Exploring Complete Blood Count to Improve Disease Resilience in Pigs

by

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Abstract

Disease resilience is defined as an animal's ability to maintain a high level of performance in the face of disease challenges caused by multiple pathogens, as is often the case in commercial pig farms. Therefore, disease resilience is anticipated to be critical to the profitability of the pig industry. However, it is difficult to make genetic improvement for disease resilience because it is not expressed in the purebred nucleus herds housed in high-health environments where the selection of elite breeding animals occurs. In addition, disease resilience to the challenge of multiple pathogens in commercial pig production systems is a complex trait that is hard and expensive to measure. Complete blood count (CBC) is a relatively inexpensive, robust, and routinely used blood test in veterinary laboratories to evaluate overall health and detect disorders. Exploring CBC traits for genomic selection could be a promising approach to address the above issues of making genetic improvement of disease resilience. Two strategies have been proposed for genomic selection of disease resilience based on CBC traits. The first strategy is to explore indicator CBC traits of disease resilience that can be directly collected from the nucleus herds, and the second strategy is to explore practical CBC phenotypes of disease resilience in commercial farms when the disease challenge is present for genomic selection. Therefore, the overall objective of this thesis was to explore the opportunity to use CBC traits collected under the high-health nucleus farms condition and a model of the polymicrobial challenge faced in commercial farms to improve disease resilience in pigs.

A wean-to-finish natural disease challenge model (NDCM) consisting of a high-health quarantine barn and a polymicrobial challenge barn was established for the project. Three sets of blood samples were collected to determine CBC: the first was collected from high-health pigs in

the quarantine barn, a second (termed Blood 3) and third (Blood 4) were collected in the challenge barn at 2- and 6-week after exposure to the polymicrobial challenge.

Firstly, most CBC traits in Blood 1, 3, and 4 were heritable, with heritability estimates ranging from 0.06 ± 0.04 to 0.53 ± 0.05. A few CBC traits in Blood 3 and 4 were found to be genetically correlated with the resilience traits of grow-to-finish growth rate (GFGR) and antimicrobial and anti-inflammatory treatment incidence (treatment rate, TR) (-0.38 ± 0.18 to -0.82 ± 0.47; 0.50 ± 0.23 to 0.89 ± 0.26). No significant genetic correlation was identified for CBC in Blood 1 with GFGR or TR. Then, genome-wide association studies (GWAS) of CBC traits and gene expression analysis of animals with divergent CBC traits in response to the challenge were used to investigate the genetic control of disease resilience. GWAS found that CBC traits were polygenic traits controlled by a large number of genes with small effects. Gene expression analysis suggested that up-regulation of genes involved in apoptosis might associate with the decreased lymphocyte concentration from Blood 1 to Blood 3 and resulted in lower disease resilience. Lastly, moderate genomic prediction accuracies (0.12 ± 0.04 to 0.28 ± 0.03) were found for three CBC traits under disease (lymphocyte concentration in Blood 3, neutrophil concentration and red blood cell distribution width in Blood 4) that had moderate to high genetic correlations (−0.38 ± 0.18 to 0.89 ± 0.26) with TR. Genomic selection on these three CBC traits may lead to a desirable decrease in TR to reduce antimicrobial use and antimicrobial resistance in the swine industry.

Overall, the results reported in this thesis suggest that genomic selection of CBC traits collected from high-health nucleus farms cannot improve disease resilience regarding the resilience traits of GFGR and TR. However, CBC traits collected in commercial farms when the disease challenge is present can be used as practical disease resilience phenotypes and have the

potential to be used to help develop a selection index for nucleus animals to make genetic improvement for disease resilience.

Preface

The research conducted for this thesis forms part of research collaboration (Project name "Alberta Livestock Genome Program–ALGP2"). This project was carried out in accordance with the Canadian Council on Animal Care guidelines. The protocol was approved by the Animal Protection Committee of the Centre de Recherche en Sciences Animales de Deschambault (15PO283) and the Animal Care and Use Committee at the University of Alberta (AUP00002227). The project was fully overseen by the Centre de Développement du Porc du Québec and the herd veterinarian together with project veterinarians. This project was funded by Genome Canada, Genome Alberta, PigGen Canada, Swine Innovation Porc, and Alberta Agriculture and Forestry. PigGen Canada also intimately involved in the design of the project and the development of protocols for the natural disease challenge model. This research is also part of the AMR – One Health Consortium, funded by the Major Innovation Fund program of the Alberta Ministry of Economic Development, Trade and Tourism.

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the genotype data and provided the genomic relationship matrix for the project. All authors helped with the interpretation of results and reviewed and approved the final manuscript.

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Dedication

I would like to dedicate this to my beloved parents, who have been my source of inspiration, strength, and support, who show me the value of hard work.

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Table of contents

List of tables

List of figures

Abbreviations

AI	Artificial insemination
AMR	Antimicrobial resistance
APCs	Antigen-presenting cells
BASO	Basophil concentration
Blood 1	Blood samples collected at 2-weeks before exposure to the challenge
Blood 3	Blood samples collected at 2-weeks after exposure to the challenge
Blood 4	Blood samples collected at 6-weeks after exposure to the challenge
CBC	Complete blood count
cNur	The challenge nursery stage
cNurGR	The growth rate during the challenge nursery stage
CPM	Counts per million
CSFV	Classical swine fever virus
DE	Differential expression
DEAD	A group of dead animals due to infectious reasons
Δ13	Change from Blood 1 to Blood 3
Δ34	Change from Blood 3 to Blood 4
Δ14	Change from Blood 1 to Blood 4
dpi	Days post infection
FDR	False discovery rate
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EOS	Eosinophil concentration
EOSB3	Eosinophil concentration in Blood 3
EP	Enzootic pneumonia
ETEC	Enterotoxigenic *Escherichia coli*
ETEC F18	Enterotoxigenic *Escherichia coli* expressing F18 fimbriae
FC	Fold change
FDR	False discovery rate
FinADG	Finishing average daily gain
GBLUP	Genomic best linear unbiased prediction
GBPs	Guanylate binding proteins
G × E	Genotype-by-environment interaction
GEBV	Genomic estimated breeding value
GF	The challenge grow-to-finish stage
GFGR	The growth rate during the challenge grow-to-finish stage
G matrix	Genomic relationship matrix
GO	Gene Ontology
GRM	Genomic relationship matrix

GWAS	Genome-wide association studies
h^2	Heritability
HA	Hemagglutinin
HCT	Hematocrit
HGB	Hemoglobin concentration
H matrix	Pedigree-genomic relationship matrix
HSC	Hematopoietic stem cell
IBS	Identity-by-state
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukin-6
IPA	Ingenuity Pathway Analysis
IQR	Interquartile range between the first and the third quartile
LD	Linkage disequilibrium
LYM	Lymphocyte concentration
LYMΔ13	The changes of lymphocyte concentration from Blood 1 to Blood 3
MC	Medcouple
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDS	Multidimensional scaling
MHC	Major histocompatibility complex
MID	A group of average animals
MONO	Monocyte concentration
MONOΔ13	The change of monocyte concentration from Blood 1to Blood 3
MPV	Mean platelet volume
NA	Neuraminidase
NDCM	Natural disease challenge model
NEU	Neutrophil concentration
NEUΔ34	The changes of neutrophil concentration from Blood 3 to Blood 4
NF- κB	NF-kappaB
Non-SPF	Non-specific pathogen-free
PBML	Peripheral blood mononuclear leucocytes
PCV2	Porcine circovirus type 2
PED	Porcine epidemic diarrhea
PLT	Platelet concentration
PRDC	Porcine respiratory disease complex
PRRS	Porcine reproductive and respiratory syndrome
PRRSV	Porcine reproductive and respiratory syndrome virus

Q1	The first quartile
Q3	The third quartile
qNur	The quarantine nursery stage
qNurGR	The growth rate during the quarantine nursery stage
r_g	Genetic correlation
Q-Q plot	Quantile–quantile plot
QTL	Quantitative trait loci
RBC	Red blood cell concentration
RDW	Red blood cell distribution width
RES	A group of resilient animals
RIN	The RNA integrity number
RT-PCR	Reverse transcription-polymerase chain reaction
SNP	Single nucleotide polymorphism
SPF	Specific pathogen-free
SRCR5	Scavenger receptor cysteine-rich domain 5
SSC	*Sus scrofa* chromosome
SSGWAS	Single-step genome-wide association studies
STEC	Shiga toxin-producing *Escherichia coli*
SUS	A group of susceptible animals
TGEV	Transmissible gastroenteritis virus
TNF	Tumor necrosis factor
TR	Individual treatment rate or incidence
TRT180	Treatment rate adjusted to 180 days for animals that reached 65 days of age
WBC	Total white blood cell concentration
WGCNA	Weighted Correlation Network Analysis
WUR	WUR10000125 SNP

Chapter 1. General introduction

The breeding pyramid (**Figure 1**) with specialized dam and sire lines, together with the separate nucleus, multiplier, and commercial farms, has been adopted in pig breeding programs since the 1960s and 70s. Most phenotype recording and selection occur in the purebred populations in nucleus farms to genetically improve the profitability and efficiency of the swine industry. The genetic improvement made through the selection at the nucleus level is then passed on to the multiplier and then to the commercial levels over a period of several years (genetic lag) by selling high-ranking nucleus gilts and boars to lower tiers (Visscher et al., 2000). The genetic improvement ($\Delta G = \frac{r \times i \times \sigma_A}{L}$) in a breeding program depends on the accuracy of breeding value (r), selection intensity (i), genetic variation (σ_A), and generation interval (L). Thus, increased selection intensity, accuracy and genotypic variation but decreased generation interval improve the genetic improvement.

By applying artificial insemination (AI) in pigs, breeding companies change the marketing strategy from selling live boars to selling semen to multiplier farms and commercial farms in the lower tiers (Visscher et al., 2000). Thereby, genetically superior nucleus boars can be used extensively to improve genetic gain with increased selection intensity at nucleus level (Visscher et al., 2000). In addition, AI makes it possible to accelerate genetic gain by decreasing the generation interval between the nucleus herds and the commercial population (Visscher et al., 2000). Multiplier and commercial farms in the lower tiers multiply and cross animals from different purebred nucleus populations (or lines) to take advantage of heterosis and breed complementarity (Smith, 1964; Sellier, 1976). The pyramid breeding structure has been beneficial in the swine industry because only a relatively small number of animals need to be phenotyped and genotyped at the nucleus level to make genetic improvement for the very large

populations of commercial pigs raised for pork. For example, only 50 elite AI boars were selected out of the 1700 boars tested annually in the Norsvin Duroc (sire line) and the Norsvin Landrace (dam line) nucleus populations, respectively (Topigs Norsvin Canada & USA, 2014). Semen from these elite Duroc AI boars were used annually to inseminate 700 Duroc sows to produce 1450 litters per year. Similarly, the Landrace AI boars were used with 2000 Landrace sows to produce approximately 4400 litters per year (Topigs Norsvin Canada & USA, 2014). For all traits in the breeding goal, the genetic improvement was $4 to $5 per slaughter pig per year for Norsvin Duroc and $4 for Norsvin Landrace from 2008 to 2012 (Topigs Norsvin Canada & USA, 2014).

However, a significant limitation of the pyramid structure is the difference in management and environment between the top and bottom of the pyramid, termed as genotype-by-environment interaction (G × E). Nucleus purebred populations at the top of breeding pyramids are highly managed and kept in a high health environment to: firstly, maximize the additive genetic values that flow down the pyramid to commercial farms, leading to higher productivity and efficiency at the commercial level; secondly, avoid vertical transmission of infectious diseases to the multiplier farms and commercial farms at the lower tiers of the pyramid; lastly, avoid horizontal transmission of diseases to other countries when the pigs and/or semen are transported across borders to other countries for breeding companies. Strict movement licensing requirements and biosecurity adopted by the pig industry have ensured a clean environment for nucleus herds in the face of many disease challenges (Pritchard et al., 2005; British Pig Association, 2019). However, health status commonly degrades as we go down the pyramid. Due to the intensification and globalization of the swine industry, endemic and emerging pathogens can be spread rapidly in commercial farms by frequent movements of pigs,

feed, and pork products at local, national, and international scales (VanderWaal and Deen, 2018). Pig performance in commercial farms typically declines when the disease challenge is present compared to nucleus farms, and the ranking of animals at the genetic level may also change.

Conventional methods, including strict biosecurity, vaccines, and antimicrobials, are being used in commercial farms to manage diseases and maintain animal performance and health. However, they are not always effective. The gaps in knowledge of epidemiology for different diseases and diverse ways for pathogen transmission are major challenges for successful biosecurity; and co-infections by multiple pathogens, and the high recombination rate for some of the viruses and their interaction with the host immune system (e.g. porcine reproductive and respiratory syndrome virus) impair vaccine efficacy; and there are also concerns of antimicrobial resistance from the overuse of antimicrobials for prevention (prophylaxis) and treatment of infections in the swine industry. Therefore, an additional strategy, breeding for disease resilient animals that maintain a high level of performance and productivity regardless of pathogen burden when challenged by infection, becomes a desirable attribute in the pig breeding program (Knap and Doeschl-Wilson, 2020). However, easy and inexpensive strategies are needed to improve disease resilience in the pig breeding program because 1) disease resilience is not expressed in the purebred nucleus herds housed in high-health environments where the selection of elite breeding animals occurs; and 2) disease resilience to the disease challenge of multiple pathogens that exist in commercial pig production systems is a complex trait, which can be hard and expensive to measure.

Complete blood count (CBC) is a robust and relatively inexpensive blood test, which measures concentrations and relative proportions of circulating blood cells (leukocytes, erythrocytes, and platelets) that play essential roles in immune response, hematocrit,

hemoglobin, and several erythrocyte indices (George-Gay and Parker, 2003). It has been routinely used in veterinary laboratories to evaluate overall health and detect a wide range of disorders (e.g. infection, anemia). Several CBC traits have been found to be heritable and genetically correlated with pig performance in response to the disease challenge caused by specific types of pathogens (Henryon et al., 2006; Clapperton et al., 2008, 2009; Flori et al., 2011; Mpetile et al., 2015). Therefore, exploring CBC traits for genomic selection could be a promising approach to address the above issues of making genetic improvement of disease resilience.

Two strategies have been proposed for genomic selection of disease resilience in the face of these difficulties. The first strategy is to explore indicator traits of disease resilience that can be directly collected from the nucleus herds to select for disease resilience, and these indicator traits need to be heritable and genetically correlated with animal performance in response to the disease challenge. The second strategy is to explore highly heritable traits as practical phenotypes of disease resilience in commercial farms where the disease challenge is present. The single nucleotide polymorphisms (SNPs) marker effects over the whole genome can be estimated as a regression of indicator traits or phenotypes of disease resilience on genotypes of animals. Therefore, the genomic estimated breeding value (GEBV) of an animal can be predicted by summing up all SNPs marker effects over the whole genome, which allows for the genomic selection of elite nucleus breeding animals based on GEBV for disease resilience.

To this end, the overall objective of this thesis was to explore CBC traits collected under the high-health environment (nucleus farms condition) and the polymicrobial challenge environment (commercial farms situation) as indicator traits and phenotypes of disease

resilience, respectively, to make genetic improvement for disease resilience in the swine industry. The short-term objectives corresponding to each study of this research were to:

1) Explore indicators and phenotypes of disease resilience from CBC traits and estimate genetic parameters of CBC traits (**Chapter 4**);

2) Identify genomic regions associated with CBC traits and disease resilience through a genome-wide association study (**Chapter 5**);

3) Explore the blood transcriptomic signature and immune mechanisms associated with disease resilience (**Chapter 6**);

4) Estimate genomic prediction accuracy of disease resilience based on CBC traits (**Chapter 7**).

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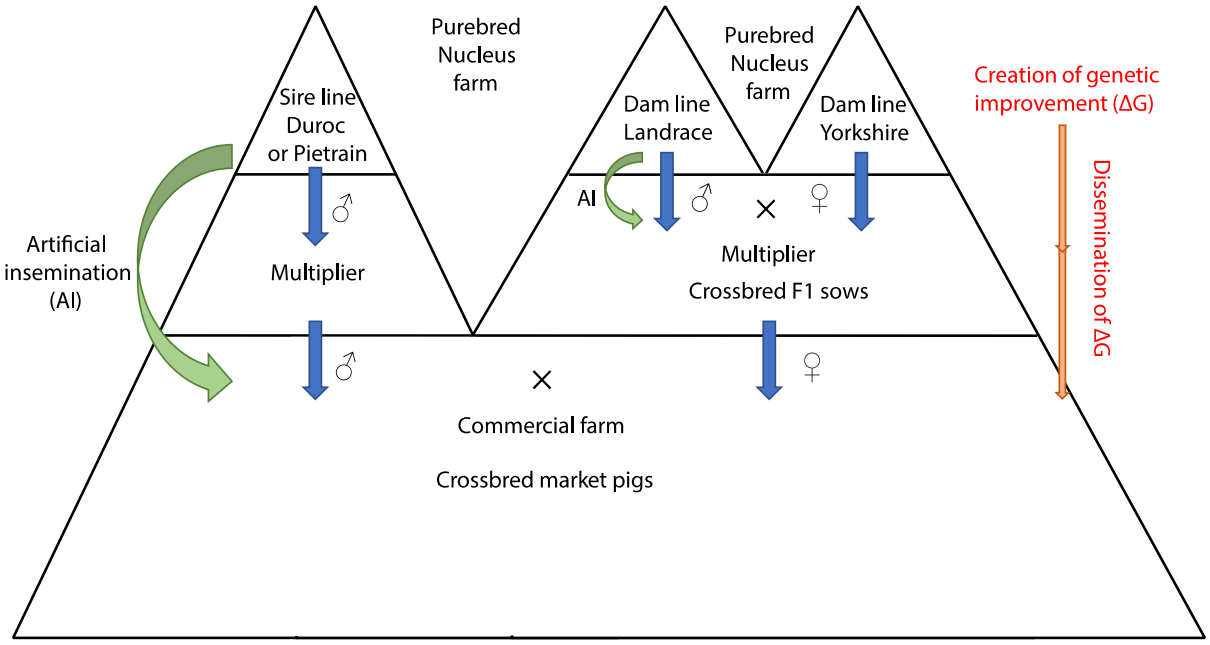

Figure 1. Pyramid breeding structure with an example of the typical three-way cross scheme.

Nucleus farms: the top tier, contain specialized purebred dam lines (e.g. Landrace and Yorkshire, selected for a combination of good female reproductive performance and growth performance) and the sire line (e.g. Duroc or Pietrain, selected for the production traits of economic importance, such as growth rate, feed efficiency, and meat quality). Sell animals (blue arrows) and semen (green arrows) to multiplier and commercial farms. **Multiplier farms**: the middle tier, multiply purebred lines from nucleus farms and cross dam lines to produce and multiply crossbred F1 sows that are sold to commercial producers. **Commercial farms:** the bottom tier cross the F1 sows with purebred sires to produce the final crossbred animals. The commercial farms contain most animals in the production pyramid.

Chapter 2. Literature review

2.1. Impact of infectious diseases in the swine industry

Nowadays, the challenge of infectious diseases in the swine industry is caused by a multitude of infectious agents that exist around the world (Zimmerman et al., 2012; VanderWaal and Deen, 2018). The emergence and global spread of pathogens are caused by the intensification and globalization of the swine industry, driven in part by frequent movements of pigs, feed, and pork products at local, national, and international scales (VanderWaal and Deen, 2018). The prevalence of infectious diseases has steadily increased morbidity and mortality in commercial herds, as well as the need for treatment programs. In addition to the loss of productivity, infectious diseases also constitute a threat to food safety, animal welfare, and international trade restrictions (Davies et al., 2009; Tomley and Shirley, 2009). The constant threat of infectious diseases results in significant economic losses for the swine industry, which in some instances (e.g. Influenza, *Streptococcus suis*, *Salmonella* spp*., Escherichia coli*) also impacts human health (VanderWaal and Deen, 2018).

VanderWaal and Deen (2018) provided a global overview of research work on swine pathogens. From 2006 to 2016, porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), influenza A virus, *Salmonella* spp., and *Escherichia coli* were recognized as the most important pathogens of swine that have been reported from nearly every country with significant losses to the swine industry (VanderWaal and Deen, 2018).

2.1.1 PRRSV

Porcine reproductive and respiratory syndrome (PRRS) caused by PRRS virus, is one of the most economically significant swine infectious diseases. PRRSV is an enveloped RNA virus, which belongs to the family *Arteriviridae*, genus *Arterivirusis* (Zimmerman et al., 1997). The

PRRSV targets the porcine monocyte/macrophage lineage, where CD163 (a macrophage-specific protein) is the essential receptor for the PRRSV infection (Welch and Calvert, 2010). PRRSV primarily replicates in pulmonary alveolar macrophages, but it has also been identified in macrophages located in other tissues, including lymph nodes, thymus, spleen, Peyer's patches, and liver of pigs (Rossow et al., 1996; Duan et al., 1997; Kavanová et al., 2017). PRRSV is highly mutagenic and resistant to low temperatures (Meulenberg, 2000). Also, it is highly transmissible and can persist for a long period (more than 100 days) in infected animals and the environment (Meulenberg, 2000; Rowland et al., 2012). Multiple methods are involved in PRRSV transmission, including aerial transmission (either short or long distance), coitus or insemination, ingestion, and contact with infected animals (Pileri and Mateu, 2016).

PRRS infection was first recognized as a distinctly new swine disease in 1987 in America, followed in Europe and Asia in 1990 to 1992, and still causes numerous outbreaks in all age groups of pigs nowadays (Zimmerman et al., 1997; Meulenberg, 2000; Schweer et al., 2017; Valdes-Donoso et al., 2018; Hancox, 2020). Clinical signs, including pyrexia, anorexia, coughing, lethargy, skin discolouration, and death, can be observed in all age groups (Hopper et al., 1992). PRRS in sows and gilts can cause reproductive failure, shown as increased abortions, mummified piglets, stillbirth levels, very weak piglets at birth, and pre-weaning morbidity (Done et al., 1996; Valdes-Donoso et al., 2018). Infected boars show loss of libido and a reduction in semen quality as PRRSV has been detected as early as 2-3 days post infection (dpi) and up to 92 dpi in semen (Hopper et al., 1992; Schulze et al., 2013). The respiratory syndrome is more often observed in young growing pigs but also occurs in naïve finishing pigs and older breeding stock associated with severe pneumonia and increased mortality (Hopper et al., 1992; Done et al., 1996). In 2006, a new PRRSV variant known as highly pathogenic PRRSV, led to a devastating

destruction to the swine industry and resulted in the culling of over 20 million pigs in China (An et al., 2010). The highly pathogenic PRRSV was rapidly spread to and affected many other countries in Southeast, East, and North Asia (An et al., 2011). In the United States, the annual cost of PRRS was estimated to be approximately $664 million, including $302.06 million in breeding herds and $361.85 million in growing pigs based on production records (2005 to 2016) from commercial farms (Haltkamp et al., 2013). The Guelph based George Morris Center on behalf of the Canadian Swine Health Board estimated PRRS was costing the Canadian swine industry a minimum of CA$130 million per year (Mussell, 2010).

2.1.2 PCV2

PCV2 is a non-enveloped DNA virus, a member of the family *Circovoridae*, genus *Circovirus*. It has a strong resistance to both chemicals (lipid-dissolving disinfectants based on alcohol, chlorhexidine, iodine, and phenol) and temperatures and is able to remain in the environment for extended periods, thus increases the risk of infection caused by the contamination of farm facilities (Segalés et al., 2005). PCV2 can be shed in respiratory and oral secretions, urine, and feces of infected pigs (Gillespie et al., 2009). The main routes of viral transmission involve oral and nasal contact with infected feces and secretions, and direct contact with infected pigs (Bolin et al., 2000; Magar et al., 2000). PCV2 infection resulting in severe porcine circovirus associated disease (PCVAD) characterized by high morbidity, high mortality, and decreased growth efficiencies has been reported from nearly every country with a significant commercial production industry (Madson et al., 2009). In addition, PCV2 has also been occasionally identified to cause reproductive failure (Madson and Opriessnig, 2011). In the United States, the disease has cost producers an average of $4/pig, with peak losses ranging up to $20/pig (Gillespie et al., 2009). In the United Kingdom, the cost was estimated to range from

£8.1/pig (~$11.26, subclinical pigs that reach slaughter age) to £84.1/pig (~$116.92, infected pig that die) (Alarcon et al., 2013). In 2007, Agriculture and Agri-Food Canada (AAFC) announced a contribution of CA$76 million from 2007 to 2014 to combat PCVAD and support activities related to biosecurity, research, and long-term disease risk management solutions (AAFC, 2015).

2.1.3 Influenza A viruses

Influenza A viruses cause a zoonotic viral disease that represents health and economic threats to both humans and animals worldwide (Vincent et al., 2008). Influenza A viruses are a group of closely related RNA viruses with the segmented genome, belong to the family *Orthomyxoviridae*, genus *Alphainfluenzavirus* (Suarez, 2016). The segmented nature of the virus genome is a key feature of the viruses to infect a variety of species (e.g., humans, pigs, birds), which supports the continued molecular evolution and the generation of new antigenic variants (Cheung and Poon, 2007). In other words, when the cells are infected with multiple influenza viruses, RNA segments can be exchanged between the viruses (reassortment), which allow the generation of viruses containing a novel combination of genes and result in the changes of surface hemagglutinin (HA) and neuraminidase (NA) antigens (Vincent et al., 2008; Steel and Lowen, 2014).

Swine influenza was first recognized in pigs in the United States in 1918, coinciding with the human influenza pandemic known as the “Spanish flu” (Vincent et al., 2008). Afterwards, the swine influenza caused by multiple strains, including H1N1, H1N2, H3N2, has become widely established in the swine population throughout the world, and is characterized by high morbidity (approaching 100%) and generally low mortality (< 1%) to the swine industry (Olsen, 2002). Studies indicated that the 1918 human strain was closely related to the classical swine H1N1 strain of avian origin; H3N2 contained genes derived from human, swine and avian viruses; and

H1N2 resulted from reassortment between H3N2 and H1N1 (Olsen, 2002; Reeth et al., 2004; Olsen et al., 2006). It is clear that swine influenza viruses can be transmitted between species as zoonotic agents, in addition to the "Spanish flu", a second human influenza pandemic in 2009 was found to be caused by a new strain of H1N1 resulted from reassortment of avian, swine, and human viruses (Trifonov et al., 2009). Therefore, the impact and concern of influenza A viruses are not limited to pigs, but also avian and human, and the transmission and reassortment in between. The viruses will continue to change, and newly emerging strains remain risks and potentials to cause a pandemic.

2.1.4 *Salmonella* spp. and *Escherichia coli*

Salmonella spp. are rod-shaped (bacillus), Gram-negative, zoonotic bacteria causing subclinical to severe clinical infection shown as diarrhea, dehydration and death in pigs, and also severe diarrhea and death in humans (Letellier et al., 2000; Evangelopoulou et al., 2015). Foodborne *Salmonella* serovars *enteritidis* and *typhimurium* infections are the most prevalent serovars in humans that occur worldwide, and pork is one of the important sources in European countries (Evangelopoulou et al., 2015). *Salmonella* serovar *choleraesuis* is an example of the "host restricted or pig-adapted" serovars that only affect pigs (Jajere, 2019). The serovar *choleraesuis* infection is less common today due to effective vaccination protocols, but it still causes sporadic issues shown as a porcine post weaning disease with septicaemia, enterocolitis, and pneumonia (Foley et al., 2008). Accordingly, *Salmonella* in commercial pigs has also become an important research priority over decades, although pork is not considered a major source for human salmonellosis in North America (Wilkins et al., 2010). Swine acquire *Salmonella* infection from the contaminated environment, feed, or through direct contact with infected animals as infected pigs can be carriers of *Salmonella* and shed the bacteria via the

feces intermittently for many months (Rber et al., 2016). Consuming undercooked pork and cross-contamination of consumer products during pork processing are high-risk factors for human infection (Prendergast et al., 2009). Every year, about 155,000 deaths in humans were estimated to be due to salmonellosis worldwide, and pigs were confirmed to be responsible for 10% to 20% of the cases (Hill et al., 2010; Evangelopoulou et al., 2015). In Canada, the number of reported human deaths caused by *Salmonella* was 4,953 in 2004 (Funk, 2008).

Escherichia coli are also rod-shaped Gram-negative flagellated bacteria belonging to the family *Enterobacteriaceae*, genus *Escherichia* (Nataro and Kaper, 1998). Shiga toxin-producing *Escherichia coli* (STEC, e.g., serotype O157:H7) strains are foodborne zoonotic pathogens that cause public health concerns (García et al., 2010; Tseng et al., 2014). Cattle are the most important STEC reservoir, but swine also play a role in STEC transmission and human disease as there are a few cases indicate that pork has been involved in outbreaks of STEC infection in human (CDC, 1995b, 1995a; Williams et al., 2000; MacDonald et al., 2004; Conedera et al., 2006; Trotz-Williams et al., 2012; Tseng et al., 2014). Of note, during July to October of 2014, an outbreak of 119 cases of human infections associated with the exposure to STEC (serotype O157:H7) contaminated pork products occurred in Alberta, Canada (Honish et al., 2017). In the swine industry, enterotoxigenic *Escherichia coli* (ETEC) are not regarded as zoonotic pathogens (Wasteson, 2002), but intestinal infection with ETEC in pigs is a significant concern. ETEC enter the animal by ingestion, their fimbriae adhere to specific receptors on porcine intestinal brush border epithelial cells (enterocytes) to start the process of enteric infection, and then the bacteria produce one or more enterotoxins inducing diarrhoea after the colonization (Luppi, 2017). Depending on the expression of receptors on porcine enterocytes, the ETEC infection results in neonatal colibacillosis (fimbriae F4, F5, F6, F41) and post-weaning diarrhoea (F4 and

F18), which are important causes of death occurring worldwide in suckling and weaned pigs, respectively (Francis, 2002). Outbreaks of ETEC neonatal and post-weaning diarrhea can affect a large proportion of pigs and are often recurrent in the same herds, resulting in significant economic losses due to mortality, decreased weight gain, increased costs for treatments, vaccinations, and feed supplements (Luppi, 2017).

2.1.5 Concurrent infections (Co-infections)

The diseases discussed above are caused by a particular type of infectious agent. However, co-infections associated with multiple infectious agents contributing to a more insidious condition are more frequent in pig farms. For example, the porcine respiratory disease complex (PRDC) results from combinations of infectious agents in particular viruses (e.g., PRRSV, PCV2, swine influenza virus, coronavirus) and bacteria (e.g., *Mycoplasma hyopneumoniae*, *Haemophilus parasuis*, *Streptococcus suis*, *Bordetella bronchiseptica*, *Actinobacillus suis*, *Actinobacillus pleuropneumoniae*) and frequently occurs in pigs reared under confined conditions (Opriessnig et al., 2011). When PRDC outbreaks occur, typically 30% to 70% of pigs will be affected, resulting in significant reductions in health and performance of pigs and increases in medication costs and mortality (Kavanová et al., 2017; Ouyang et al., 2019; Saade et al., 2020).

In practice, combinations of infectious agents may change over time and with new emerging pathogens, which may further complicate disease severity and management. Some pathogens act primarily and infect pigs as the first unique pathogen during the co-infection and then facilitate infection by secondary pathogens (Opriessnig et al., 2011). Many of these secondary infection pathogens can induce the production of excessive pro-inflammatory cytokines and the exacerbation of the disease during co-infections (Reeth and Nauwynck, 2000;

Thanawongnuwech et al., 2004; Brockmeier et al., 2008; Saade et al., 2020). Different types of co-infections, including virus-virus, virus-bacterium/bacterium-virus, and bacterium-bacterium (in the format of primary-secondary pathogens), have been observed in pig farms.

Virus-virus and bacteria-bacteria co-infections

PRRSV, PCV2, and swine influenza virus have been studied a lot for the virus-virus co-infections as they are ubiquitous in farms. No matter which virus acted as a primary pathogen during the dual or co-infections, higher viral loads and more severe diseases were always identified (Rovira et al., 2002; Kitikoon et al., 2009; Ramamoorthy et al., 2011; Dong et al., 2015; Sun et al., 2016; Ouyang et al., 2019).

For bacteria-bacteria co-infections, experimental studies identified that pulmonary colonization by *Mycoplasma hyopneumoniae* predisposes pigs to secondary bacterial infections, such as *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*, and resulted in more severe pneumonic lesions compared to the single infections (Amass et al., 1994; Marois et al., 2009). *M. hyopneumoniae* are essential airborne bacteria that cause the insidious bronchopneumonia known as enzootic pneumonia (EP), and also the outbreak of PRDC (Maes et al., 2011). *M. hyopneumoniae* infect only pigs and are ubiquitously found in nearly all countries. *M. hyopneumoniae* infection produces long-term colonization of ciliated epithelium in the respiratory tract, which causes damage to cilia, induces production of excessive thick mucus, reduces phagocytic efficiency of neutrophils and macrophages, suppresses innate and acquired pulmonary immunity, therefore, allow secondary bacteria to proliferate in the lungs and contribute to disease (Pieters and Maes, 2019). *Mycoplasma hyopneumoniae* co-infections with *A. pleuropneumoniae*, *P. multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis*,

Trueperella pyogenes, and *Streptococcus suis* have also been commonly observed in field outbreaks of EP (Pieters and Maes, 2019).

