19.48	9.73	0.50	6.02	-0.32	0.00	4.03
Index2 $$$↑	21.01	11.32	0.54	5.99	-0.38	0.00	5.71

The results in Table 4 demonstrate that when relationships between Immuno and liveweight traits are unfavourable (Index 1 $$↓ and Index2 $↓), it is easier to achieve higher profit by putting more emphasis on sale weight as is reflected in the trait response for sale weight. However, with favourable relationships, the emphasis on Immuno increases and therefore responses, accompanied by little decreased response for sale weight. The annual net profit value (NPV, Figure 1) emphasises the same trends that were observed in the index responses per round of selection over an 11-year time frame. Index1 $$↓ and Index2 $$↓ had the lowest NPV and the positive effect of higher economic values for Immuno is highlighted in the increase in NVP (Index2 $↑, $$↑ and $$$↑) (Figures 1 and 2).

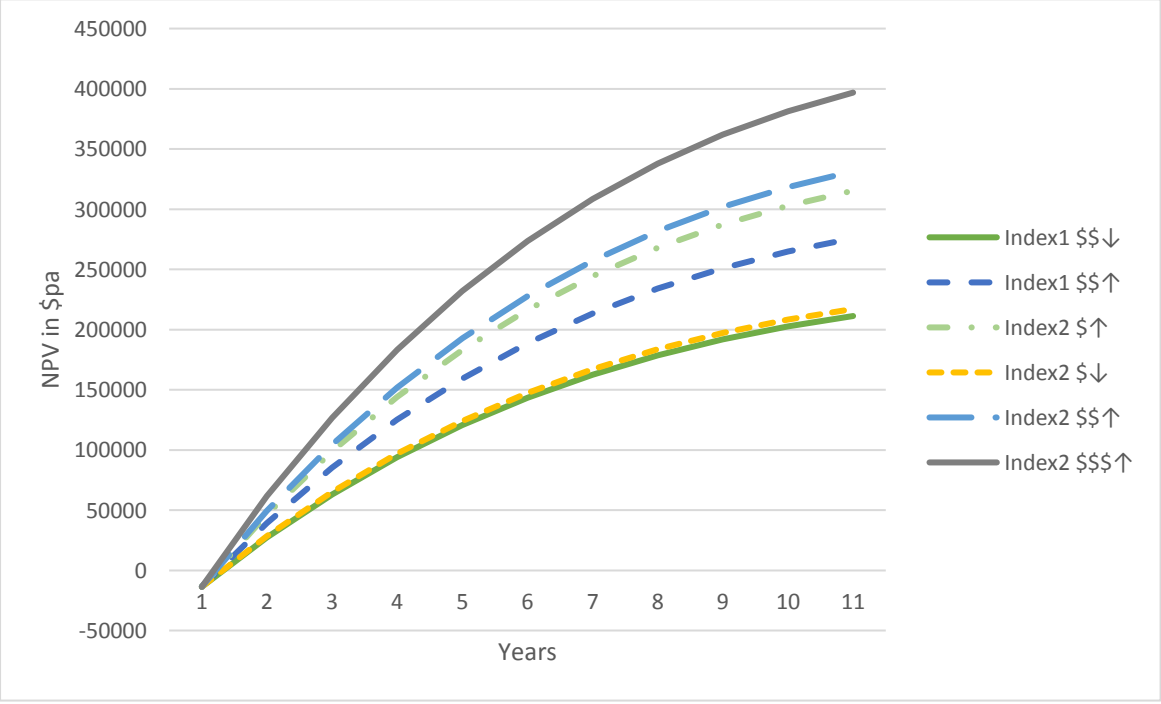

Figure 1. Annual net profit value (NPV) over 11 years for various selection index scenarios for a herd of 450 Angus breeding females

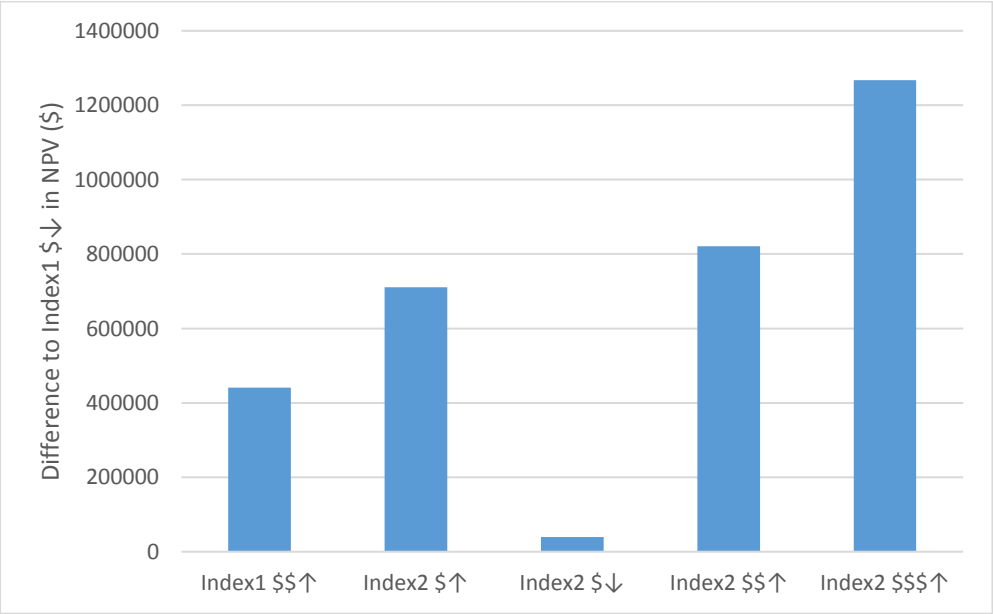

Figure 2. Difference in net profit value (total NPV in $) between Index1 $$↓ and other selection index scenarios