Virus-bacteria/bacteria-virus co-infections

For virus-bacterium co-infections, it is well-known that PRRSV infection often predisposes pigs to secondary bacterial infections, such as *Streptococcus suis*, *Haemophilus parasuis*, *M. hyopneumoniae*, *Actinobacillus pleuropneumoniae* and *Salmonella* spp. (Pol et al., 1997; Solano et al., 1997; Thanawongnuwech et al., 2000, 2004; Wills et al., 2000). It has been proposed that the destruction and decreased function of pulmonary alveolar and intravascular macrophages, the damage to the mucociliary apparatus, and the decreased function of antigen-presenting cells (dendritic cells and macrophages, vital for effective adaptive immune response) associated with PRRSV infection are likely to cause secondary bacterial infection (Brockmeier et al., 2002). However, it will need to be further explored as the pathogenesis of PRRSV is not yet fully understood. In addition to PRRSV, infection of swine influenza virus was also found to promote secondary infection by *Streptococcus suis* serotype 2 (Wang et al., 2013; Meng et al., 2015). *S. suis* are zoonotic bacteria, and serotype 2 is the most virulent one for both pigs and humans (Gottschalk and Segura, 2019). The sialic acid moiety on the capsular polysaccharide surface (tightly packed polysaccharide which forms a barrier around the bacterial cell wall) of *S. suis* serotype 2 can directly interact with swine influenza virus or the virus-infected cells, acting as a "bacterial receptor" and leading to increased bacterial adhesion to and invasion of tracheal epithelial cells (Wang et al., 2013). Although swine influenza is typically self-limited with high morbidity but low mortality, the secondary bacterial infection of *S. suis* serotype 2 can substantially increase illness and death (Dang et al., 2014; Lin et al., 2015).

Bacterium-viral co-infections are also ubiquitous in farms. For instance, *M. hyopneumoniae* infection in pigs has been found to increase the host susceptibility to PRRSV and swine influenza virus infections (Thacker et al., 1999; Yazawa et al., 2004). Moreover, *M. hyopneumoniae* was also found to potentiate the severity and the duration of pneumonia in pigs induced by the later PRRSV and swine influenza virus infections (Thacker et al., 1999; Yazawa et al., 2004).

2.1.6 Parasites

Parasites, another major type of pathogens identified in the swine industry in addition to viruses and bacteria, have not been discussed yet. Parasites infections frequently occur in the swine industry worldwide but have generally received little attention, presumably because most of them seldom cause severe clinical diseases (Roepstorff et al., 2011). However, it is essential to control and eliminated parasites infections in the swine industry because many parasites are foodborne zoonoses (Djurković-Djaković et al., 2013). Besides, parasites infections in pigs can reduce food utilization and growth rate, resulting in economic losses to the industry. *Ascaris suum* is by far one of the most prevalent pig parasites (Roepstorff et al., 2011). In addition to the above concerns, *A. suum* has been found to compromise the effect of *M. hyopneumoniae* vaccination significantly (Steenhard et al., 2009). *M. hyopneumoniae,* as mentioned above, can result in severe pneumonic lesions and respiratory disease in pigs by themselves and co-infections with other viruses and bacteria if they are not timely or adequately controlled.

2.2. Conventional control of diseases

Therefore, as the swine industry faces disease challenges caused by multiple infectious agents, disease prevention and management are essential for both animal and human health, animal welfare, and economic productivity. Conventional methods, including strict biosecurity,

vaccines, and antimicrobials, are being used to control infectious diseases. However, they are not always effective.

2.2.1 Biosecurity

Biosecurity can be defined as applying measures aimed to reduce the probability of the introduction and transmission of pathogens (Levis, 2011). Numerous factors are involved in developing and maintaining external biosecurity that protects the farm from the "threats coming from the outside" and internal biosecurity that reduces the spread of pathogens once the farm has been infected (Alarcón et al., 2021). For example, physical barriers, rules banning and restricting the introduction of certain animals, people, and vehicles are some necessary external biosecurity measures (Levis, 2011; Alarcón et al., 2021). In addition, the farm location is also an essential part of external biosecurity related to the spatial clustering of infections, as the transmission can also occur among neighbours, especially for airborne pathogens (e.g., PRRSV) (Laffan et al., 2011; Rosendal et al., 2014). Feed and water are also important factors that need to be included in external biosecurity as they could also be sources of pathogen introduction (Dee et al., 2016; Cochrane et al., 2017; Silva et al., 2018; Alarcón et al., 2021). Management of herd, hygiene of facilities, cleaning and disinfection, and experienced workers who know and follow the work routines and biosecurity measures are examples of internal biosecurity (Alarcón et al., 2021).

Nowadays, the swine industry is developing towards high concentration and globalization, seen as bigger farms and larger commercial pig populations in fewer hands together with an increasing need of movements of pigs, feed, and pork products at local, national, and international scales (Alarcón et al., 2021). Within this frame, the emergence and introduction of new pathogens into farms and countries currently not affected and can have serious and even catastrophic consequences (Alarcón et al., 2021). For example, even with

biosecurity in pig farms, the porcine epidemic diarrhea (PED) virus spread from China to the United States in 2013 (Stevenson et al., 2013). The PED virus had impacted ~50% of the United States breeding herds within one year, resulting in the deaths of at least seven million piglets (Stevenson et al., 2013). More recently, millions of pigs were culled in Asian countries to stop the spread of the African swine fever virus that emerged in China in 2018, which was devastating to the swine industry (Sánchez-Cordón et al., 2018; Zhou et al., 2018). Therefore, effective design of a cost-effective biosecurity program and its continuous improvement are still challenging many pig farms due to the gaps in knowledge of epidemiology of different diseases, including how diseases are transmitted, their risks and importance, and which measures are thought to be more effective and how to evaluate the biosecurity (Alarcón et al., 2021).

2.2.2 Vaccines

For diseases that threaten the swine industry, effective vaccination is an essential tool for herd immunity and disease control. However, vaccines cannot always protect all animals. There are many reasons why vaccines fail, such as the limited knowledge of epidemiology of diseases, the continuous change of genetic makeup of viruses, the impact of co-infections, and environmental and management causes (e.g. vaccine handling and storage, programmatic management, and disease surveillance).

For instance, as discussed above, PRRSV has caused massive losses to the swine industry, and effective vaccines are not yet found, although at least 20 PRRSV vaccines are commercially available worldwide (Rowland et al., 2012). Like other RNA viruses, PRRSV are constantly evolving to adapt to existing immunity and re-emerging as new variants to cause new outbreaks continuously, making it hard to develop effective vaccines (Nan et al., 2017). Inactivated virus and modified-live virus are two approaches used for developing PRRS vaccines

(Rowland et al., 2012). However, the inactivated virus vaccines are generally not effective (Rowland et al., 2012). The modified-live virus vaccines can only effectively protect pigs challenged with a genetically similar (homologous) virus and provide little or even no protection against heterologous PRRSV isolates that are genetically diverse (Huang and Meng, 2010; Murtaugh and Genzow, 2011). The effectiveness of a vaccine against heterologous strains largely depends on the antigenic and genetic relatedness of the virus strain to which the vaccinated animals were exposed. Therefore, given the high degree of genetic diversity among PRRSV, it is unlikely that a vaccine based on a single strain will confer adequate protection against the antigenically and genetically diversified PRRSV strains currently circulating in swine herds worldwide. The genetic diversity of PRRSV will continue to be the major obstacle for PRRS control (Huang and Meng, 2010). Furthermore, the immunosuppressive properties of PRRSV to circumvent immune responses can also interfere with vaccine efficacy (Kimman et al., 2009). For example, the increased production of interleukin (IL)-10, a potent immunosuppressive cytokine that can interact with immune cells, including monocytes and lymphocytes, has been observed following PRRSV infection (Thanawongnuwech and Suradhat, 2010). Some strains of modified-live PRRSV vaccines also induced IL-10 production and resulted in inhibition of immune responses in vaccinated pigs (Royaee et al., 2004; Thanawongnuwech and Suradhat, 2010). Meanwhile, the practical experience of currently licensed modified-live PRRSV vaccines has revealed numerous safety concerns, such as shedding of modified-live virus, reversion of live-attenuated vaccines to virulence, and recombination between field strains and modified-live virus (Nan et al., 2017).

Although PCV2 vaccines have high efficacy in reducing associated clinical signs, it is of concern whether the current vaccines would be sufficient to eliminate a series of new PCV2

variants that have also emerged during the same period (Afghah et al., 2017). The co-infection with other pathogens is also a challenge for the efficacy of vaccines. For example, swine influenza virus vaccination in the absence of PRRSV significantly reduced pneumonia and viral load, but the presence of PRRSV can reduce vaccine efficiency significantly when co-infection occurs (Kitikoon et al., 2009). Moreover, the effect of *M. hyopneumoniae* vaccination has also been found to be significantly compromised by the presence of the parasite *A. suum* (Steenhard et al., 2009).

2.2.3 Antimicrobials

Antimicrobials, including antibiotics, antivirals, antifungals, and antiparasitics, are medicines used to prevent and treat infections in humans, animals, and plants (WHO, 2020). Antimicrobials have been used routinely in farm animal production since the 1950s for not only prevention (prophylaxis) and treatment of infections but also for controlling the spread of infection (metaphylaxis) and improvement of feed efficiency and promotion of animal growth (Lekagul et al., 2019). However, antimicrobial resistance (AMR) occurs when pathogens change over time and no longer respond to antimicrobials (WHO, 2020). Nowadays, the AMR is recognized as a "One Health challenge" (**Figure 2**) because of the rapid emergence and dissemination of drug-resistant pathogens and genes among humans, animals, and the environment on a global scale (King et al., 2008; Lammie and Hughes, 2015; Rousham et al., 2018). This AMR threatens our ability to treat infections, especially the rapid global spread of multi- and pan-resistant bacteria (known as "superbugs") that are not treatable with existing antimicrobials (WHO, 2020). In animals, many countries, including the United States, Canada, and Australia, have implemented policies and regulations that medically important antimicrobials for veterinary use in animals can only be sold by prescription only, and the use of

antibiotics for growth promotion has been banned in the European Union since 2006 (Lekagul et al., 2019).

Nevertheless, antimicrobials that have been recognized as critically and highly important in humans by WHO are still commonly used for prophylaxis and treatment in the swine industry. Penicillins (highly to critically important in the WHO list) and Tetracyclines (highly important in the WHO list) are the most used antimicrobials in pigs worldwide due to their cost-effectiveness compared to other antimicrobials (Lekagul et al., 2019; WHO, 2019). This continues to raise global concerns and requires the swine industry to use antimicrobials with great care by employing principles of antimicrobial stewardship and utilizing alternatives where possible (Lammie and Hughes, 2015).

Overall, the impact of infectious diseases and the concerns of using conventional control of diseases highlight the potential vulnerability of the swine industry worldwide and the importance of managing infectious diseases. With the emergence and re-emergence of difficult-to-control diseases, conventional methods need to be continuously improved. Meanwhile, additional methods need to be explored to improve the cost-effectiveness of preventing and controlling infectious diseases in the swine industry.

2.3. Infection and host immune responses

To explore methods for the prevention and control of infectious diseases, it is important to have a general understanding of the epidemiology of infectious diseases and host immune responses here since simple interventions may break the chain of transmission. In epidemiology, infection is defined as the entrance and development of infectious agents in an animal body, whether it develops into clinical infection with apparent symptoms or subclinical infection that does not produce noticeable symptoms (Barreto et al., 2006). Infectious agents or pathogens,

including bacteria, viruses, fungi, parasites, and prions, can be transmitted vertically from an infected individual to its offspring or horizontally from an infected organism to a susceptible contemporary animal by a variety of mechanisms, including: direct contact transmission with infected individuals; indirect contact transmission with inanimate objects, called fomites, that become contaminated by pathogens from infected individuals and or reservoirs; vehicle transmission of pathogens through water, food, and air; and vector-borne transmission that living organisms can carry and transmit pathogens from one host to another (Fèvre et al., 2006). The negative consequences and illness caused by pathogens and their toxic products that result from infection are defined as infectious diseases (Barreto et al., 2006; Bishop and Woolliams, 2014). The outcome of infection in vertebrates largely depends on the immune system to prevent the invasion of pathogens into the body and induce efficient immune defence to expel and eliminate infective pathogens that have invaded the body.

The immune system is comprised of two distinct but interrelated branches: innate and adaptive (acquired) immunity. Both branches involve a complex of cellular and humoral components that work in concert for defence against the pathogens (Nelson and Williams, 2014; Hermesch, 2014).

2.3.1 Innate immunity

The innate immune system is the first line of defence against pathogens. It plays a crucial role in maintaining homeostasis, preventing microbe invasion, eliminating a great variety of pathogens, and contributing to the activation of the adaptive immune response (Romo et al., 2016).

The skin and mucosal surfaces are external barriers in the innate immune system that provide physical (stratified epithelial layers with tight junctions), chemical (lysozyme and

antimicrobial peptides), and cellular (epithelial cells thickening and cellular hyperplasia) effectors to prevent most pathogens from gaining access to the body (Delves and Roitt, 2000; Parham, 2014; Romo et al., 2016). Also, immature dendritic cells (Langerhans cells) and intraepithelial T lymphocytes found in the epithelial layer are cellular effectors that could prevent pathogen invasion, capture antigens, and prevent local infection (Hayday et al., 2001; Matsui and Amagai, 2015; Romo et al., 2016).

When pathogens breach the skin and mucosa barriers, the innate immune system is designed to mount immediate and antigen-non-specific cellular and humoral responses within minutes (Turvey and Broide, 2010; Hoffmann and Akira, 2013). The cellular responses of the innate immunity are provided by a variety of leukocytes (white blood cells), including myeloid phagocytic cells and non-specific cytotoxic cells (Janeway, 2001; Abbas et al., 2015; Romo et al., 2016). Myeloid phagocytic cells, including monocyte-macrophages, neutrophils, basophils, and eosinophils, are professional phagocytes that phagocytose and destroy pathogens and secrete immune mediators, such as cytokines, histamine, reactive oxygen or nitrogen species, lysozyme, and antimicrobial peptides (Abbas et al., 2015; Romo et al., 2016). Natural killer cells are non-specific cytotoxic cells, which play an essential role in antiviral defence and immune surveillance by secreting cytokines and chemokines and inducing apoptosis of virus-infected cells (Caligiuri, 2008). Besides, humoral responses are provided by the complement system, cytokines, natural antibodies, and acute phase proteins, which augments cellular defences to deliver innate immunity (Turvey and Broide, 2010). The complement system comprises a large number of distinct plasma proteins that react with one another to opsonize pathogens (the immune process which uses opsonins to tag pathogens for elimination by phagocytes), enhance inflammatory responses, and form the membrane attack complex on the cell wall of the bacteria

that helps to fight infection (Janeway, 2001). Cytokines, including interferons, interleukins, tumour necrosis factors, and transforming growth factors, are small secreted proteins released by many cell populations, but the predominant producers are leukocytes, including helper T lymphocytes and macrophages (Zhang and An, 2007). Both pro-inflammatory cytokines and anti-inflammatory cytokines can mediate the communication between cells and are essential for immune cell development, immunoregulation, and immune effector functions (O'Shea et al., 2002). Natural antibodies and acute phase proteins circulate in the plasma and play crucial roles in innate immunity and host defence. The natural antibodies are pre-existent antibodies of the immunoglobulin M (IgM) isotype synthesized by B lymphocytes in the absence of pathogens, which provide immediate and broad reaction against pathogens (Baumgarth et al., 2005; Romo et al., 2016). Acute phase proteins are a class of proteins whose plasma concentrations increase or decrease in response to inflammation (Jain et al., 2011). Acute phase proteins are primarily synthesized by hepatocytes, which are a part of the acute phase response involved in a complex systemic reaction to re-establish homeostasis and promote healing in response to stimuli, including infection, stress, and inflammation (Cray et al., 2009).

Various mechanisms work together in the innate immune system to provide the rapid sensing and elimination of pathogens. However, sometimes the innate immunity is insufficient to eradicate infection. For example, the overwhelming variability of antigen structures and some pathogens have also devised many mechanisms to evade the innate immune system through an evolutionary "arms race" with the hosts (Bailey et al., 2013).

2.3.2 Adaptive (acquired) immunity

Regarding the insufficiency of innate immunity, adaptive immunity with specificity and memory for any individual agent has evolved in jaw-vertebrates, including all terrestrial

vertebrates, to express itself when the infection cannot be controlled or eradicated by the innate immune responses (Rolff, 2007; Chaplin, 2010).

The adaptive immunity takes days or even weeks to be established, which is much longer than the innate immune response, but is more specific to individual pathogens and provides memory for rapid response in the event of re-exposure to the specific pathogens (Molnar and Gair, 2015). The cell-mediated immune response and the humoral immune response are the two arms of adaptive immunity carried out by T lymphocytes and B lymphocytes, respectively. Cytotoxic T cells are major players in the adaptive cell-mediated immune response, which directly kill infected cells by apoptosis and release cytokines to amplify the immune response (Janeway, 2001). B lymphocytes are activated and differentiate into antibody-secreting plasma cells involved in the humoral immune response (Janeway, 2001). The specificity of the adaptive immune response is based on the antigen-specific receptors expressed on the surface of T and B lymphocytes (Chaplin, 2010). These antigen-specific receptors are encoded by genes assembled by somatic rearrangement of germline gene elements to form millions of different T-cell receptors and immunoglobulin (B-cell antigen receptor) genes, each with potentially unique specificity for an antigen (Chaplin, 2010). The memory of the adaptive immune system is handled by producing long-lived and antigen-specific T and B memory cells that persist in a dormant state but can re-express effector functions rapidly after re-encountering their specific antigens (Chaplin, 2010; Molnar and Gair, 2015).

Although the innate and adaptive immune systems are described separately here, they communicate and cooperate to enhance the chance of eliminating a great variety of pathogens. The innate immune system is not just a primitive and first-line defence but also an “ingenious doorbell” that awakens the adaptive immune response (Yatim and Lakkis, 2015). The innate

immune system contains antigen-presenting cells (APCs, e.g., macrophages and dendritic cells) that detect and engulf pathogens and then inform the adaptive immune response about the presence of antigens (Molnar and Gair, 2015). After processing by APCs, the antigens are loaded onto major histocompatibility complex (MHC) class I in the endoplasmic reticulum or MHC class II in the specialized vesicular compartment and then are transported to the cell surface for antigen presentation (Molnar and Gair, 2015). Naïve $CD8^+$ and $CD4^+$ cells bind antigen-presenting cells via the antigen-embedded MHC class I and MHC class II molecules on cell surfaces and are simulated to become cytotoxic T lymphocytes and helper T lymphocytes, respectively (Molnar and Gair, 2015). Helper T lymphocytes are essential cells for both cell-mediated and humoral adaptive immune responses, which comprise two major populations, T_H1 and T_H2 (Janeway, 2001). T_H1 cells are involved in the cell-mediated immune response as they help the activity of cytotoxic T cells and secrete cytokines to inform more target cells about the pathogenic threat (Janeway, 2001; Abbas et al., 2015). T_H2 cells are involved in the humoral immune response and help activate B lymphocytes to defend against pathogens via antibody secretion (Janeway, 2001; Abbas et al., 2015). The adaptive immune system, in turn, amplifies its responses by recruiting the components of the innate immune response for a fully effective immune response.

2.3.3 Immune system and performance

A well-functioning and tightly regulated immune system plays essential roles in maintaining performance and preventing death from the infection by investing nutrient resources appropriately in immune defences and optimizing the competing demands for investment in other body functions when infection occurs (Calder, 2013; McDade, 2005). In the presence of infection, the immune system becomes increasingly active, which results in a significant increase

in the demand for nutrients (Calder, 2013). For example, amino acids are required to synthesise cytokines and antibodies and play important roles in the proliferation, metabolism of immune cells (Li et al., 2007). Therefore, adequate nutritional status is necessary for the normal functioning of various components of the immune system (Coop and Kyriazakis, 2001; Nelson and Williams, 2014). Any changes in resource demands by the immune system can create significant differences in the level of fitness and performance of an animal. Ecological immunologists proposed that the energetic cost associated with immune responses requires a trade-off among other physiological processes that are energy demanding (Ayres and Schneider, 2012). In addition to the endogenous energy and nutrient pools of an animal, diet is a primary exogenous source of energy to meet these demands. Although pigs are raised in controlled and nutrient-rich environments with a well-formulated diet, the course of infection could adversely affect nutritional status and cause undernutrition by infection-induced cachexia with the loss of appetite and the reduction of feed intake (Exton, 1997; Adamo et al., 2010, Calder, 2013). Moreover, the infection can further impair nutritional status by damaging the intestinal wall, causing diarrhea or vomiting, which results in nutrient malabsorption and loss (Calder, 2013).

When nutrient resources to the animal are limited, a trade-off is expected to occur between mounting an immune response and other body functions, such as growth, reproduction, and thermoregulation (Lochmiller and Deerenberg, 2000; McDade, 2005; Doeschl-Wilson et al., 2009; Rauw, 2012). The infection can become chronic if it is not eliminated quickly or effectively due to immunodeficiency in which the ability of immune system to fight against pathogens is compromised by the intrinsic defects in the immune system (primary immunodeficiency), or extrinsic factors (secondary immunodeficiency), such as limited nutrient resources in infected pigs (Bourke et al., 2016). In addition, the immunosuppressive properties of

PRRSV, such as the induction of lymphopenia and apoptosis, increased production of immunosuppressive cytokine IL-10, interferences with the induction of proinflammatory response and antigen presentation, etc., can result in persistent infection and secondary immunodeficiency (Kimman et al., 2009). Therefore, malnutrition may result from a prolonged infection that further compromises the immune system, leading to a more severe disease state and increased susceptibility to other pathogens (Nelson and Williams, 2007; Hine et al., 2014). In turn, the infection could further influence nutritional status by cachexia, reduced nutrient absorption, losses of both endogenous and exogenous nutrients, and increased nutrient requirements for immune responses (Calder and Jackson, 2000; Calder, 2013). Thus, malnutrition, immunodeficiency, and increased susceptibility to infectious diseases reinforce one another and are locked into a cyclical relationship by a bidirectional interaction (Calder and Jackson, 2000; Nelson and Williams, 2007). Such susceptible animals are expected to show poor performance or even death due to reaching a cachectic state.

Conversely, some animals can show a relatively undepressed performance in the face of infection or even maintain a healthy status in the presence of pathogens by employing appropriate immune responses and biological strategies. Advances in genetics and genomics have highlighted the potential for genetic control strategies to manage the effect of infectious disease and maintain high health and or performance level in pigs (Doeschl-Wilson and Kyriazakis, 2012). Disease resistance, tolerance, and resilience have been discussed as alternative host defence strategies for coping with pathogens and maintaining high health and or performance in response to infectious challenge, which could be tackled by genetic improvement (Doeschl-Wilson and Kyriazakis, 2012).

2.4. Disease resistance

Host disease resistance has been invariably discussed as a high priority trait that can be further investigated for infectious disease control. It is apparent that different studies and authors have different interpretations of the term "disease resistance" (Bishop, 2012). Of note, a "narrow sense" and a "broad sense" of disease resistance have been commonly used to define resistance from a livestock viewpoint. The following two sections describes the research in pigs regarding the "narrow sense" and the "broad sense" definitions of disease resistance, respectively.

2.4.1 A "narrow sense" definition of disease resistance: complete resistance

Complete resistance is regarded as a "narrow sense" definition of disease resistance because it describes a special situation where an animal has the ability to maintain completely healthy status when challenged by infection. The most apparent cause of complete resistance is the non-adhesion of the pathogen when the target tissue or cells of an animal do not express the receptors, which stops the first step of establishing the infection (Plastow, 2016). Therefore, complete resistance is the most cost-effective mechanism of preventing infection from the host's perspective. There is no need to increase energy expenditure on the immune system as resistant animals can maintain a healthy status and avoid infection when exposed to pathogens (Plastow, 2016; Burkard et al., 2017).

In pigs, complete resistance has been identified to ETEC expressing F18 (ETEC F18) fimbriae. Infection of ETEC F18 results in post-weaning diarrhea in susceptible pigs characterized by sudden death, diarrhea, dehydration, and growth retardation in surviving piglets, which are economically important diseases in pig production worldwide (Rhouma et al., 2017). A single nucleotide polymorphism (SNP) at bp 307 (G/A) in the fucosyltransferase gene (*FUT1*) was found to be associated with the complete resistance to infection with ETEC F18 (Meijerink et al., 1997, 2000; Bao et al., 2012). Pigs that had homozygous resistant alleles were completely

resistant to ETEC F18 due to the absence of receptors for attachment of the bacterium on the small intestinal epithelium (Meijerink et al., 1997, 2000; Bao et al., 2012).

In addition to the inherited ability of being completely resistant to ETEC, genome editing technology has been emerging to provide opportunities to create resistance to other pathogens in pigs. The protein encoded by the *cluster of differentiation 163* (*CD163*) gene has been identified as a definitive fusion receptor for PRRSV expressed on cells of the macrophage lineage (Calvert et al., 2007; Breedam et al., 2010; Welch and Calvert, 2010; Whitworth et al., 2016; Yang and Wu, 2018). Therefore, PRRSV-resistance pigs have been explored using CRISPR/Cas9 to modify the CD163 gene (Wells et al., 2016; Whitworth et al., 2016). CRISPR/Cas9 originally known as an adaptive immune system in bacteria and archaea to defend against the invasion of foreign genetic elements through DNA or RNA interference, has been adapted for mammalian gene editing (Gasiunas et al., 2012; Jinek et al., 2012; Wiedenheft et al., 2012). Among the *CD163*-modified pigs, those with *CD163*-null phenotype macrophages generated by the knockout of the *CD163* gene were completely resistant to several isolates of both type 1 and type 2 PRRSV (Wells et al., 2016; Whitworth et al., 2016). However, as CD163 plays multiple biological functions, including the clearance of hemoglobin in blood plasma and participation in anti-inflammation, the knockout of the *CD163* gene may have a negative impact on the animal (Onofre et al., 2009; Gorp et al., 2010). Therefore, a precision modification was used to only delete Exon 7 of the *CD163* gene, encoding the scavenger receptor cysteine-rich domain 5 (SRCR5), whereby the SRCR5 is an interaction site for PRRSV infection with no other biological functions identified (Wells et al., 2016; Burkar et al., 2017). Their results indicated that pigs lacking the *CD163* SRCR5 domain were fully resistant to both type 1 and type 2 PRRSV genotypes, and no adverse effects had been identified on growth rate or immune cell

counts of the genome-edited pigs (Burkard et al., 2017).

Selective breeding or genomic editing for complete resistance to infection seems a promising way to maintain an animal's health status and avoid infection. However, such complete resistance may only be present in the face of a single type or an isolate/strain of the infectious agent, which requires further exploration for co-infections caused by multiple pathogens in the swine industry.

Regarding the multiple pathogens in the swine industry, Xu et al. (2020) created the *CD163* SRCR5 domain and the porcine aminopeptidase N (*pAPN*) double-knockout pigs to PRRSV and transmissible gastroenteritis virus (TGEV) infections. In addition to PRRSV, TGEV is globally distributed and causes tremendous economic losses in pork production characterized by vomiting, severe diarrhea, dehydration, and a high mortality rate (~100%) of infected piglets under the age of 14 days (Xu et al., 2020). The function of the *CD163* SRCR5 domain in PRRSV infection has been discussed above. The pAPN protein on the surface of small intestinal epithelial cells is the receptor for binding TGEV glycoproteins and mediating infection (Delmas et al., 1992). *pAPN* knockout pigs have already been identified to be completely resistant to TGEV (Luo et al., 2019; Whitworth et al., 2019). The *CD163* SRCR5 domain and *pAPN* double-knockout pigs were resistant to both PRRSV and TGEV, and the genome-edited pigs reproduced and produced meat at the same level as the control pigs (Xu et al., 2020).

Overall, given the high efficiency and low cost of genome editing tools, particularly CRISPR/Cas9, genome editing holds vast promise for the future production of animals resistant to diseases, although its efficacy for more complex situations in the swine industry with other pathogens will need to be further explored (Ruan et al., 2017; Proudfoot et al., 2019). The two major hurdles in implementing genome-editing technology are consumer acceptance and the

regulatory framework (Proudfoot et al., 2019). Approval of genome-edited pigs for human consumption relies on national and multinational legislation, which is currently still at an early stage (Proudfoot et al., 2019).

2.4.2 A "broad sense" definition of disease resistance

Back to the strategy of selective breeding for resistance, since naturally occurring complete resistance is not commonly identified, the term "disease resistance" is often loosely used. Therefore, the term "disease resistance" (refers to the "broad sense" definition from now on in the thesis) is commonly defined as an animal's ability to prevent pathogen entry and to control the pathogen lifecycle within a host (Bishop and Stear, 2003; Bishop and Morris, 2007; Råberg et al., 2007, 2009; Bishop, 2012). In addition to the barriers to pathogen entry, disease resistant animals may employ active innate and adaptive immune responses which work by detection, neutralization, or destruction of pathogens to restrict the proliferation of pathogens and result in a reduction of pathogen burden (Bishop and Woolliams, 2014; Glass, 2012). Therefore, improving disease resistance could reduce disease prevalence or may have the potential to lead to disease eradication. Here, disease resistance has been recognized to be a relative rather than an absolute status as is the case for the "narrow sense" definition (section 2.4.1). In order to compare the level of disease resistance among animals, pathogen burden, including fecal egg count, viremia (viral load), or bacterial load, is often used to measure resistance for animals infected with parasites, viruses, or bacteria, respectively (Bishop, 2012). For example, the viral load for PRRSV infection in pigs was measured using a cumulative statistic quantification of repeated measures of viremia throughout the infection (Boddicker et al., 2012). Therefore, more PRRS resistant animals were expected to have a lower viral load based on cumulative measures.

Numerous studies have demonstrated host genetic variation for disease resistance. In

pigs, a major focus has been on PRRSV as it is the causative agent of the major endemic disease globally (see **Section 2.1.1**). Differences in PRRSV disease resistance were firstly found among breeds. A Hampshire × Duroc synthetic line was found to have higher viremia at 4, 7, and 14 dpi with PRRSV than a Yorkshire × Landrace line (Petry et al., 2005). Subsequently, higher PRRSV viremia was observed in Pietrain pigs than Yorkshire pigs (Doeschl-Wilson et al., 2009). These results together demonstrated the existence of a genetic basis of the disease resistance to PRRSV infection at a breed level. More studies were then conducted to further explore the variation of disease resistance among individuals. Using commercial crossbred pigs under a nursery PRRSV challenge model, Boddicker et al. (2012) and Hess et al. (2016) measured the overall viremia from 0 to 21 dpi for the PRRSV isolates of KS06 and NVSL for each pig and found that the viral loads (cumulative measures of viremia from 0 to 21 dpi) were moderately heritable (NVSL: 0.31 ± 0.06; KS06: 0.51 ± 0.09). They also identified negative genetic correlations between viral load and weight gain for NVSL (-0.74 ± 0.10) and KS06 (− 0.52 ± 0.06), suggesting that disease resistant pigs also tend to grow faster than susceptible pigs when challenged with PRRSV (Hess et al., 2016; Dekkers et al., 2017).

Genome-wide association studies (GWAS) found a region on swine chromosome 4 (SSC4) was associated with both viral load and weight gain following PRRSV infection (Boddicker et al., 2012). Multiple candidate genes within this region encode a group of guanylate binding proteins (GBPs) that were known to be involved in the innate immune response (Boddicker et al., 2012). A SNP, WUR10000125 (WUR), was found to explain 13.2% and 9.1% of the genetic variance for viral load and growth, respectively (Boddicker et al., 2012, 2014; Hess et al., 2016). Further studies about this region found the truncated GBP5 was associated with the AA genotype for the WUR locus, which is an unfavourable genotype and may reduce an

animal's ability to inhibit viral entry and replication (Koltes et al., 2015; Schroyen et al., 2016). In addition to growing pigs, the genetic basis for disease resistance has also been observed by assessing the viral load in the thymus of fetuses from pregnant gilts challenged with PRRSV and the viral load in the endometrium of challenged pregnant gilts (Yang et al., 2016). Overall, these results unravel the genetic basis of disease resistance in response to the PRRSV challenge and provide an opportunity for genetic improvement of disease resistance in the pig industry. Although the animals are not completely resistant in this case, it may still help to select pigs that are less susceptible to PRRSV. Meanwhile, the low pathogen burden could also benefit other population members by reducing the transmission of infection (Bishop and Woolliams, 2014).

Before applying selection for disease resistance in practice, one aspect that needs to be considered is the potential for increasing host disease resistance to fuel the arms race between host and pathogen and stimulate pathogen evolution toward higher virulence and multiple ways of evasion from the host immune system (Doeschl-Wilson and Kyriazakis, 2012). Moreover, studies of disease resistance are often pathogen-specific. The genetic basis for an animal to be disease resistant under the challenge caused by multiple pathogens in the field remains mostly unknown (Doeschl-Wilson and Kyriazakis, 2012). It has been suggested that selecting pigs to be more disease resistant to a specific pathogen may have some serious drawbacks for their health. For example, Hine et al. (2014) indicated that the selection of animals based on their disease resistance to a specific pathogen might inadvertently increase the susceptibility to other pathogens, which may involve the resource allocation and trade-off between the cell-mediated and humoral-mediated immune responses. An inverse relationship between antibody production and macrophage activity was identified in mice selected for resistance to *Leishmania tropica* and also in cattle selected for resistance to *Brucella abortus* (Hale and Howard, 1981; Price et al.,

1990). Subsequently, cell-mediated and antibody-mediated immune responses were found to have a negative genetic correlation in dairy cattle (Thompson-Crispi et al., 2012). The selection of resistant animals with a strong humoral-mediated immune response to extracellular pathogens might inadvertently increase their susceptibility to intracellular pathogens controlled by cell-mediated immune responses (Thompson-Crispi et al., 2012).

2.5. Disease tolerance

Concerning the potential for fueling the arms race between host and pathogen with the selection of "broad sense" disease resistance and the limitation of disease resistance mechanisms which are often pathogen-specific, disease tolerance is proposed as an alternative host defence strategy for coping with infectious challenge that could be targeted for genetic improvement. In contrast to disease resistance, disease tolerance is defined as an animal's ability to mitigate the detrimental impact and possible damages to the host caused by persistent infection and or immunopathology under a given pathogen burden but does not exert any direct negative effect on pathogens (Ayres and Schneider, 2012; Bishop, 2012; Doeschl-Wilson and Kyriazakis, 2012; Nakov et al., 2019). According to the definition, disease tolerance mechanisms may avoid stimulating pathogen evolution toward higher virulence and are more host than pathogen specific and therefore do not necessarily exert a direct effect on pathogens. Improving disease tolerance is unlikely to lead to disease eradication as disease tolerant animals can harbour high pathogen burden without showing obvious symptoms or significant drops of performance and productivity. These disease tolerant animals will act as "super-spreaders" and inadvertently infect susceptible animals on the farm, national, and international scales through direct and indirect contacts, movements of pigs, feed, and pork products or even infect humans if there are zoonotic pathogens. However, it may be more beneficial to improve disease tolerance rather than

resistance where individuals are exposed to multiple pathogens (e.g. in commercial pig farms), where high risk of pathogen evolution exists, and where disease eradication has proven difficult with the present of asymptomatic carriers (Doeschl-Wilson and Kyriazakis, 2012).

2.5.1 Possible biological and immunological mechanisms involved in disease tolerance

Since "tolerance" has also been used to describe the core property of the immune system, it is essential to not confuse "disease tolerance" with the equally important concept of "immune tolerance" (Soares et al., 2017; King and Divangahi, 2019). Although some of the mechanisms regulating immune tolerance and disease tolerance may be functionally related, these two mechanisms are distinct (Soares et al., 2017). Immune tolerance is an active process that eliminates or suppresses the activation and proliferation of antigen-specific lymphocytes based on immunoregulatory mechanisms (Soares et al., 2017). Therefore, immune tolerance plays a vital role in the immune discrimination between self and non-self and the regulation of host-commensal interactions (Soares et al., 2017; King and Divangahi, 2019). Current understanding of disease tolerance is limited but seems to revolve around evolutionarily conserved stress and damage responses that confer tissue damage control to maintain homeostasis and functional integrity of tissues in the infected host **(Figure 3)** (Soares et al., 2014, 2017; Shourian and Qureshi, 2019).

Stress responses emerged at an early stage of evolution as the means to provide adaptation and preserve host homeostasis to environmental changes (Soares et al., 2017). In the face of persistent infection, stress responses are triggered through the engagement of stress sensors that monitor the disturbance imposed on host cells, such as microbial toxins, hyperoxia and hypoxia, oxidative stress, osmotic stress, and metabolic stress caused by infection and aberrant immune responses (Soares et al., 2014). Once these parameters of stress change beyond

a certain threshold, stress sensors set off signal transduction pathways to alert cells and to trigger metabolic adaptation, which preserves core cellular functions at the expense of accessory ones while preventing macromolecular and organelle damage in host cells (Kültz, 2005; Soares et al., 2017).

Stress responses and metabolic adaption are sometimes insufficient to prevent the stress from causing cellular damage when the infection continuously persists in strength and time. Persistent infection could lead to cellular damage, including macromolecular damage of DNA, proteins, lipids, and eventually, organelles caused by both pathogens and immunopathology (Medzhitov et al., 2012; Soares et al., 2017). Therefore, the corresponding damage responses are triggered to repair the damage and maintain essential cellular functions at the expense of accessory ones (Soares et al., 2014). Meanwhile, tissue damage control is activated by stress and damage responses, which work through various mechanisms to reinforce the epithelial barrier, neutralize pathogen toxin and virulence factors, and regulate the intensity and duration of the host immune and inflammatory responses to establish disease tolerance (Medzhitov et al., 2012; Soares et al., 2014, 2017). Although the disease tolerance mechanisms cannot control the pathogen burden in an infected host, a high level of tolerance may be sufficient for the host to prevent disruptions of physiological functions and eventually establish a state of persistent but asymptomatic infection (Shourian and Qureshi, 2019).

2.5.2 Quantification of disease tolerance

The quantification of disease tolerance is originated from the ecology term of reaction norm, which describes the pattern of phenotypic expression of a given genotype across a range of environments (Simms, 2000). Therefore, disease tolerance has been quantitatively defined as the change in host performance (e.g. growth rate, feed intake) with respect to the change in within-

host pathogen burden (Simms, 2000; Schneider and Ayres, 2008; Ayres and Schneider, 2012; Doeschl-Wilson et al., 2012; Mulder and Rashidi, 2017). Although animal disease tolerance is defined at the individual level, monitoring and repeat measuring of animal performance and pathogen burden over infection on an individual level are laborious and costly. In addition, the animal performance and pathogen burden trajectories on an individual level are not always a linear pattern as there can be multiple infection stages involved over the infection (e.g. pathogens proliferate, recede, or rebound; the host gets sick, recovers or the infection becomes chronic). Empirical tolerance estimates based on a group of related individuals (e.g. breed, line, or contemporary group) had been commonly used for practical reasons (Simms and Triplett, 1994; Mauricio et al., 1997; Råberg et al., 2007; Kause, 2011). In particular, such group disease tolerance has been usually estimated using regression analysis, where performance measures of infected group members are regressed against their respective pathogen burden at a given post-infection time point, and the slope is regarded as the estimate of disease tolerance (Simms and Triplett, 1994; Mauricio et al., 1997; Råberg et al., 2007; Kause, 2011; Lough et al., 2017, 2018).

The estimate of group disease tolerance is attractive for practical reasons and has provided valuable evidence on the existence of genetic variation in disease tolerance. However, Doeschl-Wilson et al. (2012) identified three significant issues that may render group disease tolerance not ideal for pig breeding programs using field data. Firstly, the estimate of group disease tolerance relies on the assumption that all animals have been infected with the same dose and type of pathogens at the same time, which is unlikely to be true for pigs in commercial farms (Bishop and Woolliams, 2010; Doeschl-Wilson et al., 2012). Secondly, the phenotypes, both animal performance and pathogen burden for group tolerance, are cross-sectional measures (Doeschl-Wilson et al., 2012). In other words, these phenotypes are taken at one time point

during the infection, which may poorly represent the overall impact of infection on host performance over the entire time course of infection (Ayres and Schneider, 2012; Doeschl-Wilson et al., 2012). Finally, the major drawback of group disease tolerance from an animal breeding perspective is the within-group variation cannot be exploited, which could limit selection accuracy (Doeschl-Wilson et al., 2012).

2.6. Disease resilience

There is considerable debate on the relative utility of disease resistance and disease tolerance. Both disease resistance and disease tolerance have benefits and concerns for different scenarios as discussed above. Uncoupling disease resistance and tolerance may be vital as they differ in pathogens prevalence and evolution and, consequently, on breeding programs (Guy et al., 2012). However, explicit selection for disease resistance and tolerance is very difficult and has failed in many studies, as it would require extensive routine data recording for pathogen burden and animal performance in response to infection, leading to considerable investment for breeding companies (Knap and Doeschl-Wilson, 2020). Regarding this challenge, recent studies proposed to focus on disease resilience, a combination of disease resistance and tolerance, as a practical breeding goal trait rather than explicit selection for these two components traits (Mulder and Rashidi, 2017; Knap and Doeschl-Wilson, 2020). With the contribution of both disease resistance and tolerance, disease resilience is defined as an animal's ability to maintain a high level of performance and productivity in the face of disease challenge that can be caused by multiple pathogens (Albers et al., 1987). Studies of disease resilience focus more on reducing the impact of infection on performance and productivity regardless of uncoupling disease resistance and tolerance. Therefore, the effect of disease resilience on infection itself or the pathogen load remains unknown (Hermesch, 2014).

2.6.1 Exploring disease resilience phenotypes from production traits

In the context of commercial farms, production traits are economically important for the swine industry and disease resilience is commonly referred to as a relatively undepressed expression of production potential. Therefore, it has been measured as deviation of production traits in within-host longitudinal data series (Colditz and Hine, 2016; Friggens et al., 2017; Knap and Doeschl-Wilson, 2020). In this case, more resilient animals are expected to show smaller deviations compared to less resilient animals, because they are less influenced by disturbances caused by the infection (Berghof et al., 2019). For example, body weight, wool growth, and wool fibre diameter were recorded in Merino sheep with varying intervals on uninfected, infected, and recovered occasions from infection by the parasite *Haemonchus contortus* and the deviations of these traits within each host were used as phenotypes of disease resilience to measure the depression and recovery of productivity due to infection (Albers et al., 1987). However, the heritabilities of these traits were too low to make tangible genetic improvement (Albers et al., 1987). In dairy cows, the daily milk yield of each animal is the longitudinal data recorded by automatic milking systems. The resilience phenotype measured as the variance of milk yield of an individual cow per lactation was moderately heritable (0.10 to 0.24) and genetically correlated (-0.22 to -0.66) to udder health, ketosis, and longevity (Elgersma et al., 2018; Poppe et al., 2020). Multiple traits developed based on the deviation of body weights over time from an individual were investigated as resilience phenotypes in layer chickens, and these traits were moderately heritable with heritability estimates ranged between 0.09 and 0.11 (Berghof et al., 2019). In pigs, individual daily feed intake data are longitudinal data that can be automatically collected by the electronic feeding systems. The variabilities of daily feed intake and feed intake duration over time for each finishing pig in the same polymicrobial challenge model established for studies of

this thesis were estimated to be heritable (0.08 to 0.21 and 0.23 to 0.26, respectively) and genetically correlated with mortality and the standardized number of treatment events (0.12 to 0.62) (Putz et al., 2019; Cheng et al., 2020).

In addition, phenotypes obtained based on longitudinal data (e.g. growth rate or average daily gain based on body weights; treatment rate or incidence based on medicine records) and one-time measures of production traits (e.g. carcass traits) can also be used as resilience phenotypes. More resilient animals are expected to show higher productivity, for example, higher growth rate with lower treatment incidence compared to less resilient animals in the contemporary group. For instance, disease resilient sheep to nematode parasite challenge were selected based on the age when a first post-weaning anthelmintic treatment (age-at-first-drench) was required to maintain acceptable growth, which increased the average age-at-first-drench and the 6-month live weight, and decreased the breech fleece fecal soiling (Morris et al., 2010). In pigs, Cheng et al. (2020) analyzed many production traits in response to the same polymicrobial challenge describe here in **Chapter 3**, such as average daily gain, feed conversion ratio, carcass weight, lean yield, etc. and indicated the potential of further developing them as resilience phenotypes.

2.6.2 Immune traits as phenotypes of resilience

Immunocompetence defined as the ability of the body to produce effective and appropriate immune responses when exposed to a variety of pathogens, has been hypothesized to be closely correlated with both disease severity (related to disease resistance) and tissue damage (related to disease tolerance), which may be a key player in maintaining an animal's performance and productivity (disease resilience) in response to disease challenge (Wilkie and Mallard, 1999; Hine et al., 2014). Accordingly, multiple immune traits, including antibody titers and immune

cell counts, have also been investigated as resilience phenotypes in several studies (Wilkie and Mallard, 1999; Clapperton et al., 2008, 2009; Flori et al., 2011; Guy et al., 2019; Chen et al., 2020). Many innate and adaptive immune traits were demonstrated to be genetically controlled in pigs. For example, peripheral blood mononuclear leucocytes (PBML) of pigs challenged on low health status farms for 60 days were moderately to highly heritable (0.18 to 0.71) (Clapperton et al., 2008). Furthermore, negative genetic correlations of some PBML subsets with the daily weight gain of pigs measured during the challenge were identified, indicating that improved performance was associated with decreased values for PBML traits in response to the challenge (Clapperton et al., 2008, 2009). The results of these studies indicated that the changes of immune traits (PBML) in response to infection have the potential to be used as phenotypes of disease resilience and are able to capture the genetic variation to make genetic improvement of disease resilience.

Although production traits are economically important in the swine industry and they have been previously used as phenotypes for disease resilience, they can only be obtained in commercial farms when the disease challenge is present, the same problem therefore occurs as for pathogen load although it may not be the priority target for disease resilience. Therefore, the phenotypes that can be collected at early ages and even before the challenge from the nucleus farm where selection takes place are of particular interest as they may have the potential to be developed as indicators and or predictors of disease resilience in commercial pigs.

Multiple immune traits in response to vaccination or collected from healthy animals were found to be heritable and genetically correlated with animal performance in response to infection later in life. A broad range of 54 immune traits involved in innate immunity and adaptive immunity were analyzed for pigs vaccinated against *Mycoplasma hyopneumoniae*, and thirty of

them were highly heritable with heritability estimates higher than 0.4 (Flori et al., 2011). Immunoglobulin M (IgM) and IgG natural antibodies are essential parts of the innate immune system, and their titers measured from healthy pigs were estimated to be moderately heritable (0.12 to 0.53) (Chen et al., 2020). Furthermore, the IgG natural antibody titer of healthy pigs was found to have positive genetic correlations (0.18 to 0.47) with the variability of daily feed intake duration for an animal after exposure to the same polymicrobial challenge established for studies of this thesis (Chen et al., 2020). These studies further indicated that immune traits collected before infection have the potential to be used as cost-effective indicator traits of disease resilience because these traits can be directly collected from high-health nucleus breeding herds for the selection of disease resilience.

2.6.3 Complete blood count: a practical measure of immune traits

Since immune traits collected before and after infection may have the potential to be further developed as indicator traits and phenotypes of disease resilience in pigs, respectively, there is a need to further explore the most cost-effective and practical method to measure immune traits that can be applied in the swine industry. The complete blood count (CBC) is a group of flow cytometry-based tests that provide an automatic measure of cells in blood samples, including the concentrations and relative proportions of leukocytes that play multiple essential roles in the immune system (George-Gay and Parker, 2003). It is a relatively inexpensive, robust, and routinely used blood test in veterinary laboratories to evaluate overall health and detect disorders, which may have the potential to be used as a practical measure of immune traits for the selection of disease resilience in the swine industry.

The CBC analyzer uses light scattering, differential white blood cell (leukocyte) lysis, myeloperoxidase staining to determine white blood cell parameters, including the concentration

and relative proportions of total white blood cells, neutrophils, lymphocytes, eosinophils, and basophils (Harris et al., 2005). A colorimetric method is used by the CBC analyzer for hemoglobin analysis, and the light scattering is used for red blood cell (erythrocyte) and platelet analyses to quantify the hemoglobin concentration of the red blood cell, and concentration and volume of red blood cells and platelets (Harris et al., 2005).

The CBC traits measured from an individual should be in a normal range identified from the healthy population when there are no disease or perturbation challenges. Therefore, when the CBC traits are out of the normal range, either higher or lower than the normal range may provide us with information about the animal's diagnosis, prognosis, response to infection and stress, and recovery (George-Gay and Parker, 2003). Although CBC values outside the normal range can be from many sources, infection and inflammation found in animals are the most significant reasons for the increases of white blood cell traits. Significant increases in white blood cell traits can be caused by vasodilation, a manifestation of inflammation that increases blood flow to the infection areas, bringing nutrients and large amounts of white blood cells to defend against pathogens (George-Gay and Parker, 2003). Moreover, white blood cells also release cytokines to recruit more white blood cells to the area to reinforce the inflammatory response to defend against pathogens effectively (George-Gay and Parker, 2003). The primary function of red blood cells is to carry oxygen in the lungs to the cells of the body and transport carbon dioxide from the cells to the lungs for excretion (Diez-Silva et al., 2010). Besides, red blood cells carry the majority of iron of the body, which is an essential nutrient for both humans and pathogenic microbes (Cassat and Skaar, 2013). Hemoglobin is the iron-containing oxygen-transport metalloprotein in red blood cells that plays an essential role in the functions of red blood cells. The levels of red blood cell indices, including red blood cell concentration, hemoglobin

concentration, and hematocrit (the volume percentage of packed red blood cells in blood), may increase or decrease due to multiple and complex reasons. However, under the disease challenge, a significant decrease of red blood cell indices is likely caused by the challenge of bacterial pathogens, which could damage circulating blood cells and accelerate hemolysis for iron to support bacterial cellular processes of respiration and replication (Barrett-Connor, 1972; Kent, 1994; Viana, 2011; Cassat and Skaar, 2013). Although hemostasis is the primary function of platelets, they also work as important immune cells in both innate and adaptive immune response by secreting mediators and interacting with vascular endothelial cells and leukocytes (Tamagawa-Mineoka, 2015; Hottz et al., 2018). A transient increase in platelet concentration can be observed as a response to infection (George-Gay and Parker, 2003).

To date, many studies of immune traits and the relationships between immune traits and disease responses only focused on the immune traits collected at a specific time point, for example, before or after the challenge, under healthy or unhealthy conditions (**Section 2.6.2**). Such results are a single "snap-shot" and may be one-sided and confuse the understanding of the immunological mechanisms of resilience because the correlation between immune traits and performance can be either positive or negative depending on the sampling time and the health status of animals. In a study of pig responses to disease challenge with the protozoan pathogen *Sarcocystis miescheriana*, longitudinal CBC data were measured at four different time points before and after the challenge for genetic analyses of disease response (Reiner et al., 2008). Although this study only focused on a specific pathogen challenge in pigs, it indicated the potential of using longitudinal CBC data collected at different time points and health conditions, for example, healthy stage, acute disease stage, and recovery or chronic disease stage, for further genetic studies of disease resilience.

Overall, CBC provides a description of circulating blood cells that play essential roles in immune responses, which can be a practical measure of immune traits. Many studies have indicated the value of CBC traits in predicting cancer, heart diseases, and neonatal sepsis in humans (Ottolini et al., 2003; Spell et al., 2004; Anderson et al., 2007; Hornik et al., 2012; Lassale et al., 2018). However, little has been known about their value and usage as indicators and or phenotypes of disease resilience in pigs.

2.7. Summary

Hence, CBC is an excellent candidate for a practical measure of immune traits and may have the potential to be further explored to improve disease resilience for the swine industry where pigs are facing disease challenge caused by multiple pathogens. It may be possible and feasible to collect longitudinal CBC data by conducting CBC for blood samples collected before and after exposure to pathogens or at multiple time points throughout an animal's response to infection. Therefore, the following studies presented in this thesis were conducted to explore the potential of longitudinal CBC data collected before and after exposure to a polymicrobial infectious challenge (as is the case in many commercial farms) as indicator traits and phenotypes of disease resilience, respectively, to make genetic improvement for disease resilience in the swine industry.

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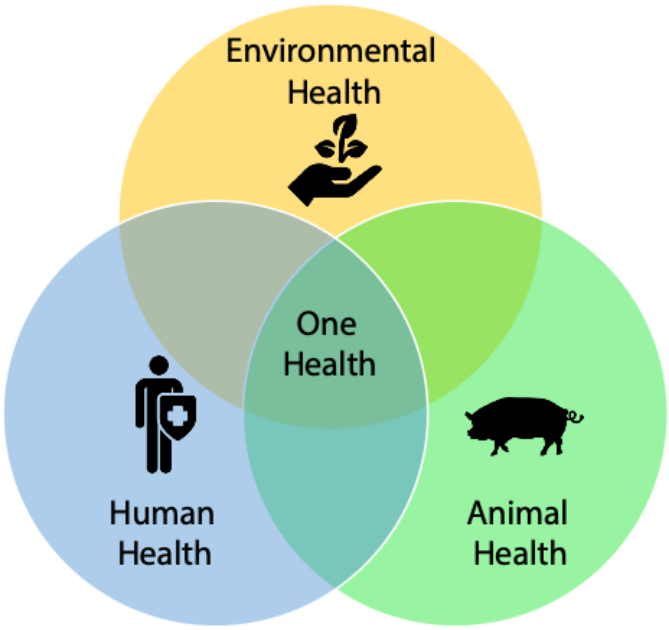

Figure 2. One health concept.

One Health is at the intersection of human health, animal health, and environmental health; it is an approach to design and implement programmes, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes; One Health approach includes food safety, the control of zoonoses, and combatting antimicrobial resistance (WHO, 2017). The figure was derived from https://en.wikipedia.org/wiki/File:One-Health-Triad-en.png, accessed March 21, 2021.

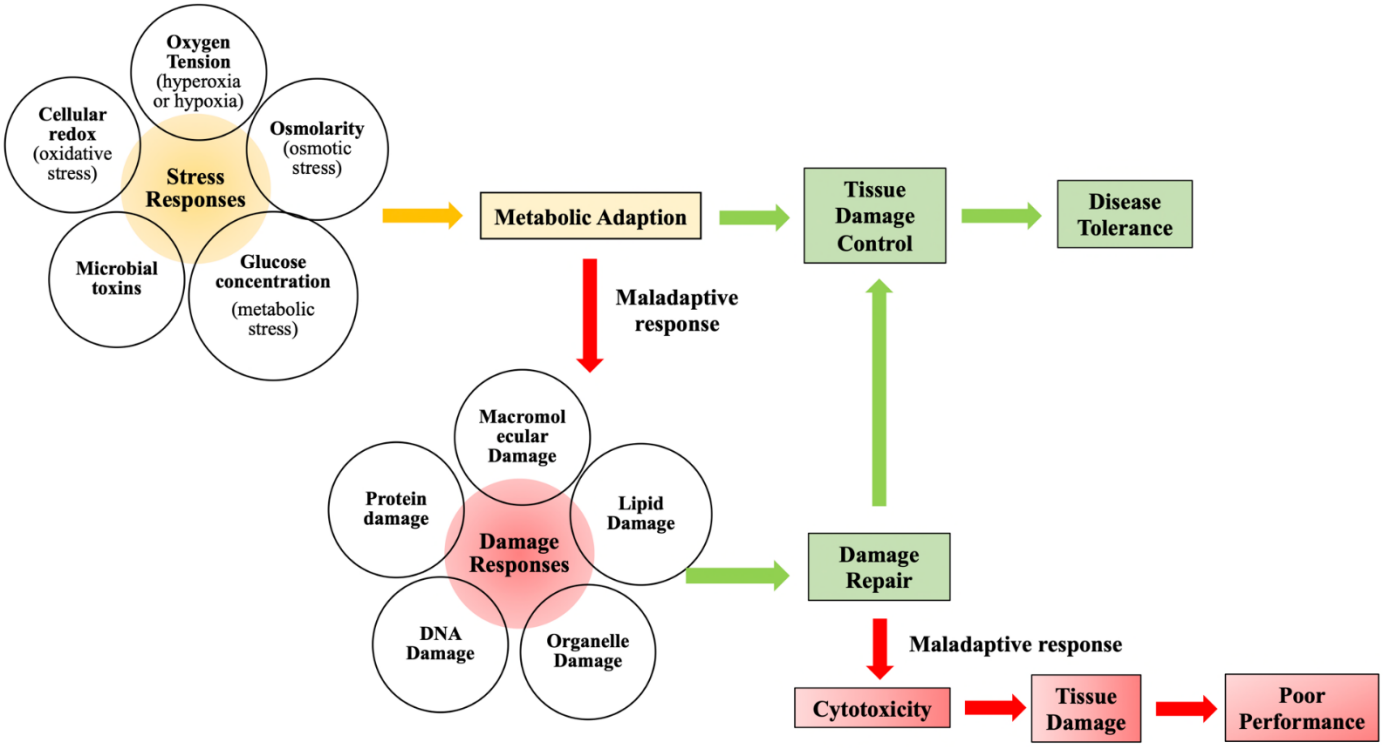

Figure 3. Mechanisms involved in disease tolerance.

Derived from Soares et al. (2014).

Chapter 3. Natural disease challenge model

To achieve the objective of exploring CBC traits collected from the high-health nucleus farm as indicator traits and from the commercial farm with a polymicrobial challenge as phenotypes of disease resilience for genetic improvement of the swine industry, a natural disease challenge model (NDCM) consisted of a healthy quarantine unit and a challenge barn was established as the basis for the studies described in this thesis.

3.1. The design of the natural disease challenge model

The NDCM for wean-to-finish pigs was established in November of 2015 at Deschambault, in Québec, Canada. There were two major facilities in the NDCM: (1) a non-challenged quarantine unit for a 3-week nursery and acclimation after weaning and transportation (qNur, 19 to 40 days old); (2) a challenge barn for a 4-week late nursery stage (cNur, 41 to 68 days of age on average) where the test pigs were first exposed to the polymicrobial challenge and a 16-week grow-to-finish stage (GF, approximately 69 to 181 days of age). Test pigs were healthy F1 crossbred (Landrace × Yorkshire) barrows (castrated male pigs) sourced from healthy multiplier farms (n = 14) of seven genetic suppliers (Alliance Genetics Canada, AlphaGene, DNA Genetics, FastGenetics, Genesus, Hypor, and Topigs), all members of PigGen Canada, in rotation. A batch of 60 or 75 test pigs from one of the multiplier farms was introduced into the NDCM every three weeks. Every seven batches constituted one cycle for cycle 1 to cycle 6, and the last eight batches (batches 43 to 50) formed cycle 7. In total, fifty batches of pigs (n = 3285) were introduced into the NDCM and were used for the studies presented in this thesis. A summary of batches and their corresponding farms, genetic suppliers, and cycles is shown in **Table 1**. Since batches were nested within farms, genetic suppliers, and cycle, and coded uniquely, the batch effect was fitted as the fixed effect in the studies of this thesis to control false

positive results due to population stratification and batch differences, and also to focus on the variance within each batch. The protocol specified that only two to four weaned barrows should be sampled per litter as test pigs. For each batch, the number of test pigs per pen was approximately 4, 7, and 13 for qNur, cNur, and GF, respectively. The quarantine unit and the challenge barn were in the same building connected by a hallway for the first cycle (batches 1 to 7), but strict biosecurity protocols were practiced. Since the biosecurity practices were found to be insufficient to stop the spread of pathogens from the challenge barn to the quarantine unit, a separate quarantine unit located approximately 1 km south of the challenge barn was set up for cycles 2 to 7 (batches 8 to 50) and kept free of disease by adhering to strict biosecurity protocols. Common disease-causing pathogens found in commercial farms were the primary target pathogens in the NDCM, including multiple strains of PRRSV and swine influenza A virus, various respiratory and enteric bacterial pathogens (such as *Mycoplasma hyopneumoniae*, *Haemophilus parasuis*, *Brachyspira hampsonii*, *Salmonella enterica* serovar *typhimurium*, and *Streptococcus suis*), and parasites including *Cystoisospora suis* and *Ascaris suum*. The challenge barn was operated as a high health status facility prior to the introduction of the disease agents. The polymicrobial challenge in the challenge barn was established by introducing naturally infected animals (seeder pigs) from strategically selected commercial farms with known disease outbreaks into the challenge barn. Four groups of 12 to 28 seeder pigs (approximately 2 to 4 seeder pig per pen with 7 test pigs in cNur) were sourced from four different commercial farms and co-introduced into the challenge barn with the first four batches of test pigs (**Table 2)**. Once the challenge was established, the pathogen circulation and the polymicrobial challenge were maintained as a continuous flow system. The new incoming batch was challenged by direct nose-to-nose contact with the adjacent proceeding batch for one week in the challenge late nursery

without repeatedly introducing seeder pigs after the first four batches (**Figure 4A**). During the periods of very high challenge pressure, as indicated by morbidity and mortality, twelve batches of test pigs were physically separated from the preceding batch that was co-housed during the week of cNur without the direct nose-to-nose contact (**Figure 4B**). The non-direct nose-to-nose challenge helped maintain the mortality rate below the target level established by the Animal Protection Committee for animal welfare.

For the data used in this thesis, every batch was confirmed to have been exposed to PRRSV in the challenge barn based on randomly sampling blood from a subset of individuals for reverse transcription-polymerase chain reaction (RT-PCR) four weeks post-challenge and enzyme-linked immunosorbent assay (ELISA) six weeks post-challenge. In addition to the target pathogens, other pathogens including PCV2, porcine rotavirus A, *Erysipelothrix rhusiopathiae*, *Staphylococcus hyicus*, and some undefined minor pathogens were also identified in the NDCM. The disease challenge was a function of these pathogens, together with the environment, management, and veterinary strategies. The disease pressure varied by batch and also on a seasonal basis. Therefore, not all pigs were exposed to all the same types or doses of pathogens, which would also be the case on a commercial farm. In the NDCM, a part of the pigs died (see details in section 3.3.4), and the other animals reaching the target slaughter weight (~130 kg) at approximately 181 days-old were slaughtered commercially and entered the food chain.

To ensure animal welfare and maintain the mortality rate below the target level established by the Animal Protection Committee, individual treatments were given on a case-by-case level. Also, the level of mortality in a batch was carefully monitored. The periodic group treatments were given through water and feed on the entire batch- or the large group-level for batches with significantly higher mortality rates. Due to significant problems in managing the

associated impact caused by PCV2 in cycle 1 (batches 1 to 7), Ingelvac CircoFLEXR® PCV2 vaccination (Boehringer Ingelheim, Ingelheim am Rhein, Germany) was administered intramuscularly as per the label instructions to pigs before being exposed to the polymicrobial challenge in the challenge barn from the second cycle onwards. The treatment protocol was established and fully overseen by the research staff and veterinarians.

3.2. Blood samples collected from the natural disease challenge model

In the NDCM, four sets of blood samples (Blood 1, Blood 2, Blood 3, and Blood 4) were collected from the jugular vein of each test pig by trained research staff following the established protocol. Blood 1 samples were collected in the qNur at an average age of 26 days, a week post-arrival from their farm of origin, and two weeks before the challenge. At the time of the collection of Blood 1, animals were expected to have recovered from acute stress, for example, weaning and shipping stress, and to acclimate to the change of diet from highly digestible liquid milk to solid dry feed that is less digestible. The Blood 2 samples were collected immediately before entry into the challenge barn, at an average age of 40 days, which were not used for the work described in this thesis. The Blood 3 samples were collected in the cNur at an average age of 54 days, four weeks after the collection of Blood 1 and also two weeks after exposure to the polymicrobial challenge, representing the acute stage of the disease. The Blood 4 samples were collected during GF at approximately 82 days old, four weeks after the collection of Blood 3 and also six weeks after exposure to the polymicrobial infectious challenge. The Blood 4 timepoint was expected to represent the chronic stage of the disease with the culmination of clinical symptoms or the convalescent stage established by disease resilience.

Blood 1, Blood 3, and Blood 4 were whole blood collected into K2 ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainer® Blood Collection Tubes, United

States) and Tempus™ Blood RNA tubes (BD Vacutainer® Blood Collection Tubes, United States). These samples were shipped overnight with ice packs to the University of Alberta. The whole blood samples in EDTA tubes were used for CBC test to evaluate the cells circulating in blood using the ADVIA® 2120i Hematology System (Siemens Healthineers, Germany). Fresh samples were necessary for CBC analysis, and most of the samples were received and processed within 24 hours. In a few cases shipping delays occurred but even in these cases the samples were usually delivered within 24 to 48 hours, and we also found the samples were relatively robust for CBC when analyzed 48 hours after collection. The whole blood samples in Tempus™ Blood RNA tubes were stored and frozen at -20 °C for future RNA extraction. Finally, Blood 2 samples were collected into Plus Serum tubes (BD Vacutainer® Blood Collection Tubes, United States) for analysis of serum for antibody-mediated responses for a separate study.

3.3. Phenotypes collected from the natural disease challenge model

Multiple phenotypes from the NDCM were monitored and collected by trained research staff following the established protocol, and individual feed and water intakes data in the finishing phase were recorded automatically using IVOG® feeding stations (Insentec, Netherlands). Of note, CBC collected before and after exposure to the challenge, bodyweights, treatments, mortality, and daily interior and outside temperature of the barn were the phenotypes monitored and analyzed for this thesis.

3.3.1 Complete blood count (CBC)

Three categories of CBC traits, including white blood cell traits, red blood cell traits, and platelet traits, were measured from Blood 1, Blood 3, and Blood 4 samples. The description and abbreviations of these CBC traits are shown in **Table 3**. White blood cell concentration traits were $\log_{10}$-transformed to reduce the skewness of the distribution. Due to the relative complexity

of the sample handling and laboratory analysis, CBC measures from “damaged samples” (e.g. from delays in transport with unfavourable environmental conditions) with clotting and severe hemolysis were regarded as outliers. Additional issues were sometimes encountered with the Hematology System used to measure CBC from blood samples. By annotating the results during sample processing and comparing the CBC data to the reference intervals, outliers potentially caused by shipping, layover, and equipment problems rather than a response of CBC to the challenge were removed from the analyses. The descriptive statistics for the CBC traits measured in Blood 1, Blood 3, and Blood 4 of all seven cycles after removing outliers are shown in **Figure 5** to **Figure 7**. Most CBC traits were recorded on all animals at Blood 1, but fewer and fewer samples were available for Blood 3 and Blood 4 because some of the animals died throughout the NDCM in response to the challenge prior to sampling. Most of the white blood cell traits in Blood 3 and Blood 4 had higher standard deviations and thinner violin plots than Blood 1, indicating that the white blood cell traits in Blood 3 and Blood 4 were spread out over a broader range. In contrast, several red blood cell traits had thinner violin plots for Blood 1 than Blood 3 and Blood 4.