The total NPV over an 11 year time frame were compared to Index1 $$↓, which yielded the lowest total NPV (Figure 2). Index2 $↓ had only a slightly higher NPV compared to Index1 $$↓, highlighting that a small increase in profit can be gained by including Immuno as selection criterion even if the relationships with liveweight are unfavourable and Immuno has a low heritability. For Index2 $$↑, the results demonstrate that by including Immuno as selection criterion, the NPV can be increased substantially if relationships between liveweight and Immuno are favourable.

Discussion

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 cattle has been estimated at between 0.15 to 0.37 (Lyons *et al*., 1991; Uribe *et al*., 1995; Van Dorp *et al*., 1998). Such findings suggest that selection for production traits in livestock with little or no emphasis on health and fitness traits has the potential to increase the incidence of disease in livestock production systems. One of the drives would have been the exponential increase in dairy cow milk production internationally over the last 50 years and a linear increase in the number of dairy cows (FAOstats, 2016). Based on this knowledge the Australian Beef industry is actively investing in research programs aimed at developing breeding strategies to improve the health, and as a consequence the welfare, of animals in their industry.

Animal health can be improved through both targeted management practices and the implementation of genetic selection strategies aimed at breeding animals with improved disease resistance. In combination, these approaches have the potential to dramatically improve animal health. Health and welfare are intimately linked and therefore improving animal health is expected to result in improved

welfare outcomes for livestock. The concept of breeding for 'general' disease resistance was first proposed by Wilkie and Mallard (1999) and has been used successfully to reduce the incidence of disease in intensively farmed pigs and dairy cattle (Mallard and Wilkie 2007, Mallard *et al.* 2014). Following extensive research to validate the benefits of breeding for improved 'general' disease resistance in dairy cattle, the global breeding company Semex Pty. Ltd. are now marketing semen from sires with estimated breeding values for immune competence (Mallard et al. 2014). Such advances have allowed dairy producers to place direct selection emphasis on traits aimed at improving the health and welfare of animals in their herds. In the current study, the potential reduction in BRD incidence in feedlot cattle that could be expected as a result of incorporating measures of immune competence in selection indexes for beef cattle was predicted based on disease incidence data from dairy farms using sires with known EBVs for immune competence.

In the absence of known parameters, this study made a first attempt at modelling potential benefits of selection for immune competence in beef breeding programs. Although a lot of assumptions had to be made, this study explores potential benefits of breeding for improved immune competence by modelling extremes of high and low opportunity to improve the trait. The key outcome of the study was that response in Immuno can be driven more strongly, if it is used a selection criterion in addition to being included in the breeding objective. Adding Immuno to a selection index results in selection response in the trait at the cost of the responses in the other breeding objective traits due to competition for selection pressure. If relationships with other breeding objective traits are unfavourable and the heritability for Immuno is low, gains in Immuno were of insufficient value to compensate for the losses in the other traits. However, favourable genetic parameters for Immuno still compromised responses in other traits due to reduced selection pressure consequently being applied to those traits, but was offset through the gain in Immuno and the overall increase in the total dollar response. Accurate estimates of heritabilities for Immuno and correlations with other traits for beef cattle are necessary to make more informed predictions and are currently being generated. However the results of the current study provide first information on the expected trends.

The economic benefits of placing selection emphasis on a particular trait drives uptake by industry. Even though substantial responses could be achieved in Immuno in this study, it is safe to assume that the economic values for Immuno were conservative estimates, since they were only derived from the economic benefit in the feedlot sector and did not take into account reduced health associated costs in the stud operation. In addition increased consumer confidence in the beef industry as a result of improved animal welfare and reduced use of antibiotics is expected to significantly increase the economic value of improving general disease resistance of beef cattle.

Changing consumer confidence can have a significant effect on the profitability of livestock industries. Consumers are increasingly conscious of the health and welfare of the animals producing their food and are demanding the highest possible standards of animal welfare through purchasing choices. For example, the number of consumers opting to purchase eggs from free-range hens in preference to eggs from caged hens, based on welfare concerns, is increasing. This change in consumer preference has been the catalyst for dramatic changes throughout the egg industry and is evidence of the influence consumers can exert on farming practices. Consumers are also increasingly concerned with the use of antibiotics in food-producing animals. As a consequence, the practice of supplementing animal feed with antibiotics to prevent disease and promote growth is under increasing scrutiny and is unlikely to continue into the future. Therefore, breeding strategies aimed at improving the health

and welfare of animals and reducing reliance on antibiotics to treat disease can be expected to also improve consumer confidence.

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