In addition to these direct measures of CBC traits from blood samples, we also calculated the changes of CBC traits between blood samples collected at different time points, which were referred to as Δ13 for the change from Blood 1 to Blood 3 (calculated by subtracting CBC in Blood 1 from CBC in Blood 3, Blood 3 − Blood 1), Δ34 for the change from Blood 3 to Blood 4 (Blood 4 − Blood 1), and Δ14 for the change from Blood 1 to Blood 4 (Blood 4 − Blood 1). Descriptive statistics for the changes in CBC traits are shown in **Figure 8** to **Figure 10**. Not all animals responded to the challenge in the same way, shown as either positive (increasing) or negative (decreasing) values in Δ13, Δ34, and Δ14 of each trait. This difference among

individuals may be associated with an animal's performance after exposure to the challenge, which will be further discussed in **Chapter 4**.

3.3.2 Bodyweight and growth rate

The bodyweights of each test pig were measured every three weeks in the NDCM. The growth rates of pigs, including the dead animals in different phases of NDCM, were estimated using the linear regression of body weights collected during each stage. The qNur growth rate (qNurGR) was assessed using the bodyweights collected at approximately 19 days of age on the first day of entry into the NDCM and 40 days-old at the end of the qNur. The cNur growth rate (cNurGR) was estimated using the body weights collected from 40 to 69 days of age. The GF growth rate (GFGR) for each animal in the challenge barn was estimated using the multiple sets of body weights collected from an average of 69 days-old to the endpoint when the test pig died or reached the target slaughter weight at approximately 181 days of age.

The growth curves of pigs in all fifty batches are shown in **Figure 11,** and the growth curves of dead animals are highlighted in red. The majority of dead animals had relatively slow growth, shown as flat growth curves. A few animals showed a significant gain of body weight but died before reaching the target slaughter weight. The highest standard deviation was identified for cNurGR (0.10) among all the animals which was greater than that for qNurGR (0.06) and GFGR (0.05). The means and standard deviations of qNurGR, cNurGR, and GFGR for each batch are shown in **Figure 12**. The cNurGR was not applicable for batches 24 and 25 because body weights were not collected in the qNur. Overall, GFGR was higher than qNurGR and cNurGR for all batches because both lean and fat accretion increase during the grow-to-finish stage of pigs. All batches showed higher standard deviations in the cNurGR and GFGR of animals after exposure to the challenge than the qNurGR of animals before the challenge. The

means of qNurGR, cNurGR, and GFGR varied among batches due to the differences in genetic background and challenge level among batches.

3.3.3 Mortality

A total of 944 pigs died in the natural disease challenge model. The cause of death is summarized in **Figure 13**, which can be classified into infectious and non-infectious reasons. The majority of animals died due to infectious diseases, according to the clinical and pathological signs observed from the animals, such as heavy breathing, lameness, scores, and fever, etc. The largest class died due to "poor/skinny/hairy/failure to thrive" (n=313), which was also classified with the group assigned infectious reasons because inappetence and failure to eat were typical symptoms of the disease caused by multiple pathogens in the NDCM. "Thumping/heavy breathing" was also a major cause of death (n=223), which was a typical symptom of pneumonia caused by respiratory pathogens in the NDCM, such as PRRSV, swine influenza A virus, *Mycoplasma hyopneumoniae*, and *Haemophilus parasuis*. Many pigs (n=129, including 7 pigs in the qNur, 57 pigs in the cNur, and 65 pigs in the GF) were recorded as "sudden death" when the pigs died unexpectedly with no pathological or warning signs that could alert the veterinarians and research staff in the NDCM. Sudden death can be found in almost all batches in the NDCM and was regarded as a non-infectious cause of death because the exact reason was unknown. In addition to the sudden death, there were 41 pigs that died due to other non-infectious and unclear reasons, including "others", "sampling/bleeding", "fraction/sprain", "fighting/tail/ear/flank biting", "allergic reaction", "hernia", and "intestinal torsion".

The mortality rates in qNur, cNur, and GF of each batch are shown in **Figure 14**. For most batches, the mortality rates were higher in cNur and GF after exposure to the challenge than

the qNur, where the animals were not yet challenged with infectious pathogens. The mortality rate varied among batches. Several successive batches had a higher mortality rate in cNur; accordingly, the mortality rate of these batches in GF was also higher than the other batches. The weekly death counts are summarized based on the scale of weeks after exposure to the challenge (**Figure 15A)**. The first three bars on the histogram indicate the weekly death counts in the qNur, three weeks before exposure to the challenge. The weekly death count increased significantly in the cNur from one to four weeks after exposure to the challenge. The peak of weekly death counts was also observed in the cNur at three and four weeks after exposure to the challenge. In the GF stage, the death count was high in the first two weeks, five to six weeks after exposure to the challenge, but dropped to a low level afterward.

3.3.4 Treatments and individual treatment rate

Individual medications were given to pigs that exhibited clinical signs, such as coughing, fever, inappetence, wasting, hairy appearance, swollen joints, and sloppy feces with a range of colours (grey, red, and yellow), which were indicative of pneumonia, diarrhea, meningitis, arthritis, erysipelas, conjunctivitis, etc. Some pigs exhibited a combination of multiple symptoms, for example, coughing and diarrhea can be observed in a pig simultaneously or at different time points, which suggested infection caused by a variety of pathogens. Antibiotics were used as per a regimented treatment protocol for each ailment. Anti-inflammatory medications were also administered for some clinical signs. Same as death counts, individual treatment counts were high in the cNur stage (**Figure 15B**). Individual medications were given intensively in the cNur, especially in the two and three weeks after exposure to the challenge, shown as significantly more counts of individual treatment events in **Figure 15B**. Test pigs did not necessarily stay in the NDCM for the same number of days because some died throughout

the NDCM, but others reached the target slaughter weight on different days, which affected the count of individual treatment events that animals received in the NDCM. For example, some animals had fewer counts of treatment events only because they died early in the NDCM due to infectious disease. Therefore, individual treatment rate or incidence (TR) was calculated for each pig to standardize the count of individual treatment events to approximately the same scale (TR = count of individual treatment events/days in the NDCM × 100%). The mean and standard deviation of the individual treatment rate of each batch is shown in **Figure 16A**, where variations of the mean and large standard deviations can be observed among batches.

The counts of group treatment events for each batch are summarized in **Figure 16B**. Twenty-five batches received group treatments, which were given to the entire batch or on a large group-level through water and feed to maintain the mortality rate below the target level established by the Animal Protection Committee. Of note, seventeen batches received group treatments multiple times throughout the NDCM, and most group treatments were given during the cNur and GF stages in the challenge barn.

3.3.5 Daily interior and outside temperatures

In addition to the challenge of pathogens, environmental conditions have also been identified as critical components for pig health, welfare, and production efficiency. In the pig industry, considerable efforts have been made to control the interior environment of the barn and maintain the thermo-neutral zone where pigs can maintain body temperature without using energy beyond the basal metabolic rate. The minimum and maximum interior temperatures were controlled and monitored on a daily basis for the qNur and challenge barn of the NDCM. The daily maximum and minimum temperatures were monitored from both east and west sides of the building to ensure the temperature was managed in the large challenge barn.

The daily interior and outside temperatures of the qNur are shown in **Figure 17**. Although the minimum interior temperature fluctuated slightly for days from October to December in 2015, it was well managed and stabilized to 23.5°C from 2016 to 2018. The interior temperature of the qNur was difficult to control for days from May to September when the outside temperature was above 20°C, based on the variation observed for the maximum interior temperature which varied from 23.1°C to 37.5°C. From May to September of each year, the daily maximum interior temperature was higher than 25°C for approximately 70% of the time and higher than 30°C for 15% of the time.

The daily temperatures (maximum and minimum) measured from the east and west sides of the challenge barn are shown in **Figure 18**. Overall, the interior temperature of the challenge barn was managed to be lower than the qNur barn. The minimum interior temperatures were maintained to 21.5°C for both sides. However, the maximum interior temperatures were also hard to control from May to September of each year for both sides when the outside temperature was higher than 20°C. Of note, the maximum interior temperatures of the east and west sides were higher than 25°C for more than 70% of the time and higher than 30°C for almost 25% of the time from May to September of each year. The interior temperature on the east side fluctuated slightly in 2019, shown as the minimum temperature ranging from 21.9°C to 18.5°C and the maximum temperature varying from 25.3°C to 19.3°C.

3.3.6 Changes in mortality and treatment events throughout the year

The prevalence of diseases has been reported to show a seasonal pattern and potentially be associated with climate factors, including ambient temperature (Sanchez-Vazquez et al., 2012; Eze et al., 2015; Lee et al., 2020). For example, Lee et al. (2020) found that the cyclical annual patterns for the prevalence of respiratory disease showing peaks in the summer months and

troughs in the winter months. Therefore, the daily death count and daily count of the group and individual treatment events in the qNur barn and the challenge barn of the NDCM are plotted in **Figures 19** to **20** to explore if they could be affected by the climate and season in addition to pathogens. As mentioned above (**Figure 15**), most death and individual treatment events happened in the challenge barn after exposure to the challenge, which can also be observed from **Figures 19** and **21**. Mortality, group treatment events, and individual treatment events happened sporadically in the qNur stage (**Figures 19A, 20A,** and **21A**).

For the qNur, relatively higher mortality was found during the winter months (January to March and October to December) in 2016 and 2017 (**Figure 19A**). During the winter months, the low outside temperature could be a challenge for the transportation of pigs from their farms of origin to the NDCM, which might result in high mortality in qNur. Group treatments in the qNur were given four times in December of 2015, three times in January and July of 2017, and four times from October to December of 2017 (**Figure 20A**). Each group treatment in the qNur was given for two to three successive days. It seems that during the winter months of 2017, although more group treatments were given in the qNur, the mortality was still relatively higher than the other months. The count of daily individual treatment events was high from the end of March to early April, and also July in 2016 compared to the other months (**Figure 21A).**

For the challenge barn, higher daily death count, daily count of group treatment and individual treatment can be observed compared to the qNur. The daily death count was higher from January to early April and from October to December of 2016 and 2017 compared to the other months (**Figure 19B**). Although the maximum interior temperature was difficult to control from May to September and resulted in a large difference between the minimum and maximum interior temperature of the challenge barn, the mortality was relatively low during these months

in the years of 2016 and 2017 (**Figure 19B**). Conversely, animal deaths were relatively low and occurred sporadically from January to March but showed a tendency of increasing from June to September at the end of the NDCM in the year of 2018 (**Figure 19B**). The group treatment in the challenge barn was only found to be relatively higher during the winter months (January to March and October to December) when the death count was high, which was also the protocol for conducting group treatment (**Figure 20B**). The individual treatment in the challenge barn showed a tendency of changing in a cyclical pattern as more individual treatments were given for a period of time and then fewer were given for the next period of time (**Figure 21B**).

The death count, counts of group treatment events, and numbers of individual treatment events were also plotted on a month scale (**Figure 22**). Group treatment seemed to be given in the months (January, March, April, and December) with higher mortality in 2016; higher counts of group treatments were also given for the months (January to May, and September to December) with higher mortality in 2017, which might indicate the animals were facing a more challenging environment during these months (**Figure 22A** and **B**). In 2017, more individual treatments also seemed to be given for the months with higher mortality (**Figure 22C**). In 2018, the mortality was low and stayed at a relatively stable level throughout the year (**Figure 22A**). Therefore, both counts of group treatment events and individual treatment events were lower and did not change dramatically among different months in 2018 compared to the other years (**Figure 22B** and **C**).

3.4. Summary

This chapter provided a detailed description of the NDCM. It aimed to help improve the understanding of the NDCM as the following studies were conducted using the data collected from the NDCM.

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Table 1 A summary of batches and their corresponding farms, genetic suppliers, and cycles for test pigs introduced into the natural disease challenge model.

Cycle	Genetic suppliers	Farm	Batch	Cycle	Genetic suppliers	Farm	Batch
1	A	I	1	4	A	E	26
	B	A	2		E	H	27
	E	H	3		D	C	28
	F	D	4	5	A	I	29
	G	B	5		B	A	30
	D	K	6		E	H	31
	C	G	7		F	D	32
2	F	D	8		G	B	33
	E	L	9		D	J	34
	A	E	10		C	G	35
	D	C	11	6	F	D	36
	D	C	12		G	B	37
	G	B	13		A	E	38
	B	A	14		C	F	39
3	E	H	15		D	C	40
	F	D	16		E	L	41
	A	I	17		B	M	42
	B	A	18	7	C	F	43
	A	E	19		F	D	44
	D	C	20		G	B	45
	G	B	21		C	F	46
4	B	A	22		B	N	47
	C	G	23		D	C	48
	F	D	24		E	L	49
	G	B	25		B	N	50

Genetic suppliers and farms are recoded using the alphabet for privacy. There are 7 cycles, 7 genetic suppliers, 14 farms (farms E and I for genetic supplier A; farms A, M, N for genetic supplier B; farms F and G for genetic supplier C; farms C, J, and K for genetic supplier D; farms H and L for genetic supplier E; farm D for genetic supplier F; farm B for genetic supplier G), and 50 batches used for this thesis.

Table 2 The scheme for introducing seeder pigs from four commercial farms with healthy test pigs to establish the polymicrobial challenge in the natural disease challenge model.

Entry date	Commercial farm[1] for seeder pigs	Batch of test pigs
2015-11-11	Commercial farm A	1
2015-12-04	Commercial farm A	2
	Commercial farm B	
2015-12-23	Commercial farm C	3
2016-01-14	Commercial farm D	4

[1]The commercial farm was recoded for privacy.

Table 3 Description of complete blood count traits analyzed in the thesis.

Abbreviation	Trait
WBC	Total white blood cell concentration (10^3/μL)
NEU	Neutrophil concentration (10^3/μL)
LYM	Lymphocyte concentration (10^3/μL)
MONO	Monocyte concentration (10^3/μL)
EOS	Eosinophil concentration (10^3/μL)
BASO	Basophil concentration (10^3/μL)
RBC	Red blood cell concentration (10^6/μL)
HGB	Hemoglobin concentration (g/dL)
HCT	Hematocrit (%). Measure the percentage of packed red blood cells volume in blood
MCV	Mean corpuscular volume (fL). Indicates the volume of the "average" red blood cell in a sample (fL; 10^{-15}L)
MCH	Mean corpuscular hemoglobin (pg). A calculated red blood cell index that indicates the average amount of hemoglobin in the red blood cells. MCH = HGB / RBC
MCHC	Mean corpuscular hemoglobin concentration (g/L). A calculated red blood cell index that indicates the mean hemoglobin concentration per unit volume in red blood cells. MCHC = (HGB / HCT)×100
RDW	Red blood cell distribution width (%). An index of the variation in cell volume within the red blood cell population
PLT	Platelet concentration (10^3/μL)
MPV	Mean platelet volume (fL)

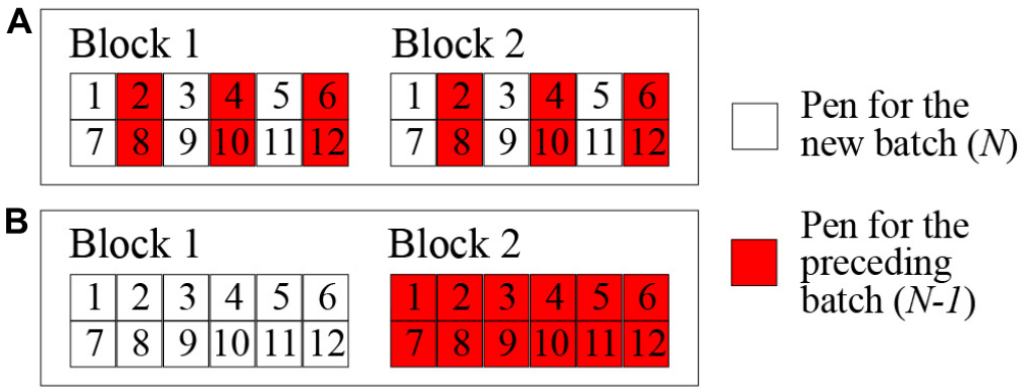

Figure 4 Pen arrangements in the challenge barn late nursery stage.

Pen arrangements for a week of nose-to-nose direct challenge (A). Pen arrangement for a week of indirect contact during the period of excessively high pressure of challenge (B). Derived from Bai et al. (2020).

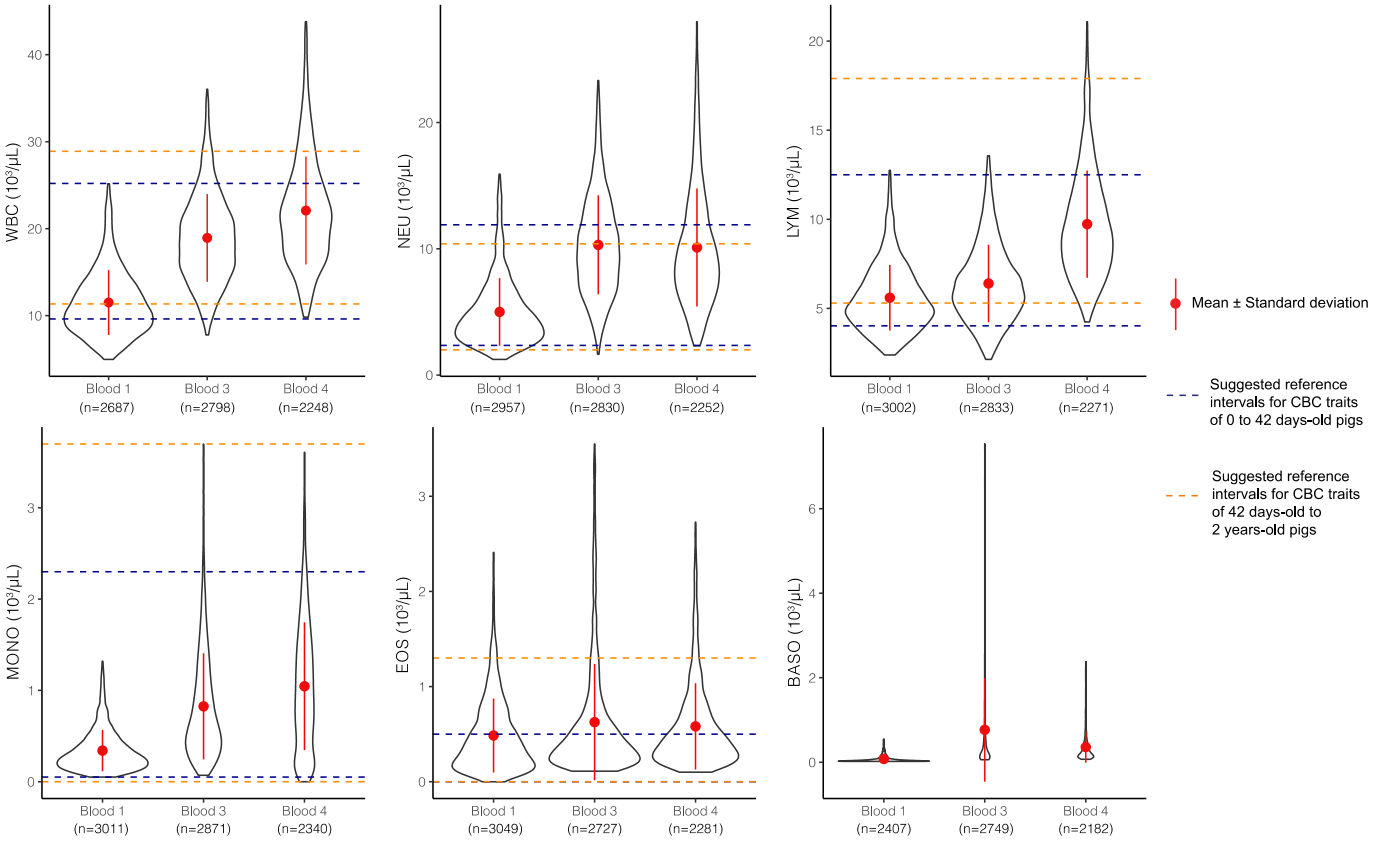

Figure 5 Violin plots for descriptive statistics for white blood cell traits of all seven cycles in Blood 1, Blood 3, and Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration.

Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability. Suggested reference intervals for CBC traits were derived from Iowa State University's Clinical Pathology Laboratory (2011). The suggested reference intervals for BASO traits are not applicable.

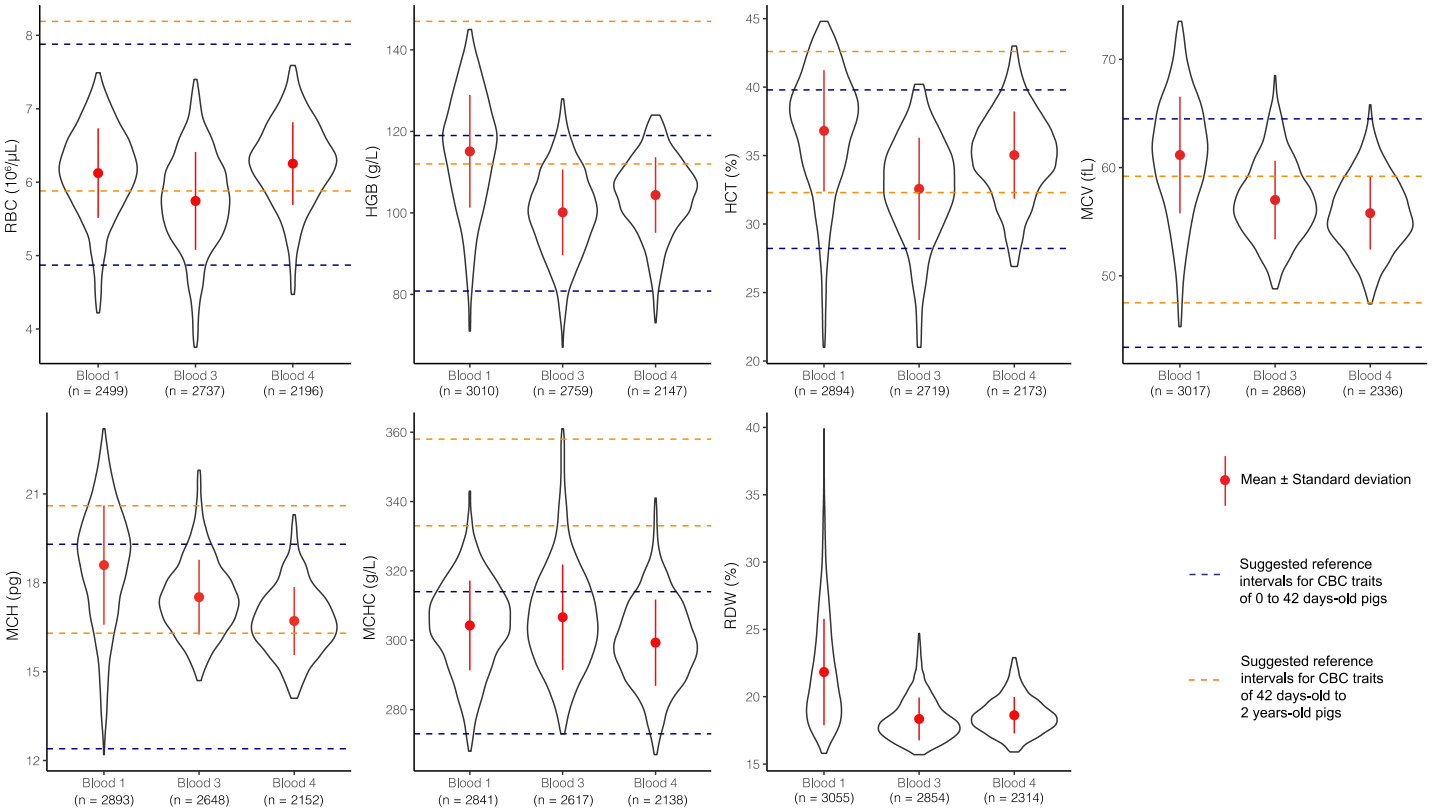

Figure 6 Violin plots for descriptive statistics for red blood cell traits of all seven cycles in Blood 1, Blood 3, and Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after the exposure to the challenge, respectively.

RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width.

Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability. Suggested reference intervals for CBC traits were derived from Iowa State University's Clinical Pathology Laboratory (2011). The suggested reference intervals for RDW traits are not applicable.

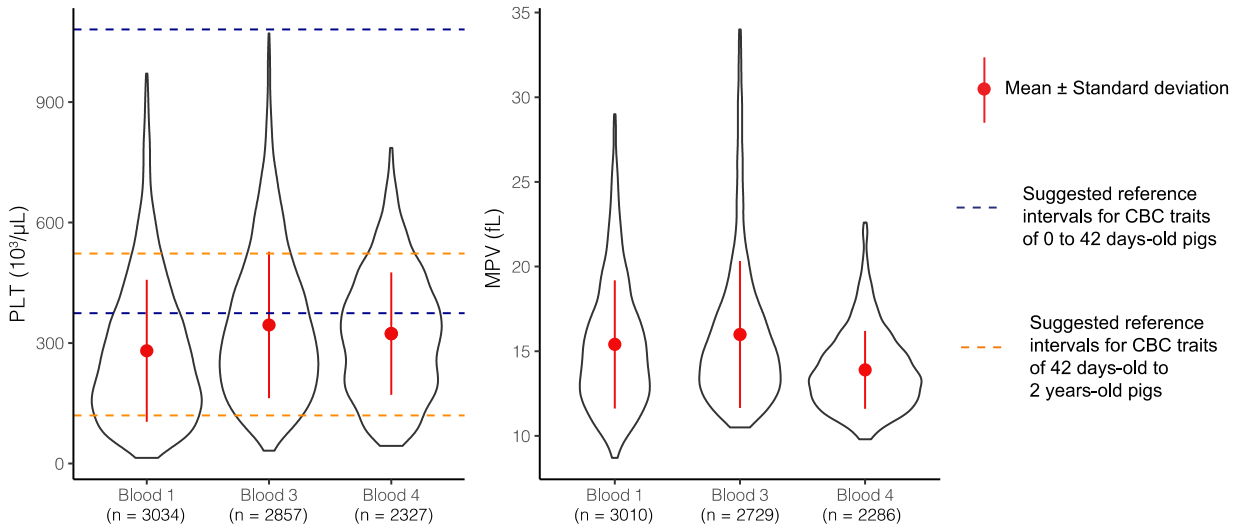

Figure 7. Violin plots for descriptive statistics for platelet traits of all seven cycles in Blood 1, Blood 3, and Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after the challenge, respectively.

PLT: platelet concentration; MPV: mean platelet volume.

Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability. Suggested reference intervals for CBC traits were derived from Iowa State University's Clinical Pathology Laboratory (2011). The suggested reference intervals for MPV traits are not applicable.

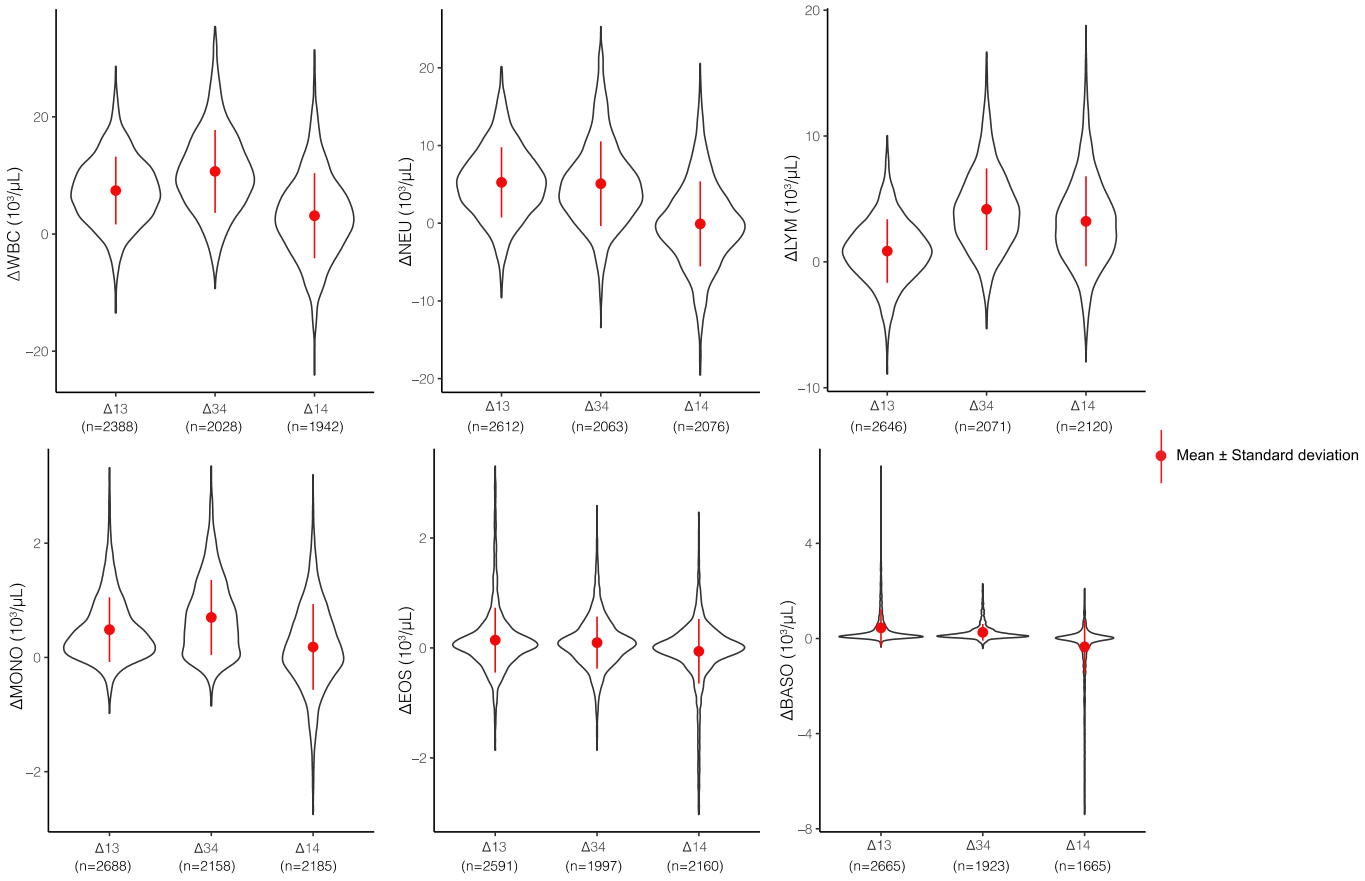

Figure 8. Violin plots for descriptive statistics for white blood cell traits in Δ13, Δ34, and Δ 14 of all seven cycles.

Δ13 for the change from Blood 1 to Blood 3; Δ34 for the change from Blood 3 to Blood 4; Δ14 for the change from Blood 1 to Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after the challenge, respectively.

WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration.

Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability.

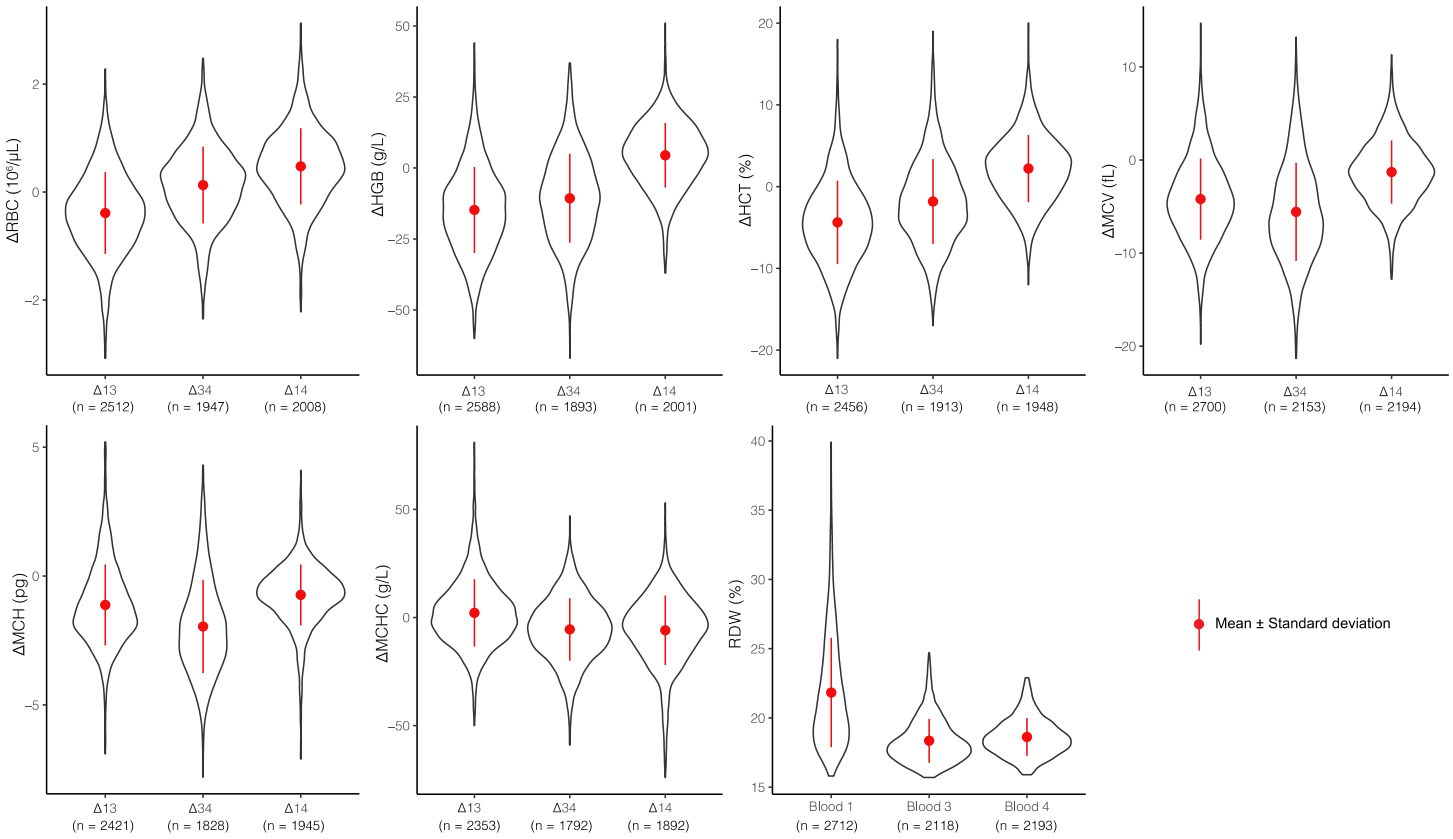

Figure 9. Violin plots for descriptive statistics for red blood cell traits in Δ13, Δ34, and Δ14 of all seven cycles.

Δ13 for the change from Blood 1 to Blood 3; Δ34 for the change from Blood 3 to Blood 4; Δ14 for the change from Blood 1 to Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after the challenge, respectively.

RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width.

Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability.

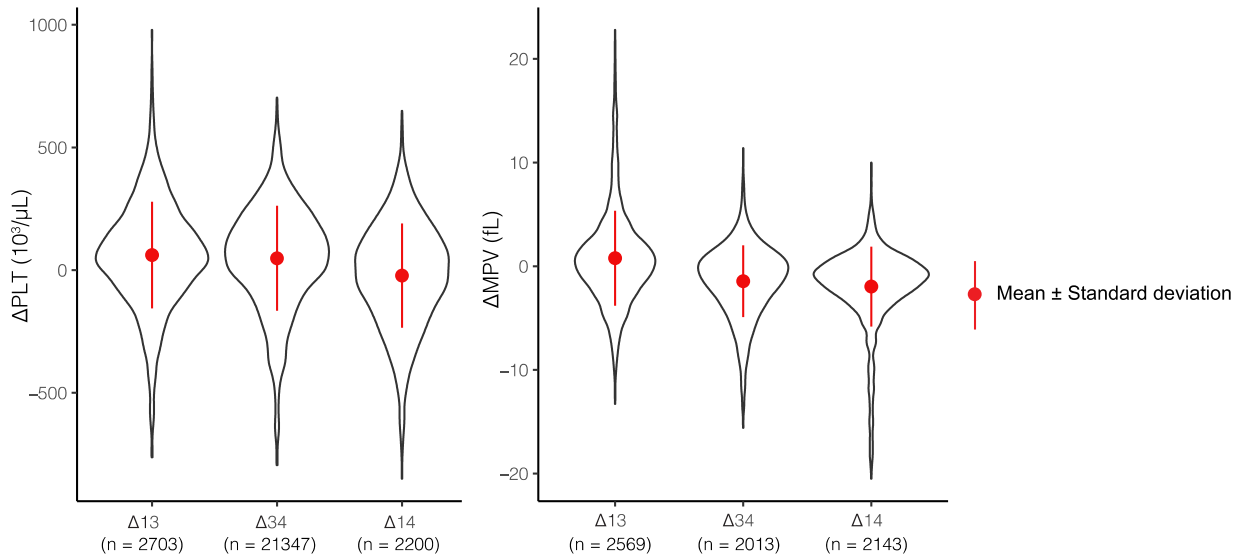

Figure 10. Violin plots for descriptive statistics for platelet traits in Δ13, Δ34, and Δ14 of all seven cycles.

Δ13 for the change from Blood 1 to Blood 3; Δ34 for the change from Blood 3 to Blood 4; Δ14 for the change from Blood 1 to Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after the challenge, respectively.

PLT: platelet concentration; MPV: mean platelet volume.

Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability.

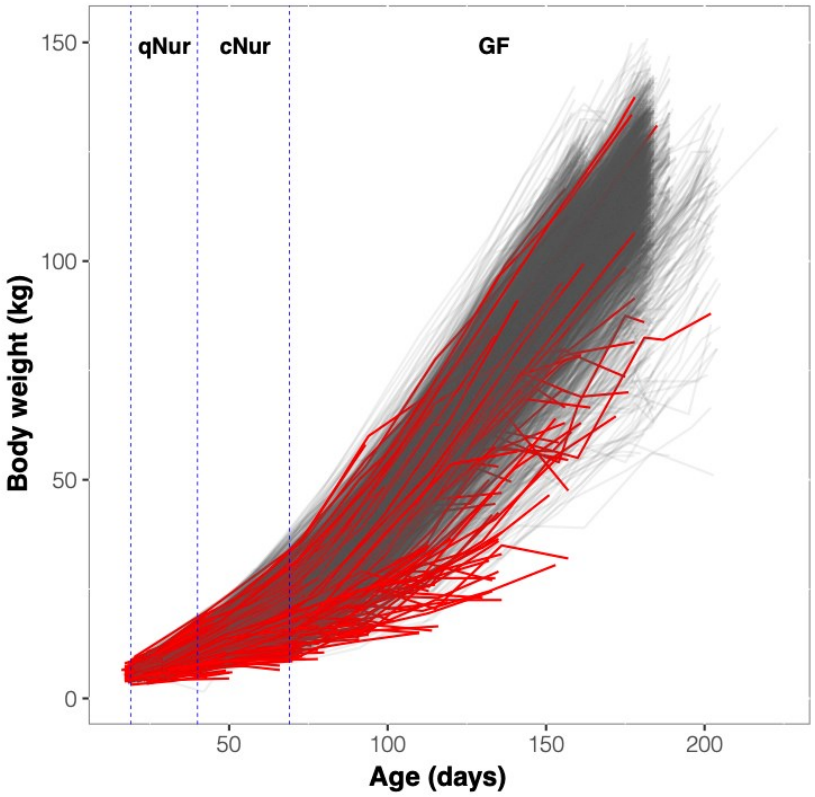

Figure 11. Growth curves of animals in the natural disease challenge model.

Growth curves of dead animals are highlighted in red. The blue dashed lines indicate the average ages of animals entering into and leaving from the quarantine nursery (qNur), the challenge nursery (cNur), and the challenge grow-to-finish barn (GF).

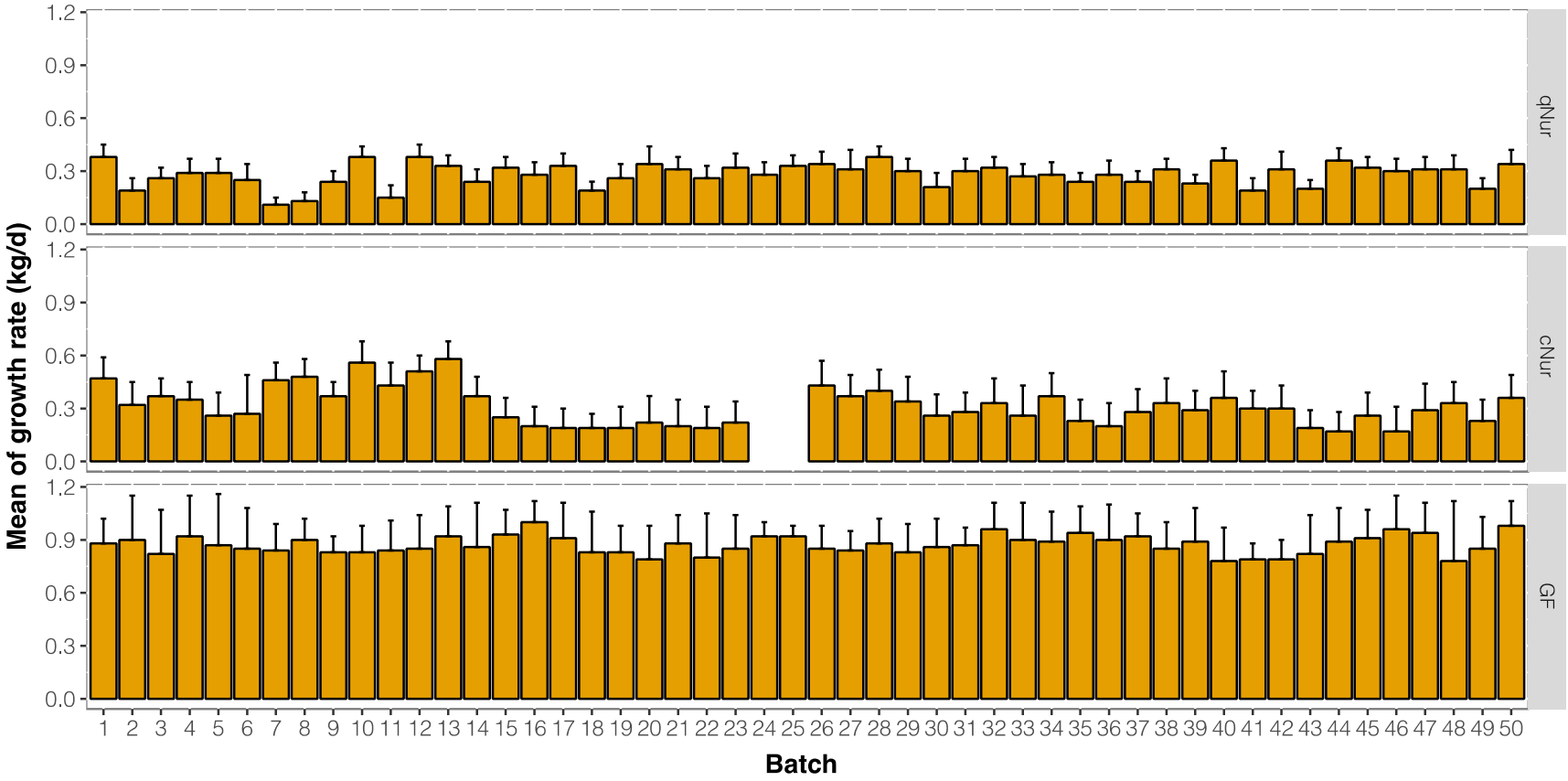

Figure 12. A summary of growth rate for each batch of animals in the natural disease challenge model.

Mean of growth rate (kg/d) in the quarantine nursery (qNur), challenge nursery (cNur), and challenge grow-to-finish stage (GF) of each batch. The error bar indicates the upper standard deviation of the growth rate of each batch.

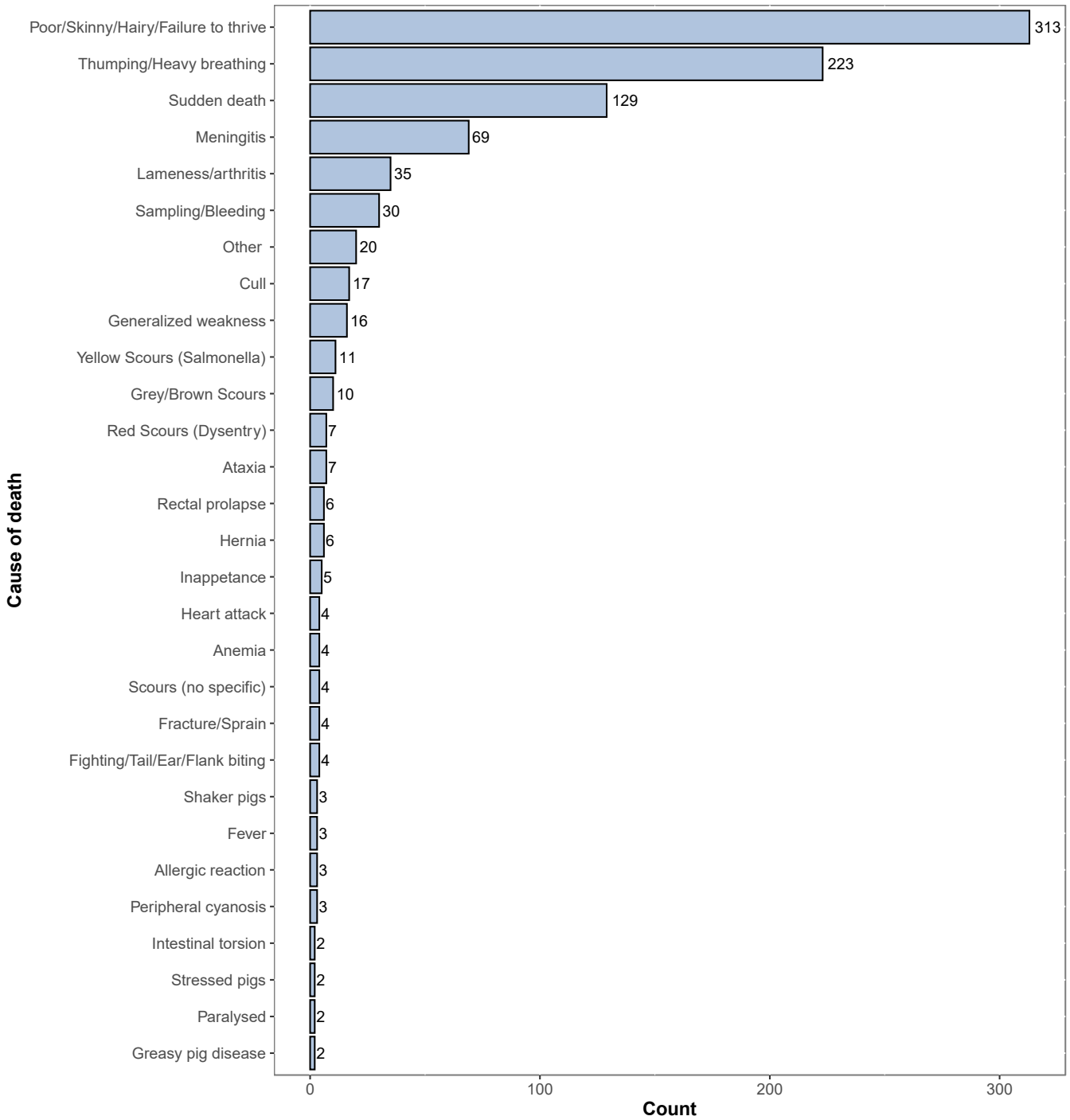

Figure 13. A summary of the cause of death in the natural disease challenge model.

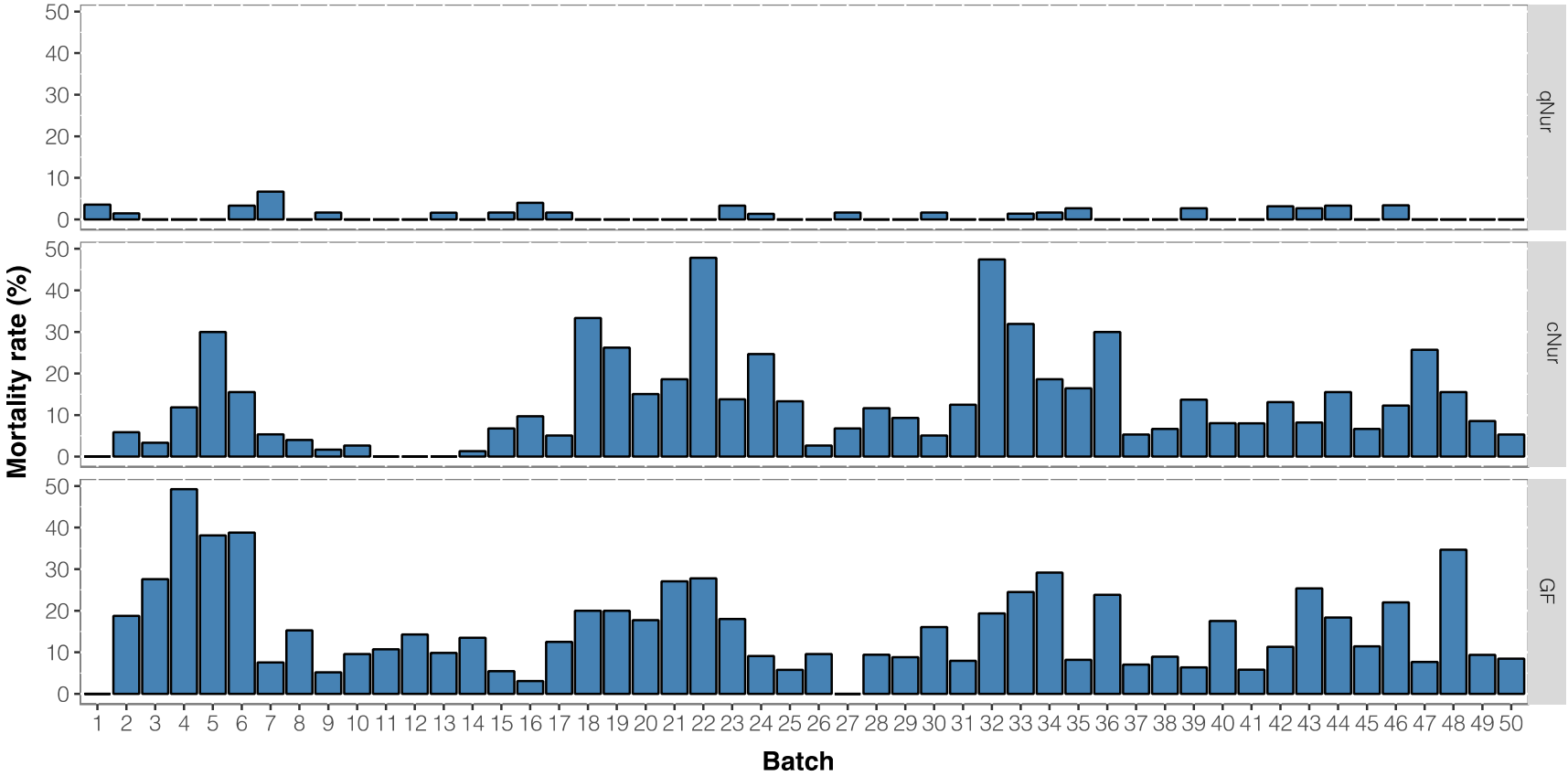

Figure 14. A summary of the mortality rate for each batch of animals in the natural disease challenge model.

Mortality rate (%) in the quarantine nursery (qNur), challenge nursery (cNur), and challenge grow-to-finish stage (GF) of each batch.

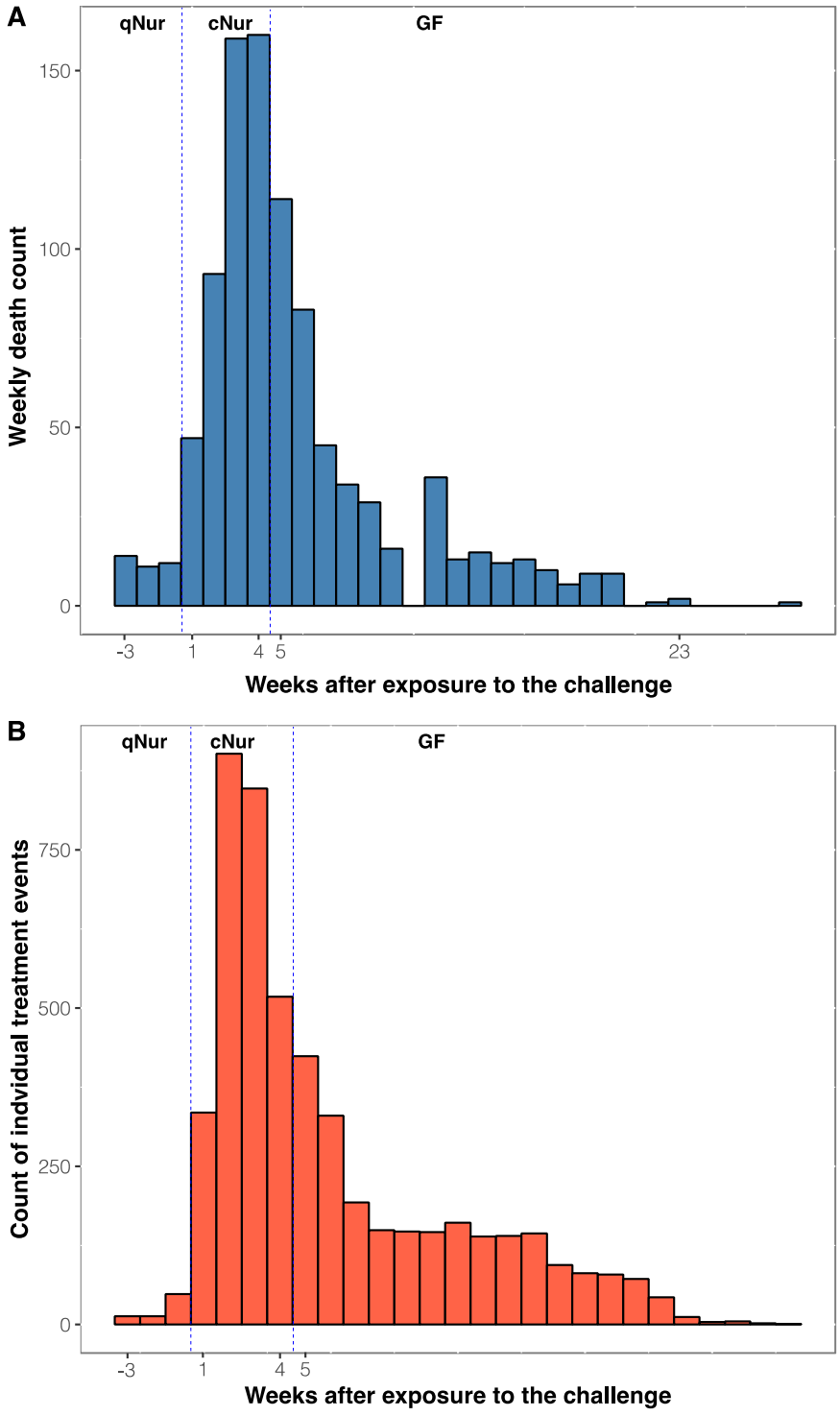

Figure 15. Weekly death count (A) and count of individual treatment event (B) in the natural disease challenge model.

qNur represents for quarantine nursery stage, cNur represents for challenge nursery, GF represents for challenge grow-to-finish stage.

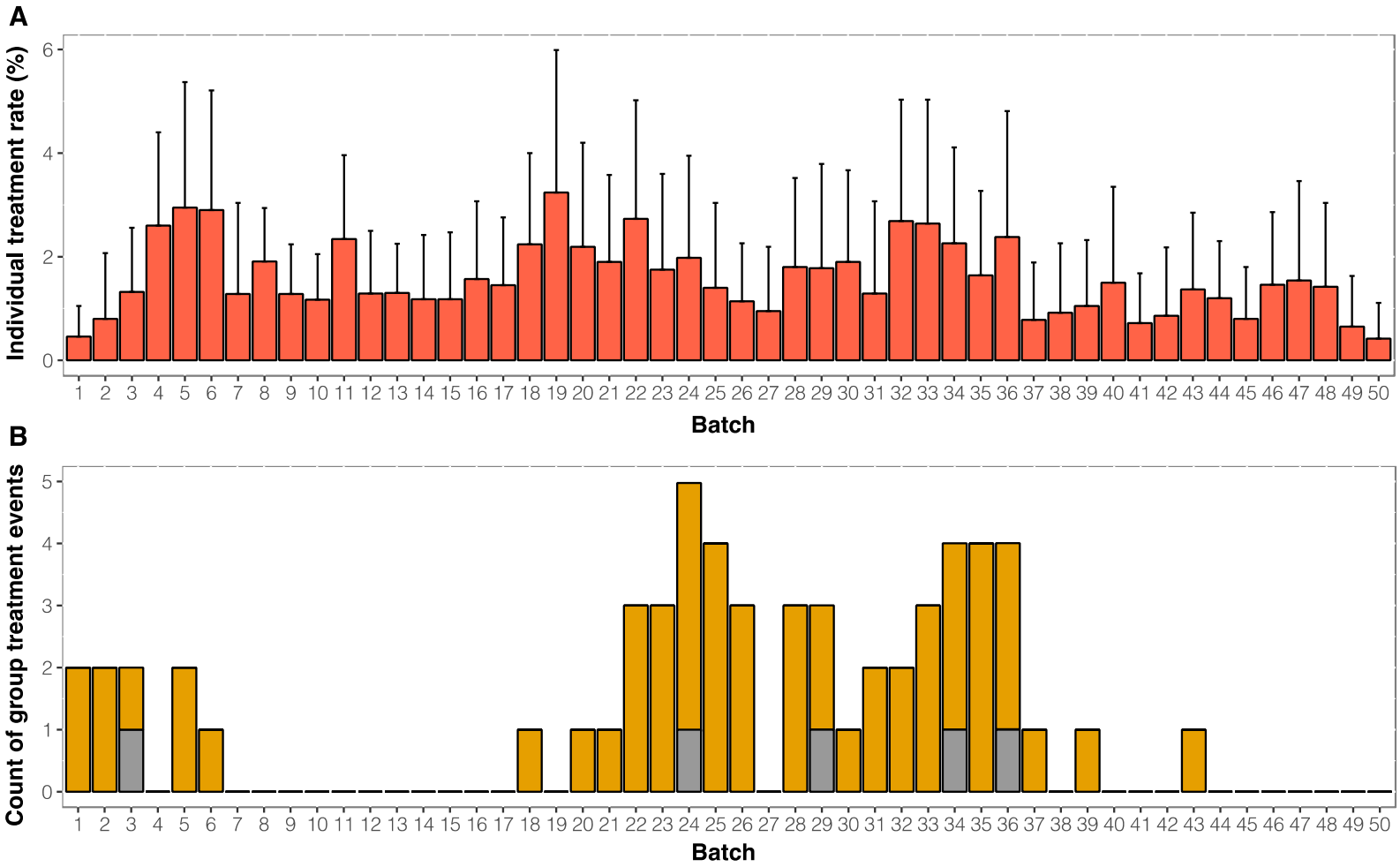

Figure 16. A summary of treatment for each batch of animals in the natural disease challenge model.

Mean (red bar) and standard deviation (error bar) of individual treatment rate in each batch **(A)**. The count of group treatments given to each batch during the quarantine nursery stage (qNur, grey bar) and in the challenge barn (yellow bar) during the late nursery (cNur) and grow-to-finish (GF) stages **(B)**.

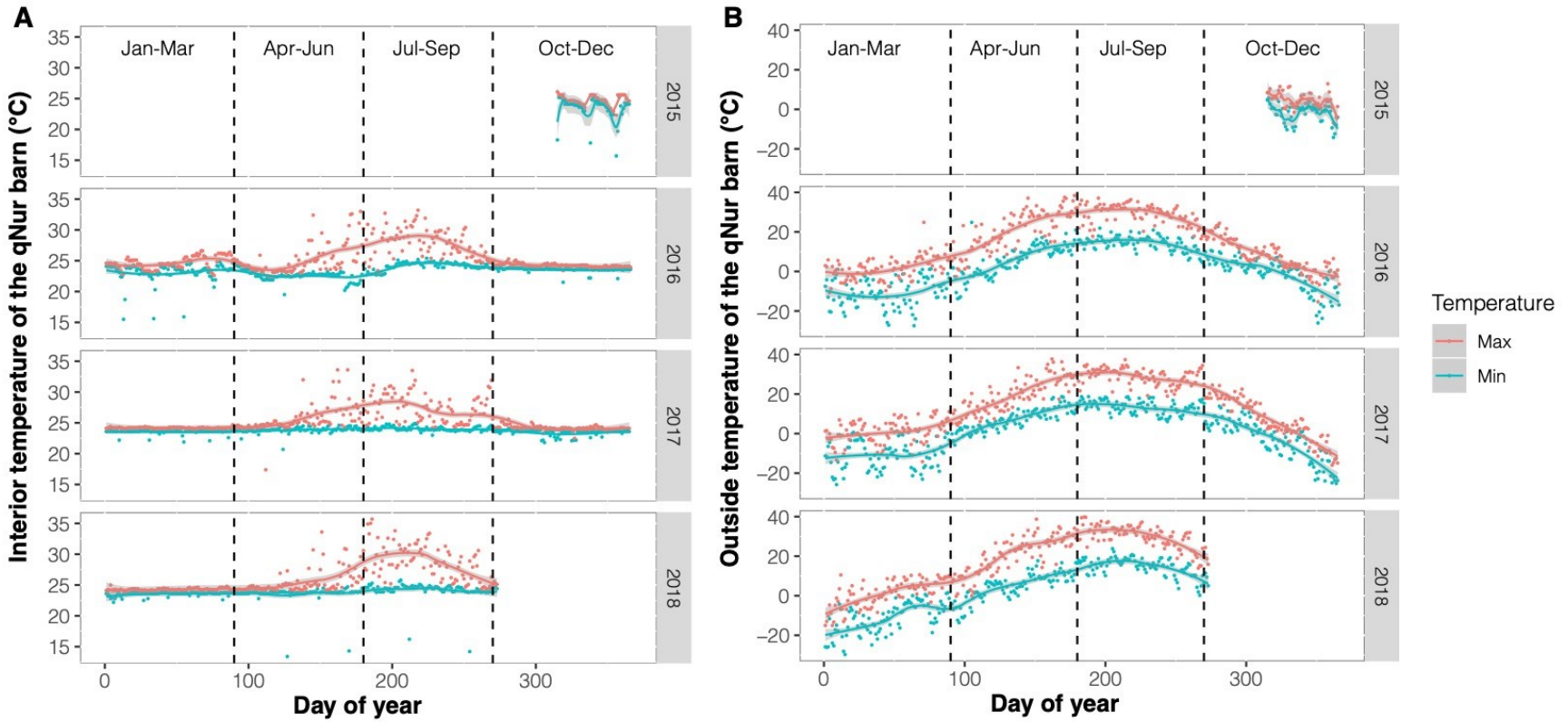

Figure 17. Daily temperatures of the quarantine nursery barn (qNur).

Maximum (Max) and minimum interior temperatures **(A)** and outside temperatures **(B)** of the quarantine nursery (qNur) barn.

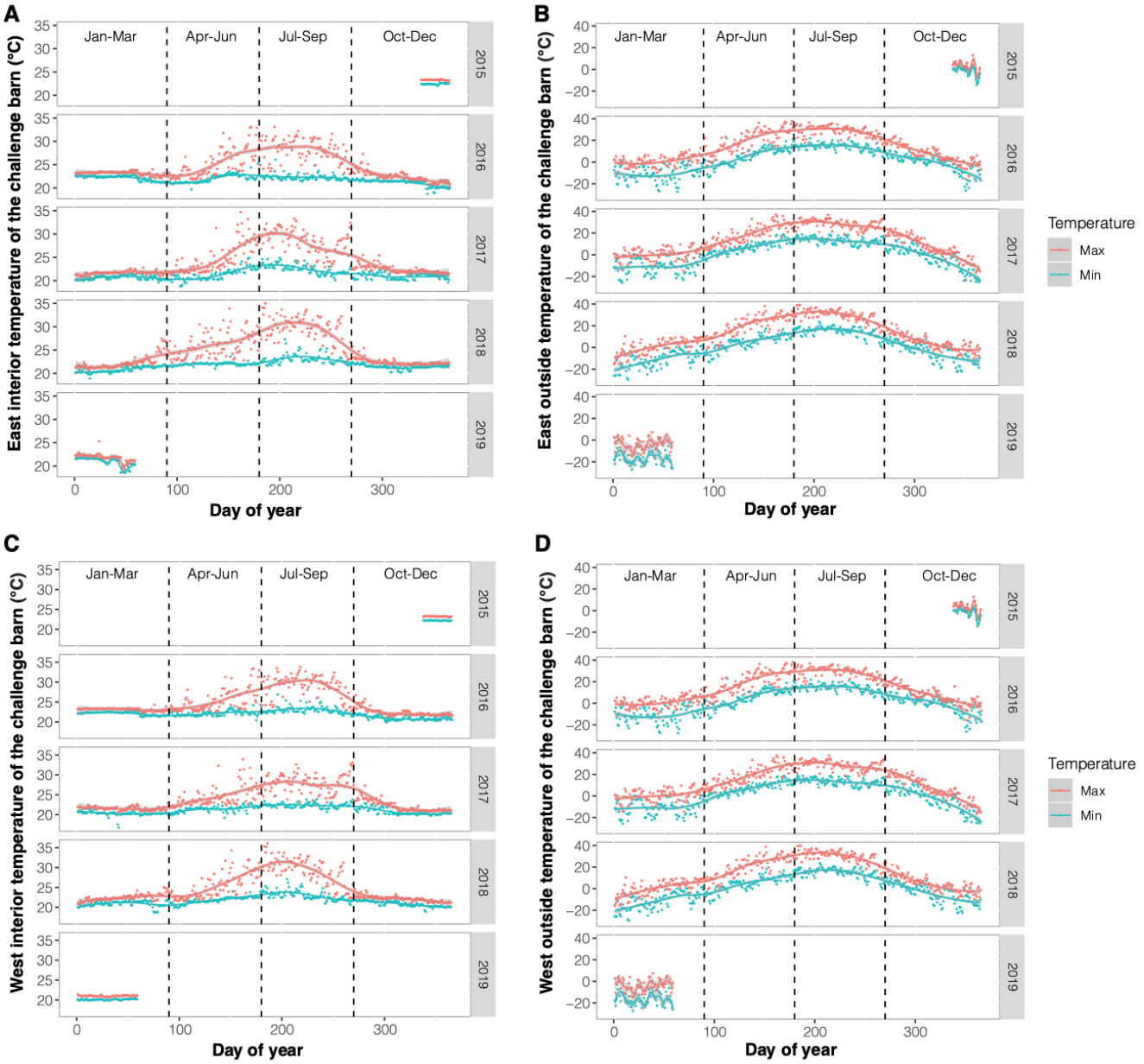

Figure 18. Daily temperatures of the challenge barn.

Maximum (Max) and minimum (Min) interior **(A)** and outside **(B)** temperatures measured from the east side of the challenge barn. Daily Max and Min interior **(C)** and outside **(D)** temperatures measured from the west side of the challenge barn.

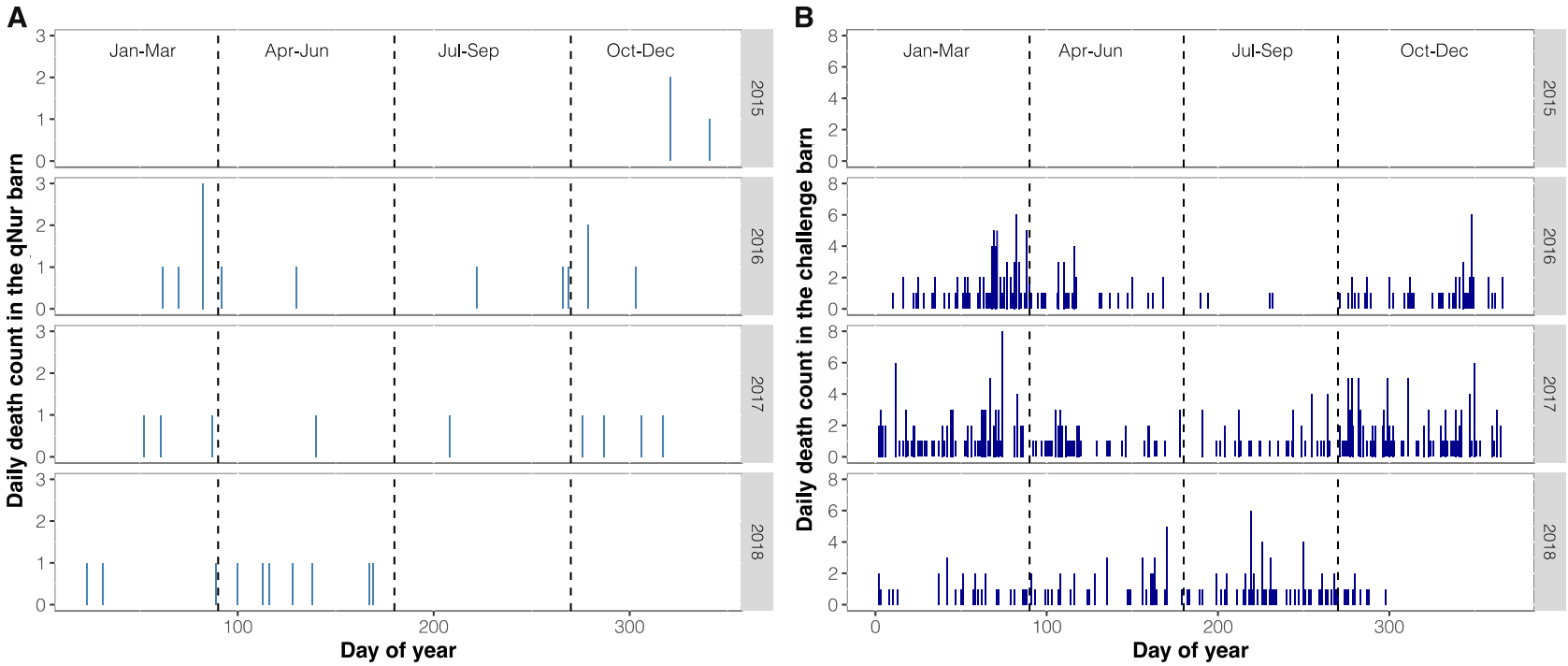

Figure 19. Daily death count in the quarantine nursery barn (qNur) (A) and the challenge barn (B).

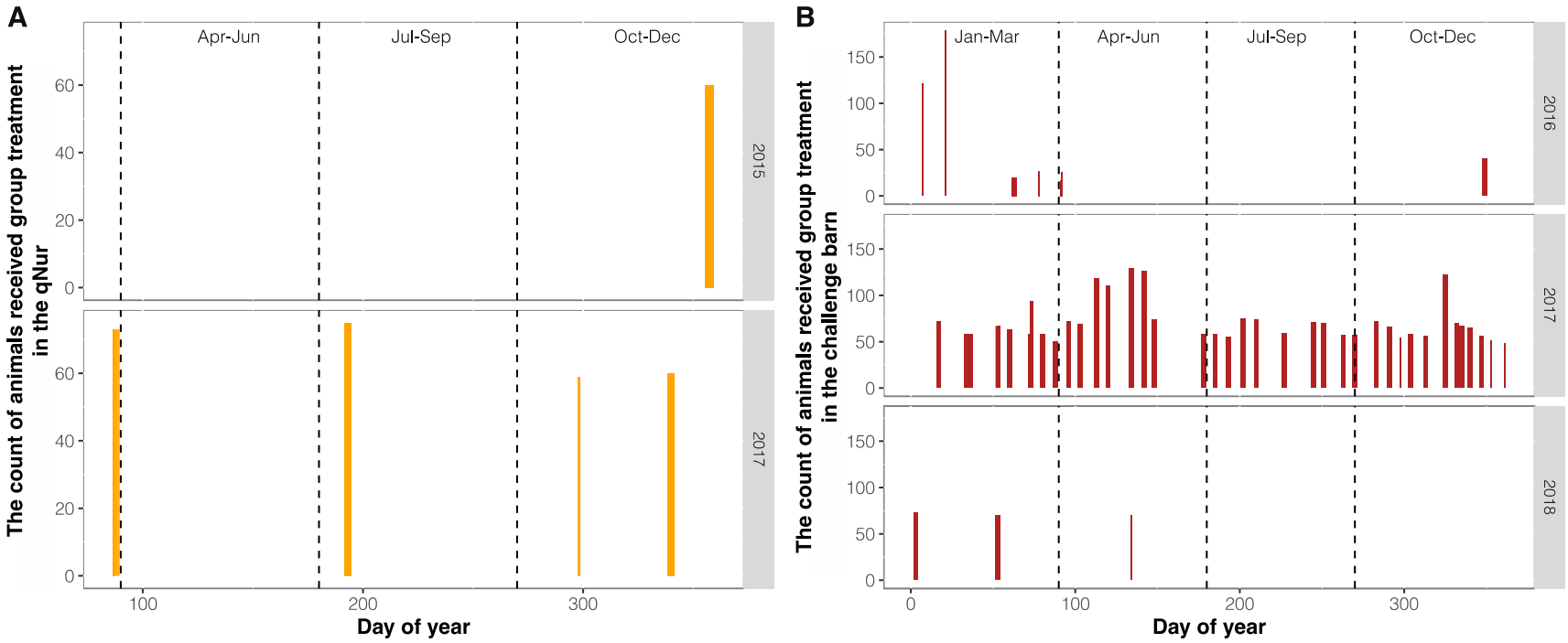

Figure 20. Daily count of group treatment events in the quarantine nursery barn (qNur) (A) and the challenge barn (B).

The thickness of the bar indicates the duration of group treatment that was given for successive days.Therefore, the thicker bar represents the group treatment was giveb for more successive days.

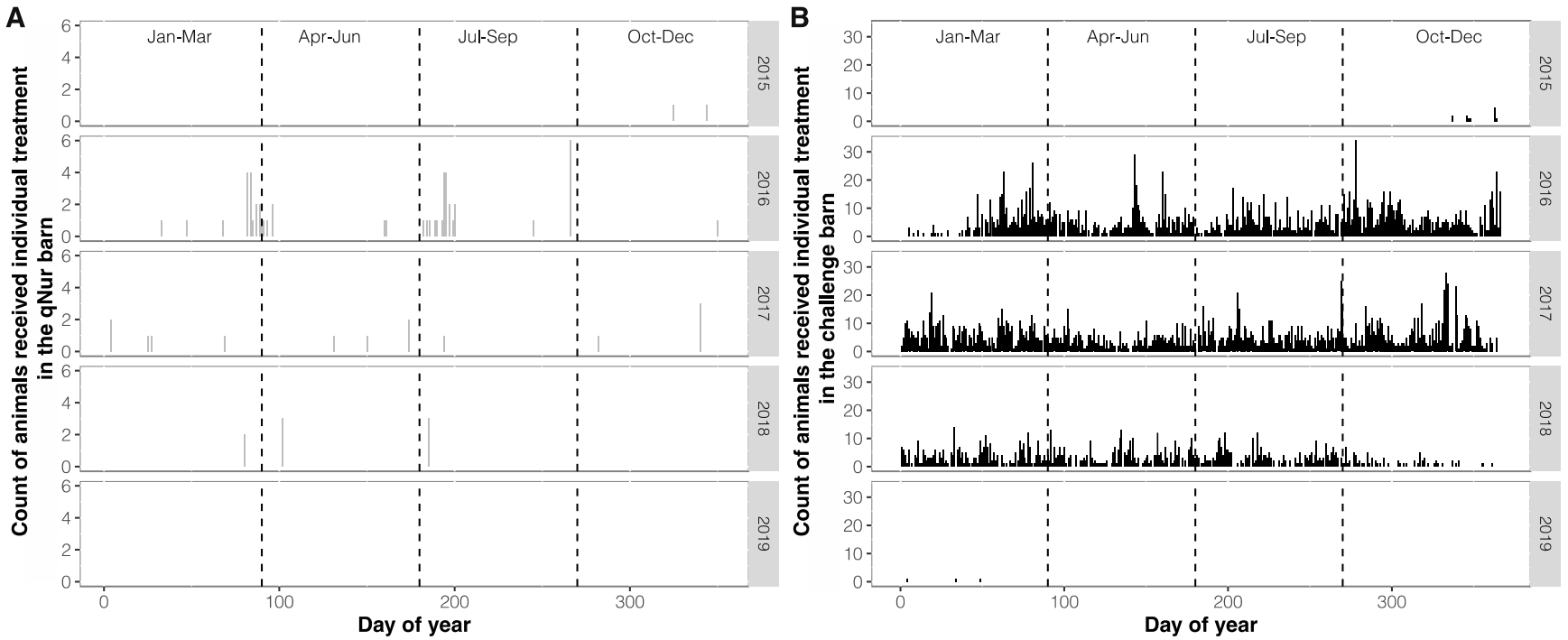

Figure 21. Daily count of individual treatment events in the quarantine nursery (qNur) barn (A) and the challenge barn (B).

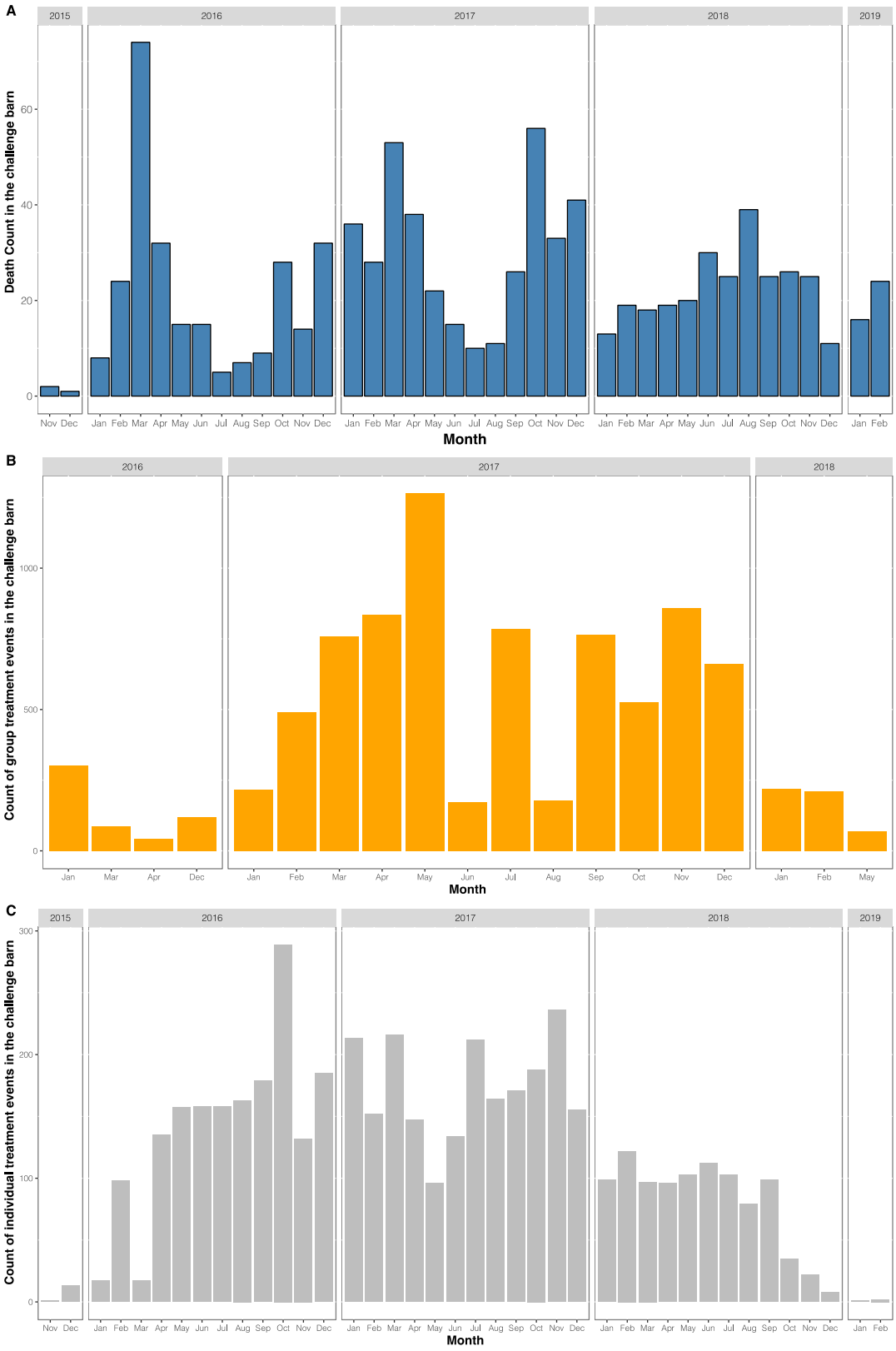

Figure 22. Count of mortality (A), group treatment events (B), and individual treatment events (C) in the challenge barn on the monthly basis.

Chapter 4. Exploring Phenotypes for Disease Resilience in Pigs Using Complete Blood Count Data[1]

4.1. Introduction

Disease resilience is defined as an animal's ability to maintain a relatively undepressed performance in the face of infection (Albers et al., 1987; Mulder and Rashidi, 2017). In pig breeding, disease resistance, which is defined as the ability to suppress establishment and subsequent development of infection, has been generally discussed in terms of making genetic improvement of herd health (Albers et al., 1987; Bishop and Steer, 2003; Guy et al., 2012). For example, the discovery of a polymorphism at bp 307 (G/A) in the fucosyltransferase gene (*FUT1*) associated with susceptibility/resistance to infection with F18 fimbriated *Escherichia coli* (ECF18) made it possible to select for ECF18 resistant pigs (Meijerink et al., 1997; Meijerink et al., 2000). Pigs that are homozygous for the resistant allele are resistant to ECF18 due to the non-adhesion of ECF18 in the small intestine (Meijerink et al., 1997; Bao et al., 2012). However, such complete resistance to a pathogen is not common, and selection for disease resistance to a specific pathogen may have unfavourable consequences for defending against other pathogens (Wilkie and Mallard, 1999; Guy et al., 2012). Currently, the challenge of infectious diseases in the pig industry is caused by a multitude of pathogens exists around the world (Zimmerman et al., 2012). Some pathogens, including porcine reproductive and respiratory syndrome virus (PRRSV), can also modulate the immune system to increase susceptibility to other pathogens while suppressing the immunologic memory of the host for the

[1]This chapter has been published as Bai, X., Putz, A. M., Wang, Z., Fortin, F., Harding, J. C. S., Dyck, M. K., Dekkers, J. C. M., Field, C. J., Plastow, G. S., and PigGen Canada. (2020). Exploring phenotypes for disease resilience in pigs using complete blood count data from a natural disease challenge model. Frontiers Genetics 11, 216. doi:10.3389/fgene.2020.00216.

same pathogen (Zhu et al., 2010). Therefore, selective breeding for resilient animals that can maintain a relatively undepressed performance in a commercial system that typically harbors abundant infectious agents could be a pragmatic way to help maintain or even improve the productivity of the swine industry.

Direct selection for disease resilience is generally not feasible, because it is impractical to obtain heritable measures of resilience in the high health nucleus herds where the selection of elite breeding animals takes place (Wilkie and Mallard, 1999). Moreover, it is also challenging to appropriately characterize resilience because it is a complex trait composed of multiple biological functions, such as production, health, nutrient status, and other dynamic elements, including the efficiency of immune response and the rate of recovery from infection (Friggens et al., 2017). Many studies have explored the relationship of immune traits with performance. These include the use of white blood cell traits (**Figure 23**), which are reported to be moderately to highly heritable and genetically correlated with an animal's performance (Henryon et al., 2006; Clapperton et al., 2008, 2009; Flori et al., 2011; Mpetile et al., 2015). In addition to white blood cells, red blood cells and platelets have also been shown to play multiple roles in the immune system to help defend against pathogens, and these also have the potential to be genetically correlated with an animal's performance (Gershon, 1997; Liepke et al., 2003; Jiang et al., 2007; Rondina and Garraud, 2014; Hottz et al., 2018). Complete blood count (CBC) is a clinical measure used to evaluate the concentration and relative proportion of circulating blood cells and may be a practical measure of immune response and, therefore, could be a candidate phenotype for disease resilience. Moreover, CBC also evaluates the volume and concentration of red blood cells and hemoglobin to provide information about oxygen-carrying capacity and

anemia, which are of concern during the disease process, with further impacts on animal performance (George-Gay and Parker, 2003).

Therefore, the objectives of this study were: 1) to assess CBC profiles of pigs that exhibited divergent performance in terms of growth and individual treatment in response to a polymicrobial infectious challenge; and 2) to estimate heritabilities of CBC traits and genetic correlations of CBC with growth and treatment rates following the disease challenge.

4.2. Materials and methods

4.2.1 Natural disease challenge model

Details of the natural disease challenge model (NDCM) that provided a polymicrobial infectious challenge environment to test pigs and the data collected from the NDCM can be found in **Chapter 3**. The first six cycles of 2743 pigs were used for this study. All of these pigs were introduced in 42 batches at three-week intervals into the NDCM.

4.2.2 Genotyping

The genotyping of animals was performed at Delta Genomics (Edmonton AB, Canada) using the 650K Affymetrix Axiom ® Porcine Genotyping Array. In total, 658,692 single nucleotide polymorphisms (SNPs) were included on the chip. Raw Affymetrix SNP data for each cycle were processed separately at Delta Genomics with the Axiom Analysis Suite, using all defaults. Missing genotypes were imputed using FImpute (Sargolzaei et al., 2014). Sscrofa 11.1 was used as the reference genome. Quality control was performed using the preGSf90 software from the BLUPF90 family of programs to remove SNPs with a minor allele frequency lower than 0.01 and call rates lower than 0.90. Overall, genotypes for 2593 animals from all six cycles were used, with 475,839 SNPs remaining after processing and quality control.

4.2.3 Traits

A detailed description of CBC traits, the growth rate of each animal in the grow-to-finish phase (GFGR), and the individual treatment rate (TR) for each animal and group treatments can be found in **sections 3.3.1**, **3.3.2**, and **3.3.4** of **Chapter 3**, respectively. Group treatments given on the batch-level were not included in the data analyses because their effects would be accounted for in the model by fitting the fixed effect of batch. The TR for animals that died before receiving any treatment was set to missing.

4.2.4 Classification of pigs based on resilience

Based on resilience indicated by phenotypes of GFGR and TR, pigs were classified into four groups as "resilient (RES)", "average (MID)", "susceptible (SUS)", and "dead (DEAD)" by batch. Within each batch, slaughtered pigs that had equal or higher GFGR than the third quartile (Q3, 75% quartile), and equal or lower TR than the first quartile (Q1, 25% quartile) of all slaughtered pigs in the batch were classified as RES; slaughtered pigs that had equal or lower GFGR than the Q1 and equal or higher TR than the Q3 of all slaughtered pigs in the batch were regarded as SUS; the rest of the slaughtered animals, which had moderate TR and GFGR, were classified as MID (**Figure 24**). The influence caused by the environmental changes and differences among batches were controlled and minimized by classifying animals within each batch. Among 2593 genotyped pigs, mortalities (n = 160) caused by hernia, fighting, fracture, sampling, or sudden death due to unclear reasons were excluded from the analysis. Of the remaining 2433 pigs, 505 (21%) pigs that died as a result of infectious disease were classified as DEAD. For the 1928 pigs that were slaughtered at market body weight in the six cycles, 213 (9%) pigs were in the RES group, 1505 (61%) pigs were in the MID group, and 210 (9%) pigs were in the SUS group.

4.2.5 Statistical analyses

Removal of Outliers

Due to the relative complexity of the sample handling, shipping conditions, and laboratory analysis, outliers for the measures of CBC traits could be the result of damaged samples with hematological issues including hemolysis and clotting, or mechanical problems of the Hematology System used to measure CBC from blood samples. Such outliers were detected and removed using the Adjusted Boxplot in R (R Core Team, Package 'robustbase'). It is a robust measure of skewness in the determination of thresholds for the removal of outliers and can avoid erroneously declaring points as outliers in a skewed distribution (Hubert and Vandervieren, 2008). The skewness of a CBC trait was measured using Medcouple (Brys et al., 2004). Thresholds for removing outliers for CBC measures were determined by several parameters, including Medcouple (MC), first quartile (Q1), third quartile (Q3), and interquartile range between Q1 and Q3 (IQR). The lower and upper bounds for a right-skewed distribution ($MC > 0$) were $Q1 - 1.5(-4MC) \times IQR$ and $Q3 + 1.5(3MC) \times IQR$; for a left-skewed distribution ($MC < 0$), the lower and upper bounds were $Q1 - 1.5(-3MC) \times IQR$ and $Q3 + 1.5(4MC) \times IQR$; and for a symmetric distribution ($MC = 0$), the outliers were removed using Tukey's boxplot (lower bound $Q1 - 1.5 \times IQR$, upper bound: $Q3 + 1.5 \times IQR$) (Seo, 2006; Hubert and Vandervieren, 2008). All CBC measures outside of the upper and lower bounds were removed as outliers.

Models

The likelihood ratio test in ASReml 4.1 was used to determine the significance of different environmental random terms for litter and pen effects by comparing the full model,

including batch, bleed age, litter, pen, and genetic effects to reduced models without each litter or pen effect (Hagger, 1998; Gilmour et al., 2015).

The CBC phenotype data were analyzed using linear mixed effects models to estimate the least-squares means for CBC traits by group (RES, MID, SUS, and DEAD), and the Tukey-Kramer test was applied for pairwise comparisons of the difference between groups in R (R Core Team, packages 'lme4' and 'lsmeans'). White blood cell traits were log10-transformed because of residual heterogeneity. In the mixed model, batch was fitted as a fixed effect to control and minimize the influence of the environmental changes among batches, group was also fitted as a fixed effect, and bleeding age was fitted as a covariate. Of note, for the changes of CBC between time points, bleeding age of Blood 1 was fitted for Δ13 and Δ14, and Blood 3 bleeding age was fitted for Δ34 since the four-week interval between each blood sampling was the same for all animals. Random terms, including the litter and pen effects were fitted if significant (*p-value* < *0.05*).

Heritabilities and genetic correlations of CBC traits with resilience traits were estimated in ASReml4.1 using pairwise bivariate models, with batch, bleed age, litter, and pen effects as described above for estimating the difference between resilience groups. Analyses for GFGR and TR included the fixed effect of batch for both traits, and random effects of litter and pen if significant (*p-value* < *0.05*). Animal genetic effects were fitted using the genomic relationship matrix for 2593 animals, rather than the pedigree-based relationship matrix because the complete pedigree was unavailable due to the use of pooled semen in some batches. The genomic relationship matrix was constructed using $\boldsymbol{ZZ'}/2\sum p_i(1-p_i)$, where $\boldsymbol{Z}$ contains centered genotypes codes and p_i is the minor allele frequency for locus i (VanRaden, 2008). The average estimate of corresponding pairwise bivariate analyses was reported as the heritability for each

trait. In the bivariate models, batch was fitted as a fixed effect for both traits. The likelihood ratio test was applied to test the significance of estimates for heritabilities and genetic correlations in ASReml 4.1, where the log-likelihood of full models were compared to restricted models that constrained the genetic variance and the genetic covariance to zero, respectively (Gilmour et al., 2015).

The model used in ASReml 4.1 can be written as

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{g_1} \\ \boldsymbol{g_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_3} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z_4} \end{bmatrix} \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix}$$

where $\mathbf{y_1}$ and $\mathbf{y_2}$ denote vectors of observations for traits 1 and 2; $\mathbf{X_1}$ and $\mathbf{X_2}$ are incidence matrices relating fixed effects to $\mathbf{y_1}$ and $\mathbf{y_2}$, $\mathbf{b_1}$ and $\mathbf{b_2}$ are vectors of fixed effects for traits 1 and 2; $\mathbf{Z_1}$ and $\mathbf{Z_2}$ represent design matrices that associate observations of traits 1 and 2 to vectors of animal genetic effects $\mathbf{g_1}$ and $\mathbf{g_2}$; $\mathbf{c_1}$ and $\mathbf{c_2}$ are vectors of random effects, including litter and pen effects when they were significant (*p-value* < *0.05*); $\mathbf{Z_3}$ and $\mathbf{Z_4}$ are incidence matrices relating $\mathbf{y_1}$ and $\mathbf{y_2}$ to random effects $\mathbf{c_1}$ and $\mathbf{c_2}$; $\mathbf{e_1}$ and $\mathbf{e_2}$ are vectors of unknown and random residuals for traits 1 and 2 (Miar et al., 2014a; Miar et al., 2014b; Gilmour, 2015).

When random effects c and residuals errors e are uncorrelated, and identically distributed following a normal distribution, the (co-)variances of random effects are assumed to be

$$\boldsymbol{Var}\begin{bmatrix} \boldsymbol{g_1} \\ \boldsymbol{g_2} \\ \boldsymbol{c_1} \\ \boldsymbol{c_2} \\ \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{G\sigma^2_{g_1}} & \boldsymbol{G\sigma_{g_1g_2}} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{G\sigma_{g_1g_2}} & \boldsymbol{G\sigma^2_{g_2}} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I\sigma^2_{c_1}} & \boldsymbol{I\sigma_{c_1c_2}} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I\sigma_{c_1c_2}} & \boldsymbol{I\sigma^2_{c_2}} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I\sigma^2_{e_1}} & \boldsymbol{I\sigma_{e_1e_2}} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I\sigma_{e_1e_2}} & \boldsymbol{I\sigma^2_{e_2}} \end{bmatrix}$$

where G is the genomic relationship matrix, I is the identity matrix, $\boldsymbol{\sigma^2_g}$ is the additive genetic variance, $\boldsymbol{\sigma^2_c}$ is the random effect variance, and $\boldsymbol{\sigma^2_e}$ is the residual variance. $\boldsymbol{\sigma_{g1g2}}$, $\boldsymbol{\sigma_{c_1c_2}}$,

and $\boldsymbol{\sigma_{e_1e_2}}$ are covariances between two traits due to the additive genetic effects, common random effects, and residual effects, respectively. Heritability (h^2) of a trait was estimated using variance components obtained from the bivariate analyses, and the average estimates of corresponding pairwise bivariate analyses were reported as the heritabilities:

$$\boldsymbol{h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_c^2 + \sigma_e^2)}$$

and the genetic correlation (r_g) between two traits was estimated as:

$$\boldsymbol{r_g = \sigma_{g1g2}/\sigma_{g1}\sigma_{g2}}$$

4.3. Results

4.3.1 Descriptive statistics for CBC traits

Table 4 summarizes the descriptive statistics for the CBC data of 2593 genotyped animals after removing outliers. Most traits were recorded on all animals in Blood 1, but some samples for Blood 3 and Blood 4 were unavailable for animals that died prior to the sampling. Relevant random effects fitted in the models for CBC traits are presented in **Table 5**. The random effect of litter was fitted for GFGR, and pen effects in the challenge barn late nursery and the grow-to-finish stage were fitted for TR.

4.3.2 Group differences in CBC traits

White Blood Cell Traits

Results comparing the least-squares means of white blood cell traits in groups with different responses to the natural disease challenge are shown in **Table 6**. In Blood 1, no significant difference was found between groups for any of the white blood cell traits. However, in Blood 3, the RES group had a significantly higher LYM, and the LYM for the MID group was also significantly higher than for the DEAD group (*FDR = 0.0003*). In Blood 4, the RES and MID groups had significantly lower NEU levels than both the SUS and DEAD groups (*FDR* =

0.0002). For the count of LYM in Blood 4, the DEAD group was significantly lower than both the RES and MID groups (*FDR = 0.0012*).

Results comparing the least-squares means of changes in white blood cell traits between groups are summarized in **Table 7**. All white blood cell traits increased from Blood 1 to Blood 3 shown as positive Δ13. The increase of LYM was significantly higher for the RES group than for the other groups (*FDR = 0.0002*), but no significant difference was found among the MID, SUS and DEAD groups. Changes of white blood cell traits from Blood 3 to Blood 4 were not as dramatic as those from Blood 1 to Blood 3, except for LYM, which had a higher increase from Blood 3 to Blood 4 for all groups. The WBC, LYM, and MONO levels increased continuously for all groups based on positive Δ13 and Δ34, but EOS and BASO decreased from Blood 3 to Blood 4 based on negative Δ34. NEU showed a tendency to decrease in the RES and MID groups, which was opposite to the positive NEU in the SUS and DEAD groups for Δ34 (*FDR <0.0024*). Additionally, a significant difference in NEU among groups was also identified for Δ14, which represents the overall change of NEU from Blood 1 to Blood 4. Δ14 for NEU were positive for all groups, but the SUS and DEAD groups had significantly higher increases in NEU than the RES and MID groups (*FDR = 0.0002*). Compared with Blood 1, which was collected in the quarantine unit, the other white blood cell traits, including WBC, LYM, MONO, EOS, and BASO, also increased significantly in Blood 4, although no significant differences based on Δ14 were found between groups.

Red Blood Cell and Platelet Traits

Results of comparing red blood cell and platelet traits in the RES, SUS, MID, and DEAD groups are summarized in **Table 8**. No significant differences were identified between groups for either red blood cell or platelet traits in Blood 1. However, for Blood 3, RDW and MPV were

significantly higher in the DEAD group than in the RES and MID groups (*FDR < 0.002*). For Blood 4, several red blood cell traits showed significant differences between groups. Notably, HGB, HCT, and MCH were found to be significantly lower in the SUS and DEAD groups than in the RES and MID groups (*FDR < 0.0005*). Moreover, RBC was significantly higher in the RES and MID groups than in the SUS and DEAD groups (*FDR = 0.0036*), and MCV was significantly lower in the DEAD group than in the others. In contrast, RDW and MPV were found to be significantly higher in the DEAD group than in the RES in Blood 4.

Table 9 summarizes the results of comparing the least-squares means of changes in red blood cell and platelet traits between groups. In contrast to the increase in white blood cell traits, all red blood cell traits decreased from Blood 1 to Blood 3, except for MCHC, which increased significantly in the DEAD group. Apart from MCHC, the drop for the other red blood cell traits from Blood 1 to Blood 3 did not show a tendency of being different between groups. The MPV in the SUS group was the only platelet trait that did not show a significantly positive Δ13 due to a relatively large standard error. Changes of platelet traits based on Δ13 did not show significant differences between groups. In contrast to the decreasing trend of red blood cell traits from Blood 1 to Blood 3, RBC and HCT increased significantly from Blood 3 to Blood 4 for all groups based on positive Δ34. Moreover, HGB also increased for both the RES and MID groups from Blood 3 to Blood 4, and Δ34 for HGB of these groups was significantly different from Δ34 for the SUS and DEAD groups (*FDR = 0.0002*), which were not found to be significantly different from zero. The MCV decreased continuously based on negative Δ34, and the DEAD group showed a more dramatic drop in MCV than the RES and MID groups (*FDR = 0.0003*). MCH and MCHC also kept decreasing based on negative Δ34, and the decrease of MCH for the DEAD group was significantly higher than for the RES and MID groups. Platelet traits also

reduced from Blood 3 to Blood 4 for all groups, except for PLT in the SUS group, which did not show a significantly negative Δ34 due to a relatively large standard error.

Although several traits increased slightly from Blood 3 to Blood 4, for the overall changes from Blood 1 to Blood 4, all traits decreased significantly based on negative Δ14, except for RBC and PLT. Comparing Blood 4 to Blood 1, RBC increased slightly for the RES and MID groups, but it showed a tendency to return to the same level as in Blood 1 for the SUS and DEAD groups. PLT increased significantly from Blood 1 to Blood 4 for the RES, MID, and SUS groups, with no significant change identified for the DEAD group. MCHC was the only trait that showed a significant difference between groups for Δ14, which was lower in the SUS group than in the RES group (*FDR = 0.04*).

4.3.3 Estimates of Heritability

The GFGR was estimated to be moderately heritable (0.15 ± 0.04), but the heritability estimate of TR was low (0.04 ± 0.01). Heritability estimates for CBC traits with standard errors are in **Table 10**. Most CBC traits were moderately heritable, with estimates ranging from 0.11 ± 0.03 to 0.27 ± 0.04. A few red blood cell traits showed moderate to high heritability estimates, ranging from 0.30 ± 0.04 to 0.53 ± 0.05, including RBC, MCV, and MCH in Blood 3 and 4. Estimates of heritability were low for some CBC traits, including BASO, HGB, and HCT in Blood 1, PLT in Blood 3 and Blood 4, RDW in Blood 4, and also for the changes of many CBC traits based on Δ13, Δ34, and Δ14. Genetic variances of several traits, especially MONO, and some changes of EOS, BASO, HCT, PLT and MPV were not found to be significantly different from zero based on likelihood ratio tests, which compared full models to restricted models that constrained the genetic variance to zero in ASReml 4.1 (*p-value > 0.05*) (Gilmour et al., 2015).

4.3.4 Estimates of Genetic Correlations

GFGR and TR were estimated to be negatively correlated, with a genetic correlation of -0.50 ± 0.16. Estimates of genetic correlations for CBC traits that showed significant differences among groups (RES, MID, SUS, and DEAD) and the resilience traits of GFGR and TR are summarized in **Table 11**. LYM in Blood 3 and its change based on Δ13, which had the highest levels in the RES group, showed significantly negative genetic correlations with TR of -0.38 ± 0.18 and -0.46 ± 0.24, respectively. HCT based on Δ34, which was significantly higher in the RES and MID groups, showed a high negative genetic correlation with TR (-0.82 ± 0.47). NEU in Blood 4, RDW in Blood 4, and the change of NEU based on Δ14, which all had higher counts in the SUS and DEAD groups, showed significantly positive genetic correlations with TR. Genetic correlations between these CBC traits and GFGR showed a tendency of being opposite to the positive genetic correlations with TR but had relatively large standard errors. NEU based on Δ34, which was significantly positive in the SUS and DEAD groups but not significantly different from zero in the RES and MID groups, was estimated to have a negative genetic correlation with GFGR (-0.45 ± 0.21). TR showed a tendency to have a positive genetic correlation with the NEU based on Δ34 but had a large standard error (0.44 ± 0.26). For CBC traits from Blood 1, RDW was the only trait that showed a significantly positive genetic correlation with TR (0.41 ± 0.20), while none of the other CBC traits from Blood 1 showed significant correlations with TR or GFGR due to having low estimates and relatively high standard errors. Estimates of genetic correlations for CBC traits within Blood 1, Blood 3, and Blood 4 are summarized in **Table 12**, while estimates of genetic correlations for each CBC trait between Blood 1, Blood 3, and Blood 4 are shown in **Table 13**. Genetic correlations between Δ13, Δ34, and Δ14 were also estimated for each CBC trait and are summarized in **Table 14**.

4.4. Discussion

4.4.1 CBC Traits and Disease resilience

Hematopoiesis, including the establishment and maintenance of all circulating cellular blood components, relies on the proliferation and differentiation of hematopoietic stem cells (HSCs) (Orkin and Zon, 2008; Zaretsky et al., 2014). In response to disturbances of the hematopoietic equilibrium, such as infection, extensive proliferation and increased differentiation of HSCs are required to meet the higher demand of immune effector cells (Baldridge et al., 2001; Shahbazian et al., 2004; Singh et al., 2008; Johns et al., 2009; Yáñez et al., 2009; Boettcher and Manz, 2017). In the natural challenge model, our results showed that all white blood cell traits increased significantly from Blood 1 to Blood 4, although some traits, including NEU, EOS, and BASO, decreased from Blood 3 to Blood 4 (**Tables 7**). According to the reference intervals, white blood cell traits have the tendency to increase slightly with age, except for NEU, which tends to decrease with age (**Table 4**) (Iowa State University's Clinical Pathology Laboratory, 2011). Eze et al. (2011) indicated that white blood cell traits did not vary significantly between clinically healthy piglets and adults raised under an intensive management system. Therefore, the significant increases of all white blood cell traits observed here are likely to result from recruiting phagocytes (monocytes, neutrophils), immunocytes (lymphocytes), and granulocytes (neutrophils, eosinophils, and basophils) to drive immune responses at the early stage of infection (George-Gay and Parker, 2003; Rothenberg and Hogan, 2006; Mitre and Nutman, 2006; Porwit et al., 2011).

Notably, resilient pigs had significantly higher LYM for Blood 3 and based on Δ13 compared to the other three groups. Lymphocytes are mainly indicative of initiation and execution of the adaptive immune responses due to their essential and multiple roles in adaptive

immunity (**Figure 23B**). Higher LYM in the blood of resilient pigs may indicate earlier and greater adaptive immune responses and increase the transport of lymphocytes to the infected tissues. Resilient pigs may be primed to orchestrate immune responses against a wide variety of pathogens more efficiently together with the higher concentrations of lymphocytes in infected tissues at the early stage of infection and, therefore, limiting the adverse effect caused by infectious challenges (Wilkie and Mallard, 1999, Badri and Wood, 2003, Zabriskie, 2009, Zhu et al., 2010; Luckheeram et al., 2012). This was also indicated by the negative genetic relationships of TR with LYM in Blood 3 and its change based on Δ13. A higher increase of LYM from Blood 1 to Blood 3 should favor resilience, which is related to a lower TR. Neutrophils, which increased significantly from Blood 1 to Blood 3 for all groups are both present as phagocytes and granulocytes in the innate immune response to defend against bacterial pathogens (**Figure 23A**) (Pham, 2006; Kolaczkowska and Kubes, 2013; Boettcher and Manz, 2017). However, after moving animals into the grow-to-finish stage, between Blood 3 and Blood 4, NEU showed the tendency to decrease in the RES and MID groups, which was opposite to the significant rise observed for the SUS and DEAD groups. Thus, NEU in Blood 4, and its changes based on Δ34 and Δ14 were also significantly lower for the RES and MID groups compared to the SUS and DEAD groups. Sustained high levels of NEU for the SUS and DEAD groups may be related to ongoing bacterial infection. The decrease of NEU in the blood of the RES and MID groups may indicate the recovery and resolution of inflammation when pathogens were brought under control by early initiation and efficient adaptive immune responses in resilient animals with higher increase of LYM from Blood 1 to Blood 3 (Savill, 1997; Nathan, 2006). Alternatively, it may reflect that neutrophils were already transported to the infected tissues to defend against pathogens in the RES and MID groups. These suggested processes need to be further explored

for example, by monitoring the pathogen load in animals and identifying signs of the resolution of inflammation, such as the exodus of neutrophils in infected tissues and "stop signals" or checkpoints of inflammation, including lipoxins, Resolvins, and D-series prostaglandins (Serhan et al., 2007). Positive genetic correlations of TR with NEU in Blood 4 and its change based on Δ14, and the negative genetic correlation of GFGR with NEU based on Δ34 together may indicate that higher NEU in the grow-to-finish stage has a negative relationship with resilience, which is associated with increased TR and decreased GFGR.

Unlike the situation of white blood cells, red blood cell traits declined from Blood 1 to Blood 3 to the same degree for all groups, except for MCHC, which did not show a significant decrease (**Table 9**). By comparing clinically healthy grower to finisher pigs, Ježek et al. (2018) suggested that red blood cell traits, including RBC, HGB, HCT, MCV, and MCH, increased with age. The reference intervals from Iowa State University's Clinical Pathology Laboratory (2011) also indicated a tendency for red blood cell traits to increase with age in pigs. Therefore, significant decreases in red blood cell traits from Blood 1 to Blood 3 are likely caused by the challenge of bacterial pathogens, which could damage circulating blood cells and accelerate hemolysis for iron to support bacterial cellular processes of respiration and replication (Barrett-Conner, 1972; Kent, 1994; Viana, 2011, Cassat and Skaar, 2013). This, however, changed during the late stage of infection for the RES and MID groups, for which HGB and HCT increased significantly from Blood 3 to Blood 4. Although red blood cell traits may increase with age, the significantly higher increase of HGB and HCT from Blood 3 to Blood 4 of more resilient animals may also suggest a better performance and faster recovery from infection by providing a higher level of iron and oxygen to the host (Morera and MacKenzie, 2011). Moreover, hemoglobin has been found to directly participate in immune responses as a source of bioactive

peptides that exhibit antimicrobial activity against bacteria (EL Bishlawy, 1999; Liepke et al., 2003). The higher increase of HGB from Blood 3 to Blood 4 of resilient animals are expected to enhance immune responses and work together with the other immune cells to defend against pathogens. Although relatively large standard errors are reported, highly negative genetic correlations of TR with HGB and HCT based on Δ34 and in Blood 4 may indicate that higher HGB and HCT during the late stage of infection favors resilience, which is related to lower TR. In addition, the significant increase in RDW has been identified to be a valuable index for assessing various pathological conditions, including inflammation and respiratory diseases in humans (Goyal et al., 2017). Our results also showed higher levels of RDW in Blood 3 and Blood 4 for less resilient animals. According to the highly positive genetic correlation of TR with RDW in Blood 4 (0.89 ± 0.26), higher RDW after challenge may have adverse effects associated with increasing the TR.

Significant genetic correlations of CBC traits with resilience traits suggest that a well-functioning immune system plays an essential role in resilient animals to maintain performance and prevent death from infection. An adequate nutritional status is necessary for the normal functioning of various components of the immune system because the immune system is energetically expensive (Coop and Kyriazakis, 2001; McDade, 2005; Nelson and Williams, 2007; Calder, 2013). Any changes in resource demands by the immune system can create significant differences in the level of fitness and performance that are related to resilience (Stearns, 1976). When nutrient resources are limited by decreased feed intake in response to disease challenge, a trade-off is expected to occur between the immune system and other nutrient-demands, such as growth (Lochmiller and Deerenberg, 2000; Doeschl-Wilson et al., 2009; Rauw, 2012; Putz et al., 2018). Although the negative genetic correlation between GFGR

and TR could be the result of decreasing feed intake in challenged pigs, it might further indicate the trade-off and competing demands for the investment of nutrients in growth and immune function. In susceptible and dead animals, the infection may not be eliminated effectively as a result of a weak immune response. Therefore, decreased feed intake, along with prolonged infection, may further compromise the immune system, leading to a more severe disease state, and increased susceptibility to other pathogens (Keusch, 2003; Nelson and Williams, 2007; Hine et al., 2014). Conversely, the significant changes of CBC traits over time in RES animals, including higher LYM based on Δ13, higher HGB and HCT based on Δ34, and lower NEU based on Δ34 together, are expected to indicate the allocation of more resources towards immunity during the infection stage to help limit infection in resilient animals. Once the infection is brought under control by an efficient immune response, resilient animals may recover earlier from the infection, which could allow them to allocate more resources to maintain a higher growth rate in the grow-to-finish stage (Calder, 2013; McDade, 2005).

4.4.2 Estimates of Heritabilities

Estimates of heritabilities for CBC traits have been reported in many studies (**Table 15**). Some of these were conducted under a controlled environment with limited disease challenges and types of pathogens (Clapperton et al., 2008, 2009). Others were conducted under a lower health status condition with multiple pathogens (Henryon et al., 2006; Flori et al., 2011; Mpetile et al., 2015). Heritability estimates for CBC traits in the natural challenge model in this study were within the range of estimates reported in these studies. Additionally, we were able to provide heritability estimates for novel CBC traits that capture changes of CBC in response to the challenge of infection. Heritability estimates for many CBC traits, especially red blood cells, were observed to be higher in Blood 3 and Blood 4 than in Blood 1, possibly because genetic

variances of these traits may be more fully expressed in a lower health environment when there is the challenge of infection (Clapperton et al., 2008, 2009).

Heritability estimates for GFGR and TR in this study were 0.15 ± 0.04 and 0.04 ± 0.01, respectively. Guy et al. (2018) estimated the heritability of treatments for a relatively high-health herd to be between 0.04 ± 0.03 and 0.06 ± 0.04. Putz et al. (2018) estimated the heritability of finishing average daily gain (FinADG) to be 0.25 ± 0.07 based on the phenotypes of the first three cycles of this natural challenge model. Moreover, the heritability for treatment rate adjusted to 180 days for animals that reached 65 days of age (TRT180) was estimated to 0.29 ± 0.07 by Putz et al. (2018). Our use of phenotypes and genotypes on a larger population with 2593 animals of six cycles resulted in relatively lower estimates of heritabilities and lower standard errors for both growth and treatment traits. Moreover, heritability estimates for the treatment rate were different since the definitions of this trait were not the same. In Putz et al. (2018), animals that died before the age of 65 days were excluded, but we included all animals unless they died without receiving any treatment. Moreover, we used additional batches of animals that were introduced into the natural challenge. As disease pressure varied by batch and on a seasonal basis, treatment rates could change accordingly. Moreover, treatment rates may also change with many other noninfectious factors, such as the level of stress caused by weather and transport in these batches (Bishop and Woolliams, 2014). Therefore, the heritability estimates for treatment rates are expected to change correspondingly.

4.5. Conclusions

Resilience is a valuable attribute in livestock to manage infectious diseases and sustainably increase production efficiency, as resilient animals can maintain their performance without the need for intensive treatment. Consequently, there is an increasing focus on exploring

the potential to select for resilience. Although CBC in Blood 1 is attractive as a potential predictor trait for resilience, as it is a cost-effective phenotype that can be collected from nucleus breeding herds with high health, no significant differences in CBC traits between resilience groups were identified for Blood 1 and estimates of genetic correlations of Blood 1 CBC traits with resilience were not significantly different from zero. Alternatively, for CBC under disease, resilient animals were found to have a greater increase of lymphocyte levels in the blood collected at 2-weeks after challenge, higher levels of hemoglobin and hematocrit, but a significantly lower level of the neutrophil concentration based on the changes from 2-weeks to 6-weeks. Therefore, these changes of CBC traits in response to a disease challenge could provide a measure of resilience. Several of the latter CBC traits were found to be heritable and genetically correlated with resilience. Thus, these CBC traits may have the potential to be further developed as a phenotype for prediction of resilience by collecting data from commercial systems.

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Table 4. Descriptive statistics for complete blood count (CBC) traits in Blood 1, Blood 3, and Blood 4 after removing outliers, including the number of animals per trait (n), mean, standard deviation (sd), minimum (Min), and maximum (Max) values.

Traits[1]	Blood 1					Blood 3					Blood 4					Reference Intervals[2]	
	n	mean	sd	Min	Max	n	mean	sd	Min	Max	n	mean	sd	Min	Max	0 to 42 days	42 day to 2 years
WBC, $10^3/\mu L$	2222	11.47	3.67	5.64	28.21	2284	19.11	5.09	8.28	36.53	1802	21.92	6.15	9.23	43.01	9.62 – 25.20	11.35 – 28.90
NEU, $10^3/\mu L$	2375	4.76	2.38	1.33	14.71	2322	10.34	4.01	1.64	23.61	1808	9.95	4.65	2.48	28.37	2.35 – 11.90	2.00 – 10.40
LYM, $10^3/\mu L$	2425	5.61	1.85	2.39	12.65	2326	6.47	2.21	2.06	13.57	1840	9.82	3.11	3.67	21.09	4.02 – 12.50	5.30 – 17.90
MONO, $10^3/\mu L$	2440	0.32	0.21	0.04	1.23	2364	0.82	0.59	0.05	3.70	1890	1.01	0.74	0	4.06	0.05 – 2.30	0 – 3.70
EOS, $10^3/\mu L$	2474	0.47	0.40	0	2.61	2213	0.71	0.75	0.12	4.35	1807	0.60	0.48	0.12	3.01	0 – 0.50	0 – 1.30
BASO, $10^3/\mu L$	2096	0.13	0.23	0.02	1.69	2264	0.84	1.36	0.06	8.51	1798	0.33	0.32	0.05	2.09	NA[3]	NA
RBC, $10^6/\mu L$	2373	6.15	0.60	4.27	7.52	2242	5.79	0.67	3.82	7.55	1767	6.28	0.57	4.51	7.67	4.87 – 7.88	5.88 – 8.19
HGB, g/L	2434	116.45	13.46	73	148	2239	100.59	10.35	68	126	1730	104.95	9.71	69	125	80.8 – 119	112 – 147
HCT, %	2310	37.12	4.10	24	44	2228	32.81	3.63	22.10	41.80	1723	35.25	3.14	28	43	28.22 – 39.80	32.30 – 42.60
MCV, fL	2444	61.25	5.45	44.5	73.40	2339	57.02	3.59	49.60	69.50	1879	55.78	3.42	46.80	65.40	43.40 – 64.50	47.50 – 59.20
MCH, pg	2318	18.73	2.03	12.50	23.60	2153	17.52	1.26	14.70	21.80	1719	16.72	1.19	13.40	20.10	12.40 – 19.30	16.30 – 20.60
MCHC, g/L	2245	305.88	12.06	274	340	2150	307.40	15.77	268	366	1708	300.22	13.31	264	345	273 – 314	333 – 358
RDW, %	2473	21.97	4.02	15.80	39.90	2321	18.45	1.61	15.90	25.10	1873	18.61	1.40	15.60	23.10	NA	NA
PLT, $10^3/\mu L$	2457	285.13	177.18	0	949	2351	365.46	182.69	35	1062	1872	337.08	150.87	47	784	374.3 – 1080.8	118.9 – 522.9
MPV, fL	2435	14.63	3.35	8.30	26.20	2180	15.33	3.72	10.10	30.80	1849	13.57	2.01	9.30	20.50	NA	NA

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration; RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; PLT: platelet concentration; MPV: mean platelet volume.

[2]Suggested reference intervals for CBC traits of 0 to 42 days-old pigs and 42 days-old to 2 years-old pigs (Iowa State University's Clinical Pathology Laboratory, 2011);

[3]Not applicable.

Table 5. Random effects included in the models for the analyses of complete blood count (CBC) traits.

Traits[1]	Blood 1		Blood 3			Blood 4				Δ13[2]			Δ34[3]				Δ14[4]			
	Litter	Pen1[5]	Litter	Pen1	Pen2[6]	Litter	Pen1	Pen2	Pen3[7]	Litter	Pen1	Pen2	Litter	Pen1	Pen2	Pen3	Litter	Pen1	Pen2	Pen3
WBC	√[8]	NS[9]	√	NS	√	NS	NS	NS	√	√	NS	√	NS	NS	NS	√	NS	NS	NS	√
NEU	√	NS	√	NS	√	NS	NS	NS	√	√	NS	√	NS	NS	NS	√	NS	NS	NS	√
LYM	√	NS	√	NS	√	NS	NS	NS	√	√	NS	NS	NS	NS	NS	√	NS	NS	NS	√
MONO	√	NS	NS	NS	√	√	√	NS	NS	NS	NS	NS	√	NS	NS	√	√	NS	NS	NS
EOS	√	√	NS	√	NS	NS	NS	NS	NS	NS	NS	NS	NS	√	NS	NS	√	NS	NS	√
BASO	NS	√	NS	√	√	√	√	NS	√	NS	√	√	NS	√	√	NS	NS	NS	NS	√
RBC	√	√	√	√	√	NS	NS	NS	√	√	√	NS	NS	NS	NS	√	√	√	NS	NS
HGB	√	√	√	√	√	NS	NS	NS	√	√	√	√	NS	NS	NS	√	√	√	NS	√
HCT	√	√	√	√	√	NS	NS	NS	√	√	√	NS	NS	NS	NS	√	√	√	NS	√
MCV	√	√	√	NS	NS	√	NS	NS	√	√	√	NS	√	NS	NS	√	√	NS	NS	NS
MCH	√	√	√	NS	√	√	NS	NS	NS	√	√	NS	√	NS	NS	√	√	NS	NS	√
MCHC	√	√	√	√	NS	NS	NS	NS	√	√	√	NS	NS	NS	NS	√	√	√	NS	√
RDW	√	√	√	√	√	√	√	NS	√	√	√	NS	√	NS	NS	√	√	NS	NS	NS
PLT	√	√	NS	NS	√	√	√	NS	NS	√	√	NS	NS	NS	NS	√	√	√	NS	NS
MPV	√	√	NS	NS	√	NS	NS	NS	√	NS	√	NS	NS	√	NS	NS	NS	√	NS	NS

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration; RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; PLT: platelet concentration; MPV: mean platelet volume.

[2]The change of CBC traits from Blood 1 to Blood 3; [3]The change of CBC traits from Blood 3 to Blood 4; [4]The change of CBC traits from Blood 1 to Blood 4.

[5]The pen arrangement in the healthy quarantine unit; [6]The pen arrangement in the challenge late nursery; [7]The pen arrangement in the challenge grow-to-finish stage.

[8]Significant random effect that was included in the model.

[9]Not significant.

Table 6. Least-squares means ± standard errors for white blood cell traits[1] in Blood 1, 3, and 4 of animals from the resilient (RES), average (MID), susceptible (SUS), and dead (DEAD) groups.

Blood 1, 10^3/µL	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group[2]**
$\log_{10}$ (WBC)	1.03 ± 0.01[a3]	1.04 ± 0.00[a]	1.04 ± 0.01[a]	1.03 ± 0.01[a]	0.55
$\log_{10}$ (NEU)	0.62 ± 0.01[a]	0.64 ± 0.01[a]	0.63 ± 0.01[a]	0.62 ± 0.01[a]	0.55
$\log_{10}$ (LYM)	0.71 ± 0.01[a]	0.73 ± 0.00[a]	0.73 ± 0.01[a]	0.72 ± 0.01[a]	0.29
$\log_{10}$ (MONO)	-0.61 ± 0.02[a]	-0.59 ± 0.01[a]	-0.58 ± 0.02[a]	-0.58 ± 0.01[a]	0.42
$\log_{10}$ (EOS)	-0.47 ± 0.02[a]	-0.49 ± 0.01[a]	-0.50 ± 0.02[a]	-0.50 ± 0.01[a]	0.84
$\log_{10}$ (BASO)	-1.15 ± 0.02[a]	-1.15 ± 0.01[a]	-1.14 ± 0.02[a]	-1.16 ± 0.01[a]	0.88
Blood 3, 10^3/µL	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group**
$\log_{10}$ (WBC)	1.27 ± 0.01[a]	1.27 ± 0.00[a]	1.25 ± 0.01[a]	1.26 ± 0.01[a]	0.18
$\log_{10}$ (NEU)	0.97 ± 0.01[a]	0.98 ± 0.00[a]	0.97 ± 0.01[a]	0.98 ± 0.01[a]	0.56
$\log_{10}$ (LYM)	**0.82 ± 0.01[c4]**	**0.79 ± 0.00[b]**	**0.77 ± 0.01[ab]**	**0.75 ± 0.01[a]**	**< 0.0001**
$\log_{10}$ (MONO)	-0.18 ± 0.02[a]	-0.21 ± 0.01[a]	-0.23 ± 0.02[a]	-0.22 ± 0.01[a]	0.27
$\log_{10}$ (EOS)	-0.32 ± 0.02[a]	-0.30 ± 0.01[a]	-0.33 ± 0.02[a]	-0.33 ± 0.01[a]	0.15
$\log_{10}$ (BASO)	-0.51 ± 0.02[a]	-0.51 ± 0.01[a]	-0.55 ± 0.02[a]	-0.49 ± 0.01[a]	0.15
Blood 4, 10^3/µL	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group**
$\log_{10}$ (WBC)	1.31 ± 0.01[a]	1.32 ± 0.00[a]	1.34 ± 0.01[a]	1.34 ± 0.01[a]	0.21
$\log_{10}$ (NEU)	**0.93 ± 0.01[a]**	**0.95 ± 0.01[a]**	**1.02 ± 0.01[b]**	**1.03 ± 0.02[b]**	**< 0.0001**
$\log_{10}$ (LYM)	**0.98 ± 0.01[b]**	**0.98 ± 0.00[b]**	**0.95 ± 0.01[ab]**	**0.92 ± 0.01[a]**	**0.0009**
$\log_{10}$ (MONO)	-0.16 ± 0.02[a]	-0.15 ± 0.01[a]	-0.14 ± 0.02[a]	-0.18 ± 0.03[a]	0.67
$\log_{10}$ (EOS)	-0.33 ± 0.01[a]	-0.33 ± 0.01[a]	-0.29 ± 0.02[a]	-0.30 ± 0.02[a]	0.46
$\log_{10}$ (BASO)	-0.61 ± 0.02[a]	-0.59 ± 0.01[a]	-0.57 ± 0.02[a]	-0.57 ± 0.02[a]	0.40

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration.

[2]FDR-Group: adjusted *p-values* for the significant level of group effect using the Benjamini and Hochberg correction (FDR) in R to control false positives from multiple comparisons (R Core Team, Package 'stats').

[3]Values in a column suffixed with different letters are significantly different from each other at *FDR < 0.05*.

[4]Significant differences among RES, MID, SUS and DEAD groups are highlighted in bold (*FDR < 0.05*).

Table 7. Least-squares means ± standard errors for changes of white blood cell traits[1] between Blood 1, 3, and 4 of animals in the resilient (RES), average (MID), susceptible (SUS), and dead (DEAD) groups.

Δ13[2], 10[3]/μL	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group[5]**
WBC	8.39 ± 0.39[a6]	7.68 ± 0.17[a]	7.10 ± 0.40[a]	7.74 ± 0.29[a]	0.24
NEU	5.52 ± 0.27[a]	5.52 ± 0.11[a]	5.35 ± 0.29[a]	5.93 ± 0.20[a]	0.38
LYM	**1.69 ± 0.17[b7]**	**0.85 ± 0.07[a]**	**0.67 ± 0.17[a]**	**0.51 ± 0.12[a]**	**< 0.0001**
MONO	0.56 ± 0.03[a]	0.49 ± 0.01[a]	0.42 ± 0.03[a]	0.47 ± 0.02[a]	0.08
EOS	0.23 ± 0.04[a]	0.26 ± 0.01[a]	0.18 ± 0.04[a]	0.24 ± 0.03[a]	0.38
BASO	0.85 ± 0.08[a]	0.66 ± 0.04[a]	0.63 ± 0.07[a]	0.79 ± 0.06[a]	0.08
Δ34[3], 10[3]/μL	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group**
WBC	2.13 ± 0.51[a]	2.89 ± 0.23[a]	4.06 ± 0.54[a]	3.91 ± 0.68[a]	0.08
NEU	**-0.60 ± 0.41[a]**	**-0.33 ± 0.17[a]**	**1.36 ± 0.40[b]**	**1.77 ± 0.51[b]**	**< 0.0001**
LYM	3.08 ± 0.25[a]	3.41 ± 0.11[a]	3.24 ± 0.26[a]	2.78 ± 0.32[a]	0.32
MONO	0.16 ± 0.05[a]	0.20 ± 0.02[a]	0.24 ± 0.05[a]	0.13 ± 0.06[a]	0.65
EOS	-0.08 ± 0.04[a]	-0.13 ± 0.02[a]	-0.04 ± 0.04[a]	-0.03 ± 0.06[a]	0.16
BASO	-0.54 ± 0.06[a]	-0.45 ± 0.03[a]	-0.38 ± 0.06[a]	-0.40 ± 0.08[a]	0.42
Δ14[4], 10[3]/μL	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group**
WBC	10.39 ± 0.48[a]	10.75 ± 0.19[a]	11.60 ± 0.51[a]	11.73 ± 0.63[a]	0.27
NEU	**4.75 ± 0.35[a]**	**5.11 ± 0.14[a]**	**6.83 ± 0.36[b]**	**7.32 ± 0.45[b]**	**< 0.0001**
LYM	4.63 ± 0.23[a]	4.37 ± 0.09[a]	4.06 ± 0.24[a]	3.56 ± 0.31[a]	0.08
MONO	0.72 ± 0.04[a]	0.71 ± 0.02[a]	0.66 ± 0.04[a]	0.61 ± 0.05[a]	0.37
EOS	0.12 ± 0.03[a]	0.13 ± 0.01[a]	0.22 ± 0.03[a]	0.19 ± 0.04[a]	0.12
BASO	0.15 ± 0.03[a]	0.20 ± 0.01[a]	0.24 ± 0.02[a]	0.23 ± 0.03[a]	0.12

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration.

[2]The change of complete blood count (CBC) traits from Blood 1 to Blood 3; [3]The change of CBC traits from Blood 3 to Blood 4; [4]The change of CBC traits from Blood 1 to Blood 4.

[5]FDR-Group: adjusted *p-values* for the significant level of group effect using the Benjamini and Hochberg correction (FDR) in R to control false positives from multiple comparisons (R Core Team, Package 'stats').

[6]Values in a column suffixed with different letters are significantly different from each other at *FDR < 0.05*.

[7]Significant differences among RES, MID, SUS and DEAD groups are highlighted in bold (*FDR < 0.05*).

Table 8. Least-squares means ± standard errors for red blood cell and platelet traits[1] in Blood 1, 3, and 4 of animals in the resilient (RES), average (MID), susceptible (SUS), and dead (DEAD) groups.

Blood 1	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group[2]**
RBC, 10^6/µL	6.18 ± 0.03[a3]	6.18 ± 0.01[a]	6.13 ± 0.03[a]	6.15 ± 0.02[a]	0.58
HGB, g/L	117.15 ± 0.71[a]	116.84 ± 0.31[a]	116.89 ± 0.72[a]	116.50 ± 0.50[a]	0.92
HCT, %	37.45 ± 0.23[a]	37.50 ± 0.10[a]	37.63 ± 0.23[a]	37.22 ± 0.16[a]	0.51
MCV, fL	61.31 ± 0.28[a]	61.36 ± 0.13[a]	62.07 ± 0.28[a]	61.39 ± 0.20[a]	0.20
MCH, pg	18.67 ± 0.11[a]	18.67 ± 0.05[a]	18.83 ± 0.11[a]	18.69 ± 0.08[a]	0.67
MCHC, g/L	306.86 ± 0.68[a]	305.74 ± 0.29[a]	305.70 ± 0.68[a]	305.11 ± 0.47[a]	0.32
RDW, %	21.94 ± 0.22[a]	21.77 ± 0.10[a]	21.91 ± 0.22[a]	22.12 ± 0.16[a]	0.32
PLT, 10^3/µL	281.02 ± 10.26[a]	283.85 ± 4.29[a]	290.49 ± 10.42[a]	286.12 ± 7.10[a]	0.93
MPV, fL	14.57 ± 0.16[a]	14.70 ± 0.07[a]	14.98 ± 0.16[a]	14.79 ± 0.11[a]	0.40
Blood 3	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group**
RBC, 10^6/µL	5.81 ± 0.04[a]	5.77 ± 0.02[a]	5.75 ± 0.04[a]	5.79 ± 0.03[a]	0.74
HGB, g/L	101.66 ± 0.60[a]	101.03 ± 0.25[a]	100.63 ± 0.61[a]	101.32 ± 0.44[a]	0.68
HCT, %	32.92 ± 0.20[a]	32.87 ± 0.09[a]	32.85 ± 0.21[a]	32.71 ± 0.15[a]	0.87
MCV, fL	57.15 ± 0.20[a]	57.15 ± 0.08[a]	57.22 ± 0.20[a]	56.80 ± 0.14[a]	0.29
MCH, pg	17.51 ± 0.07[a]	17.54 ± 0.03[a]	17.52 ± 0.08[a]	17.49 ± 0.06[a]	0.93
MCHC, g/L	306.66 ± 0.95[a]	306.68 ± 0.60[a]	304.65 ± 0.97[a]	307.79 ± 0.79[b]	0.14
RDW, %	**18.26 ± 0.10[a4]**	**18.40 ± 0.0[a]**	**18.58 ± 0.10[ab]**	**18.72 ± 0.07[b]**	**0.0004**
PLT, 10^3/µL	390.20 ± 11.04[a]	362.14 ± 11.10[a]	305.14 ± 0.81[a]	363.44 ± 8.12[a]	0.26
MPV, fL	**14.59 ± 0.16[a]**	**14.92 ± 0.06[a]**	**15.01 ± 0.16[ab]**	**15.51 ± 0.12[b]**	**< 0.0001**
Blood 4	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group**
RBC, 10^6/µL	**6.36 ± 0.04[b]**	**6.32 ± 0.01[b]**	**6.16 ± 0.04[a]**	**6.22 ± 0.05[ab]**	**0.0009**
HGB, g/L	**106.52 ± 0.60[b]**	**105.16 ± 0.25[b]**	**100.78 ± 0.62[a]**	**100.94 ± 0.81[a]**	**< 0.0001**
HCT, %	**35.54 ± 0.20[b]**	**35.21 ± 0.08[b]**	**34.15 ± 0.22[a]**	**34.05 ± 0.28[a]**	**< 0.0001**
MCV, fL	**56.03 ± 0.20[b]**	**55.74 ± 0.08[b]**	**55.44 ± 0.20[b]**	**54.39 ± 0.26[a]**	**< 0.0001**
MCH, pg	**16.89 ± 0.07[b]**	**16.78 ± 0.03[b]**	**16.57 ± 0.07[a]**	**16.37 ± 0.09[a]**	**< 0.0001**
MCHC, g/L	301.73 ± 0.72[a]	301.17 ± 0.30[a]	299.45 ± 0.73[a]	299.97 ± 0.94[a]	0.15
RDW, %	**18.31 ± 0.09[a]**	**18.57 ± 0.04[b]**	**18.84 ± 0.09[c]**	**18.89 ± 0.12[bc]**	**0.0001**
PLT, 10^3/µL	352.11 ± 9.84[a]	337.37 ± 4.02[a]	354.67 ± 10.25[a]	339.54 ± 13.13[a]	0.38
MPV, fL	**13.31 ± 0.11[a]**	**13.41 ± 0.04[a]**	**13.63 ± 0.11[ab]**	**14.12 ± 0.13[b]**	**< 0.0001**

[1]RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; PLT: platelet concentration; MPV: mean platelet volume.

[2]FDR-Group: adjusted *p-values* for the significant level of group effect using the Benjamini and Hochberg correction (FDR) in R to control false positives from multiple comparisons (R Core Team, Package 'stats').

[3]Values in a column suffixed with different letters are significantly different from each other at $FDR < 0.05$.

[4]Significant differences among RES, MID, SUS and DEAD groups are highlighted in bold ($FDR < 0.05$).

Table 9. Least-squares means ± standard errors for changes of red blood cell and platelet traits[1] between Blood1, Blood 3, and Blood 4 of animals in the resilient (RES), average (MID), susceptible (SUS), and dead (DEAD) groups.

Δ13[2]	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group[5]**
RBC, 10^6/μL	-0.43 ± 0.05[a6]	-0.44 ± 0.02[a]	-0.43 ± 0.05[a]	-0.33 ± 0.03[a]	0.16
HGB, g/L	-15.57 ± 0.93[a]	-15.77 ± 0.38[a]	-16.25 ± 0.96[a]	-14.72 ± 0.68[a]	0.59
HCT, %	-4.58 ± 0.31[a]	-4.68 ± 0.13[a]	-4.83 ± 0.32[a]	-4.59 ± 0.23[a]	0.93
MCV, fL	-4.11 ± 0.26[a]	-4.31 ± 0.10[a]	-4.74 ± 0.26[a]	-4.44 ± 0.18[a]	0.43
MCH, pg	-1.22 ± 0.10[a]	-1.20± 0.04[a]	-1.30 ± 0.10[a]	-1.17 ± 0.07[a]	0.81
MCHC, g/L	**-0.63 ± 1.00[a7]**	**0.91 ± 0.42[ab]**	**-0.92 ± 1.04[a]**	**3.13 ± 0.74[b]**	**0.01**
RDW, %	-3.58 ± 0.20[a]	-3.37 ± 0.08[a]	-3.32 ± 0.20[a]	-3.63 ± 0.14[a]	0.44
PLT, 10^3/μL	105.93 ± 14.20[a]	76.20 ± 5.73[a]	67.59 ± 10.26[a]	67.59 ± 10.26[a]	0.29
MPV, fL	0.29 ± 0.22[a]	0.40 ± 0.09[a]	0.10 ± 0.22[a]	0.79 ± 0.16[a]	0.14
Δ34[3]	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group**
RBC, 10^6/μL	0.56 ± 0.05[a]	0.54 ± 0.02[a]	0.40 ± 0.05[a]	0.36 ± 0.06[a]	0.01
HGB, g/L	**6.23 ± 0.79[b]**	**4.32 ± 0.34[b]**	**0.59 ± 0.81[a]**	**-0.64 ± 1.03[a]**	**< 0.0001**
HCT, %	**2.61 ± 0.27[b]**	**2.22 ± 0.12[b]**	**1.15 ± 0.29[a]**	**1.04 ± 0.38[a]**	**0.0002**
MCV, fL	**-1.01 ± 0.20[b]**	**-1.40 ± 0.08[b]**	**-1.84 ± 0.20[ab]**	**-2.55 ± 0.25[a]**	**< 0.0001**
MCH, pg	**-0.59 ± 0.07[c]**	**-0.73 ± 0.03[bc]**	**-0.94 ± 0.08[ab]**	**-1.01 ± 0.10[a]**	**0.0007**
MCHC, g/L	-4.22 ± 1.06[a]	-4.57 ± 0.45[a]	-4.61 ± 1.10[a]	-3.74 ± 1.43[a]	0.94
RDW, %	0.06 ± 0.10[a]	0.22 ± 0.04[a]	0.30 ± 0.10[a]	0.15 ± 0.13[a]	0.46
PLT, 10^3/μL	-43.81 ± 14.00[a]	-30.55 ± 5.90[a]	-2.77 ± 14.53[a]	-52.74 ± 18.46[a]	0.21
MPV, fL	**-1.34 ± 0.17[ab]**	**-1.55 ± 0.07[a]**	**-1.24 ± 0.17[ab]**	**-0.76 ± 0.22[b]**	**0.02**
Δ14[4]	**RES**	**MID**	**SUS**	**DEAD**	***FDR*-group**
RBC, 10^6/μL	0.18 ± 0.05[a]	0.16 ± 0.02[a]	0.05 ± 0.05[a]	0.09 ± 0.06[a]	0.22
HGB, g/L	-10.75 ± 0.98[a]	-11.76 ± 0.39[a]	-14.96 ± 1.04[a]	-13.42 ± 1.35[a]	0.06
HCT, %	-1.93 ± 0.32[a]	-2.22 ± 0.12[a]	-3.15 ± 0.35[a]	-2.96 ± 0.43[a]	0.06
MCV, fL	-5.38 ± 0.29[a]	-5.84 ± 0.11[a]	-6.70 ± 0.30[a]	-6.74 ± 0.39[a]	0.01
MCH, pg	**-1.81 ± 0.11[b]**	**-1.99 ± 0.05[ab]**	**-2.33 ± 0.12[a]**	**-2.20 ± 0.15[ab]**	**0.02**
MCHC, g/L	-5.53 ± 1.02[a]	-5.40 ± 0.44[a]	-7.01 ± 1.04[a]	-5.05 ± 1.27[a]	0.58
RDW, %	-3.36 ± 0.22[a]	-2.93 ± 0.09[a]	-2.84 ± 0.23[a]	-3.68 ± 0.29[a]	0.08
PLT, 10^3/μL	69.91 ± 13.48[a]	56.42 ± 5.35[a]	60.74 ± 14.30[a]	22.48 ± 18.13[a]	0.32
MPV, fL	-1.12 ± 0.18[a]	-1.24 ± 0.07[a]	-1.15 ± 0.19[a]	-0.40 ± 0.25[a]	0.05

[1]RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; PLT: platelet concentration; MPV: mean platelet volume.

[2]The change of complete blood count (CBC) traits from Blood 1 to Blood 3; [3]The change of CBC traits from Blood 3 to Blood 4; [4]The change of CBC traits from Blood 1 to Blood 4.

[5]FDR-Group: adjusted *p-values* for the significant level of group effect using the Benjamini and Hochberg correction (FDR) in R to control false positives from multiple comparisons (R Core Team, Package 'stats').

[6]Values in a column suffixed with different letters are significantly different from each other at $FDR < 0.05$.

[7]Significant differences among RES, MID, SUS and DEAD groups are highlighted in bold ($FDR < 0.05$).

Table 10. Estimates of heritability ± standard error for complete blood count (CBC) traits.

Traits[1]	Blood 1	Blood 3	Blood 4	Δ13[2]	Δ34[3]	Δ14[4]
WBC	**0.16 ± 0.04**[5]	**0.22 ± 0.04**	**0.19 ± 0.04**	**0.09 ± 0.04**	**0.14 ± 0.04**	**0.15 ± 0.04**
NEU	**0.18 ± 0.04**	**0.18 ± 0.04**	**0.13 ± 0.04**	**0.11 ± 0.04**	**0.11 ± 0.04**	**0.07 ± 0.04**
LYM	**0.21 ± 0.04**	**0.21 ± 0.04**	**0.30 ± 0.04**	**0.11 ± 0.04**	**0.20 ± 0.04**	**0.24 ± 0.04**
MONO	0.05 ± 0.03	**0.12 ± 0.03**	0.02 ± 0.03	**0.08 ± 0.03**	0.00 ± 0.00	0.05 ± 0.04
EOS	**0.22 ± 0.04**	**0.19 ± 0.04**	**0.27 ± 0.04**	**0.07 ± 0.03**	0.00 ± 0.03	**0.08 ± 0.04**
BASO	**0.08 ± 0.04**	**0.10 ± 0.03**	**0.13 ± 0.04**	**0.06 ± 0.04**	**0.06 ± 0.04**	0.06 ± 0.05
RBC	**0.27 ± 0.04**	**0.30 ± 0.04**	**0.34 ± 0.05**	**0.08 ± 0.04**	0.04 ± 0.04	**0.08 ± 0.05**
HGB	**0.08 ± 0.03**	**0.16 ± 0.04**	**0.28 ± 0.05**	**0.16 ± 0.04**	**0.11 ± 0.04**	**0.09 ± 0.05**
HCT	**0.09 ± 0.03**	**0.23 ± 0.04**	**0.23 ± 0.04**	0.04 ± 0.03	0.04 ± 0.04	**0.10 ± 0.05**
MCV	**0.19 ± 0.04**	**0.38 ± 0.04**	**0.46 ± 0.05**	**0.08 ± 0.03**	**0.22 ± 0.05**	0.06 ± 0.04
MCH	**0.18 ± 0.04**	**0.39 ± 0.04**	**0.53 ± 0.05**	**0.15 ± 0.04**	**0.13 ± 0.05**	0.06 ± 0.05
MCHC	**0.13 ± 0.04**	**0.25 ± 0.04**	**0.26 ± 0.05**	**0.17 ± 0.04**	**0.20 ± 0.05**	**0.07 ± 0.05**
RDW	**0.13 ± 0.03**	**0.14 ± 0.04**	**0.08 ± 0.04**	**0.14 ± 0.04**	**0.09 ± 0.04**	**0.18 ± 0.05**
PLT	**0.15 ± 0.03**	**0.07 ± 0.03**	**0.08 ± 0.04**	0.01 ± 0.03	0.00 ± 0.03	0.04 ± 0.03
MPV	**0.11 ± 0.03**	**0.19 ± 0.04**	**0.23 ± 0.04**	0.02 ± 0.03	**0.10 ± 0.04**	**0.08 ± 0.04**

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration; RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; PLT: platelet concentration; MPV: mean platelet volume.

[2]The change of CBC traits from Blood 1 to Blood 3; [3]The change of CBC traits from Blood 3 to Blood 4; [4]The change of CBC traits from Blood 1 to Blood 4.

[5]Significant estimates of genetic variances are highlighted in bold based on the likelihood ratio test by comparing full models to restricted models that constrained genetic variances to zero in ASReml 4.1 (*p-value < 0.05*).

Table 11. Estimates of genetic correlations ± standard errors for complete blood count (CBC) traits that showed significant differences among groups with the resilience traits of grow-to-finish growth rate (GFGR) and treatment rate (TR).

Traits[1]	GFGR	TR
Blood3		
LYM	0.10 ± 0.18	**-0.38 ± 0.18**[2]
RDW	-0.07 ± 0.21	0.39 ± 0.22
MPV	0.09 ± 0.18	0.26 ± 0.18
Blood4		
NEU	-0.31 ± 0.20	**0.50 ±0.23**
LYM	0.16 ± 0.15	-0.28 ± 0.16
RBC	0.15 ± 0.15	-0.08 ± 0.17
HGB	0.04 ± 0.16	-0.25 ± 0.18
HCT	0.10 ± 0.17	-0.33 ± 0.19
MCV	-0.08 ± 0.15	-0.16 ± 0.16
MCH	-0.03 ± 0.14	-0.21 ± 0.15
RDW	-0.12 ± 0.28	**0.89 ± 0.26**
MPV	0.09 ± 0.17	0.11 ± 0.19
Δ13[3]		
LYM	0.15 ± 0.23	**-0.46 ± 0.24**
MCHC	-0.25 ± 0.20	0.26 ± 0.21
Δ34[4]		
NEU	**-0.45 ± 0.21**	0.44 ± 0.26
RBC	-0.33 ± 0.45	-0.35 ± 0.43
HGB	0.01 ± 0.25	-0.32 ± 0.28
HCT	-0.29 ± 0.44	**-0.82 ± 0.47**
MCV	0.03 ± 0.19	0.02 ± 0.20
MCH	0.25 ± 0.25	0.14 ± 0.28
MPV	-0.15 ± 0.26	-0.27 ± 0.28
Δ14[5]		
NEU	-0.32 ± 0.26	**0.76 ± 0.29**
MCV	0.02 ± 0.33	-0.02 ± 0.35
MCH	0.00 ± 0.35	0.26 ± 0.36

[1]NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width.

[2]Significant estimates of genetic correlations are highlighted in bold based on the likelihood ratio test by comparing full models to restricted models that constrained the genetic covariance to zero in ASReml 4.1 (*p-value < 0.05*).

[3]The change of CBC traits from Blood 1 to Blood 3; [4]The change of CBC traits from Blood 3 to Blood 4; [5]The change of CBC traits from Blood 1 to Blood 4.

Table 12. Estimates of genetic correlations ± standard errors for complete blood count traits[1] within Blood 1, Blood 3, and Blood 4.

Blood 1	**WBC**	**NEU**	**LYM**	**MONO**	**EOS**	**BASO**	**RBC**	**PLT**
WBC	-				Symmetric			
NEU	**0.59 ± 0.09**[2]	-						
LYM	**0.70 ± 0.09**	0.24 ± 0.14	-					
MONO	**0.30 ± 0.12**	0.28 ± 0.24	**0.47 ± 0.18**	-				
EOS	**0.42 ± 0.14**	**0.46 ± 0.12**	0.24 ± 0.13	0.36 ± 0.21	-			
BASO	0.23 ± 0.32	**0.65 ± 0.16**	**0.70 ± 0.17**	**0.87 ± 0.27**	0.31 ± 0.22	-		
RBC	0.00 ± 0.15	-0.07 ± 0.14	-0.22 ± 0.13	-0.44 ± 0.25	0.18 ± 0.12	0.19 ± 0.21	-	
PLT	**-0.42 ± 0.18**	-0.15 ± 0.17	-0.16 ± 0.16	-0.52 ± 0.31	**-0.45 ± 0.13**	0.05 ± 0.26	0.11 ± 0.14	-
Blood 3	**WBC**	**NEU**	**LYM**	**MONO**	**EOS**	**BASO**	**RBC**	**PLT**
WBC	-				Symmetric			
NEU	**0.83 ± 0.05**	-						
LYM	**0.76 ± 0.08**	**0.36 ± 0.15**	-					
MONO	**0.65 ± 0.12**	**0.34 ± 0.16**	**0.63 ± 0.12**	-				
EOS	**0.41 ± 0.12**	0.25 ± 0.15	0.22 ± 0.14	0.21 ± 0.17	-			
BASO	**0.57 ± 0.13**	**0.53 ± 0.14**	0.23 ± 0.18	**0.43 ± 0.19**	-0.05 ± 0.19	-		
RBC	0.16 ± 0.12	0.17 ± 0.14	0.18 ± 0.13	-0.08 ± 0.14	-0.14 ± 0.13	-0.13 ± 0.16	-	
PLT	-0.08 ± 0.21	-0.11 ± 0.23	-0.24 ± 0.22	-0.12 ± 0.25	0.10 ± 0.21	0.09 ± 0.26	-0.15 ± 0.17	-
Blood 4	**WBC**	**NEU**	**LYM**	**MONO**	**EOS**	**BASO**	**RBC**	**PLT**
WBC	-				Symmetric			
NEU	**0.79 ± 0.08**	-						
LYM	**0.82 ± 0.07**	0.25 ± 0.16	-					
MONO	**1.23 ± 0.35**	**1.03 ± 0.44**	0.95 ± 0.48	-				
EOS	**0.45 ± 0.12**	**0.52 ± 0.14**	0.01 ± 0.12	0.56 ± 0.46	-			
BASO	**0.93 ± 0.09**	**0.77 ± 0.14**	**0.76 ± 0.15**	1.18 ± 0.65	**0.50 ± 0.14**	-		
RBC	**0.34 ± 0.14**	0.20 ± 0.16	**0.35 ± 0.11**	0.03 ± 0.24	-0.15 ± 0.12	0.13 ± 0.17	-	
PLT	-0.08 ± 0.26	0.00 ± 0.29	0.01 ± 0.20	-0.45 ± 1.04	**-0.15 ± 0.03**	0.10 ± 0.30	0.03 ± 0.19	-

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration; RBC: red blood cell concentration; PLT: platelet concentration.

[2]Significant estimates of genetic correlations are highlighted in bold based on the likelihood ratio test by comparing full models to restricted models that constrained the genetic covariance to zero in ASReml 4.1 ($p\text{-value} < 0.05$).

Table 13. Estimates of genetic correlations ± standard errors for each complete blood count trait between Blood 1, Blood 3, and Blood4.

Traits[1]	Blood 1 & Blood 3	Blood 3 & Blood 4	Blood 1 & Blood 4
WBC	**0.85 ± 0.13[2]**	**0.65 ± 0.12**	**0.62 ± 0.16**
NEU	**0.73 ± 0.12**	**0.80 ± 0.17**	**0.64 ± 0.16**
LYM	**0.68 ± 0.12**	**0.46 ± 0.12**	**0.57 ± 0.11**
MONO	**0.93 ± 0.25**	1.26 ± 0.93	0.88 ± 0.89
EOS	**0.40 ± 0.12**	**0.86 ± 0.12**	**0.60 ± 0.12**
BASO	**0.70 ± 0.28**	**0.49 ± 0.21**	**0.98 ± 0.29**
RBC	**0.86 ± 0.08**	**0.87 ± 0.07**	**0.82 ± 0.09**
HGB	**0.73 ± 0.23**	**0.79 ± 0.11**	**0.80 ± 0.19**
HCT	**0.60 ± 0.20**	**0.84 ± 0.11**	**0.69 ± 0.21**
MCV	**0.84 ± 0.06**	**0.81 ± 0.05**	**0.77 ± 0.08**
MCH	**0.77 ± 0.07**	**0.91 ± 0.04**	**0.79 ± 0.08**
MCHC	**0.49 ± 0.14**	**0.74 ± 0.11**	**0.74 ± 0.13**
RDW	**0.57 ± 0.13**	**0.62 ± 0.18**	-0.28 ± 0.31
PLT	**0.78 ± 0.19**	**0.87 ± 0.26**	**0.99 ± 0.18**
MPV	**1.02 ± 0.16**	**0.76 ± 0.11**	**0.66 ± 0.14**

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration; RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; PLT: platelet concentration; MPV: mean platelet volume.

[2]Significant estimates of genetic correlations are highlighted in bold based on the likelihood ratio test by comparing full models to restricted models that constrained the genetic covariance to zero in ASReml 4.1 (*p-value* < *0.05*).

Table 14. Estimates of genetic correlations ± standard errors for the changes of each complete blood count (CBC) trait between Blood 1, Blood 3, and Blood 4.

Traits[1]	Δ13[2] & Δ34[3]	Δ13 & Δ14[4]	Δ34 & Δ14
WBC	**-0.45 ± 0.21**[5]	0.36 ± 0.23	**0.67 ± 0.13**
NEU	-0.39 ± 0.23	0.52 ± 0.28	**0.62 ± 0.19**
LYM	**-0.52 ± 0.15**	0.25 ± 0.17	**0.76 ± 0.07**
MONO	-0.89 ± 0.71	0.48 ± 0.38	0.54 ± 0.72
EOS	-0.42 ± 0.38	**0.76 ± 0.23**	0.95 ± 0.60
BASO	**-0.92 ± 0.11**	-0.70 ± 0.76	0.42 ± 0.60
RBC	**-0.76 ± 0.25**	0.58 ± 0.42	0.25 ± 0.46
HGB	-0.30 ± 0.19	0.32 ± 0.26	0.44 ± 0.25
HCT	-0.93 ± 0.51	0.53 ± 0.42	-0.15 ± 0.60
MCV	-0.37 ± 0.22	0.44 ± 0.24	**0.54 ± 0.22**
MCH	**-0.58 ± 0.25**	0.02 ± 0.37	0.05 ± 0.35
MCHC	**-0.76 ± 0.12**	**0.44 ± 0.22**	0.36 ± 0.24
RDW	**0.64 ± 0.32**	**0.98 ± 0.03**	**0.87 ± 0.16**
PLT	-0.44 ± 1.07	0.82 ± 1.03	0.62 ± 1.85
MPV	**-0.77 ± 0.33**	0.25 ± 0.50	0.34 ± 0.29

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration; RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; PLT: platelet concentration; MPV: mean platelet volume.

[2]The change of CBC traits from Blood 1 to Blood 3; [3]The change of CBC traits from Blood 3 to Blood 4; [4]The change of CBC traits from Blood 1 to Blood 4.

[5]Significant estimates of genetic correlations are highlighted in bold based on the likelihood ratio test by comparing full models to restricted models that constrained the genetic covariance to zero in ASReml 4.1 ($p\text{-}value < 0.05$).

Table 15. Heritability estimates of complete blood count traits in related studies reported in the literature.

Traits[1]	Henryon et al. (2006)	Clapperton et al. (2008)				Clapperton et al. (2009)		Flori et al. (2011)	Mpetile et al. (2015)
		SPF[2]	Non-SPF[3]	Start-Test[4]	End-Test[5]	SPF	Non-SPF		
WBC	0.25 ± 0.05	0.06 ± 0.11	0.37 ± 0.16	0.24 ± 0.15	0.18 ± 0.11	0.29 ± 0.13	0.28 ± 0.11	0.73 ± 0.20	0.23 ± 0.19
NEU	0.22 ± 0.04	-	-	-[6]	-	-	-	0.61 ± 0.20	0.31 ± 0.21
LYM	0.24 ± 0.05	-	-	-	-	-	-	0.72 ± 0.21	0.15 ± 0.19
MONO	0.22 ± 0.04	0.58 ± 0.18	0.58 ± 0.18	0.52 ± 0.17	0.59 ± 0.14	0.26 ± 0.11	0.16 ± 0.13	0.38 ± 0.20	0.36 ± 0.20
EOS	0.30 ± 0.05	-	-	-	-	-	-	0.80 ± 0.21	0.58 ± 0.12
BASO	-	-	-	-	-	-	-	-	0.12 ± 0.19
RBC	-	-	-	-	-	-	-	0.43 ± 0.20	0.62 ± 0.25
HGB	-	-	-	-	-	-	-	-	0.56 ± 0.13
HCT	-	-	-	-	-	-	-	-	0.06 ± 0.14
MCV	-	-	-	-	-	-	-	-	0.47 ± 0.24
RDW	-	-	-	-	-	-	-	0.70 ± 0.20	0.34 ± 0.25
MCH	-	-	-	-	-	-	-	-	0.37 ± 0.24
MCHC	-	-	-	-	-	-	-	-	0.04 ± 0.16
PLT	-	-	-	-	-	-	-	0.56 ± 0.19	0.11 ± 0.23
MPV	-	-	-	-	-	-	-	-	0.38 ± 0.25

[1]WBC: total white blood cell concentration; NEU: neutrophil concentration, LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration; RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; RDW: red blood cell distribution width; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet concentration; MPV: mean platelet volume.

[2]Specific pathogen-free (SPF), free of all major swine pathogens;

[3]Non-specific pathogen-free (Non-SPF), lower health status condition with the challenge of enzootic pneumonia, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Leptospira Bratislava*, *Salmonella typhimurium*, and porcine multi-wasting syndrome;

[4]Blood samples collected from animals in both SPF and non-SPF farms at the average of 89 days old;

[5]Blood samples collected from animals in both SPF and non-SPF farms at the average of 148 days old;

[6]Heritability estimate of the trait was not reported in the study.

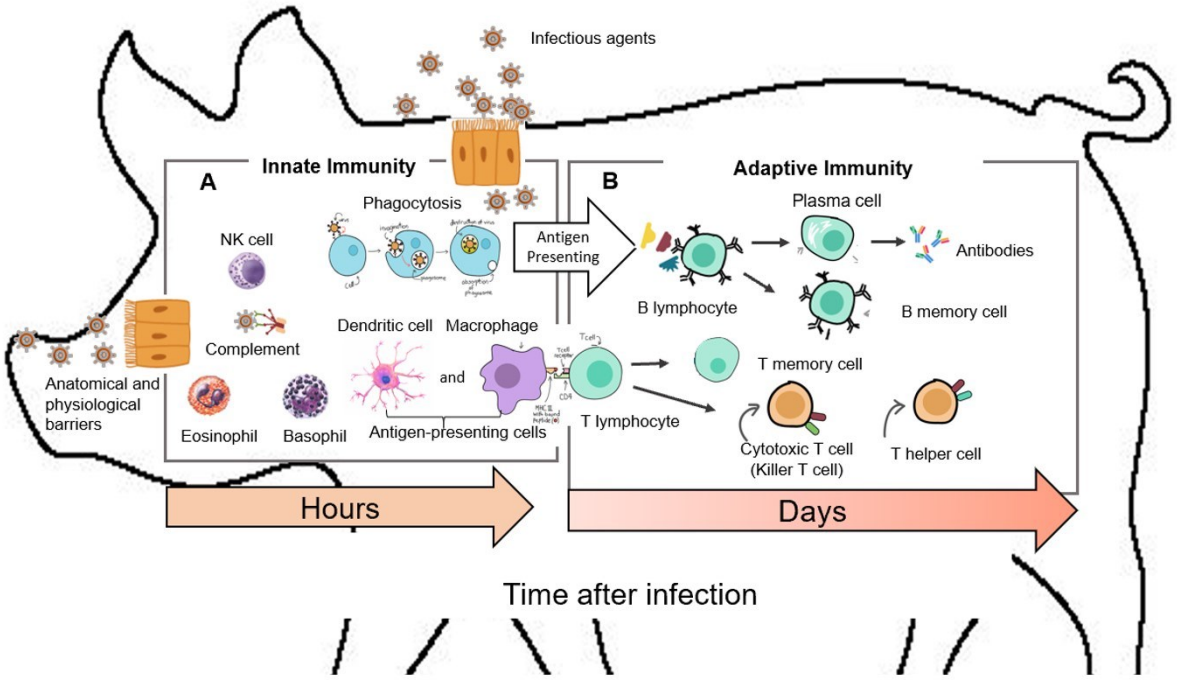

Figure 23. Roles of white blood cells in innate (A) and adaptive (B) immunity.

Roles of white blood cells in innate immunity: Phagocytosis is the process by which phagocytic cells recognize and ingest microbes for intracellular killing. Phagocytes include neutrophils, monocytes, dendritic cells, and eosinophils; Neutrophils, eosinophils, and basophils are granulocytes, the granules present in their cytoplasm contain biochemical mediators that serve inflammatory and immune functions; Eosinophils and basophils combat parasites through production of toxic proteins and histamine respectively; Dendritic cells produce cytokines that recruit white blood cells and initiate adaptive immune responses, and also present antigens to the adaptive immune system; Natural killer (NK) cells are a class of lymphocytes that recognize and kill infected cells to stop the spread of an infection; The complement system consists of a set of plasma proteins that act together to defend against extracellular pathogens.

Roles of white blood cells in adaptive immunity: B lymphocytes mediate humoral immunity by secreting antibodies into the circulation and mucosal fluid to neutralize and eliminate extracellular infectious agents; T lymphocytes characterize cell-mediated immunity and kill host cells that are harboring infectious agents in the cytoplasm.

Derived from Janeway et al. (2001), Abbas et al. (2015), and Elsevier Health Sciences and Khan Academy (2019).

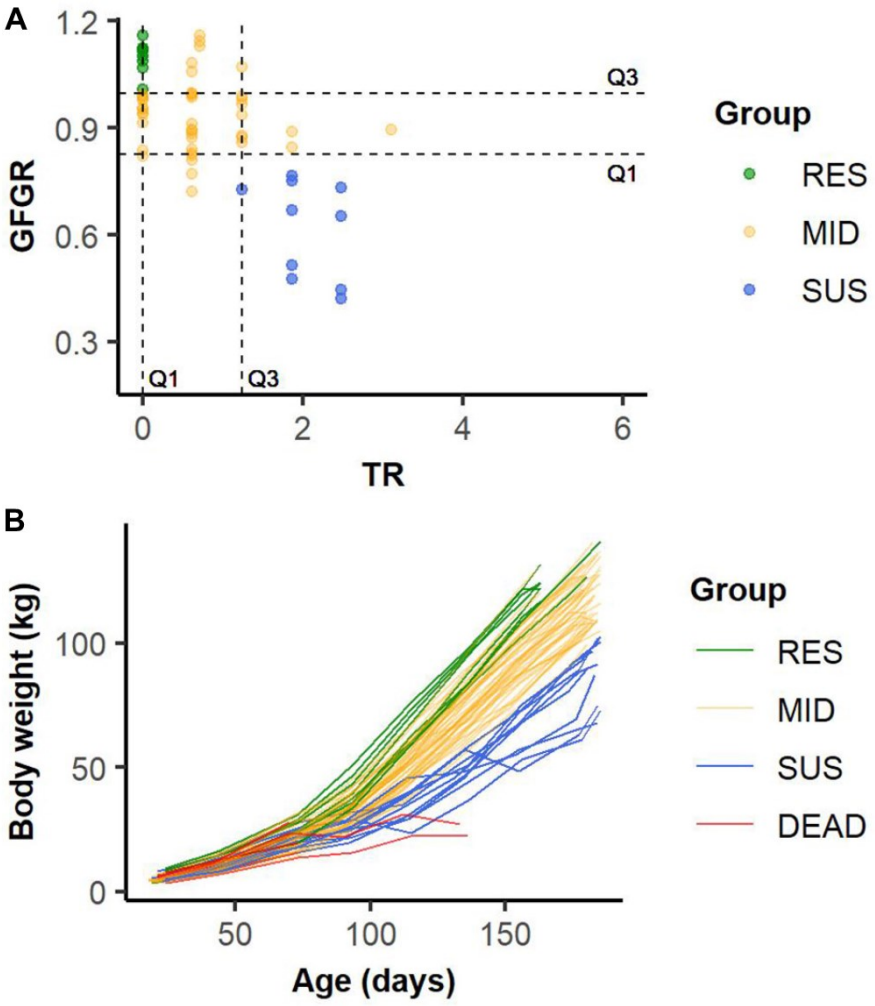

Figure 24. An example of the classification of slaughtered animals (A) and growth curves for all animals in different groups (B) in Batch 14.

Batch 14 was used as an example here. Slaughted animals were classified into resilient (RES), average (MID), and susceptible (SUS) groups based on the first (Q1) and the third (Q3) quartiles of grow-to-finish growth rate (GFGR) and treatment rate (TR).

Chapter 5. Investigating the genetic architecture of disease resilience in pigs by genome-wide association studies of complete blood count traits[2]

5.1. Introduction

The prevalence of infectious diseases caused by a multitude of pathogens results in high economic losses for the pig industry (Davies et al., 2008; Tomley and Shirley, 2009). Genetic improvement for disease resilience is a practical option to help address the problem of infectious disease as it can ensure production efficiency, because resilient animals are defined as maintaining a relatively undepressed performance in the face of disturbances caused by infection (Albers et al., 1987; Mulder and Rashidi, 2017). Disease resilience is a complex trait composed of multiple biological functions, such as growth, health, nutrient status, and other dynamic elements, including the efficiency of immune response and the rate of recovery from infection (Friggens et al., 2017). This complexity makes disease resilience hard to properly characterize and little is known about the genetic architecture that drives disease resilience. Alternatively, indirect selection of disease resilience based on immune-related traits may be a feasible breeding strategy, because the disease response of an animal largely depends upon its immunity (Knap and Bishop, 2000; Calder, 2013).

Blood cells comprise white blood cells, red blood cells, and platelets that are important elements of an animal's immune status (Abbas et al., 2015). Complete blood count (CBC) is one of the most common clinical tests performed to evaluate concentrations and relative proportions

[2]This chapter has been submitted to BMC Genomics as Bai, X., Yang, T., Putz, A. M., Wang, Z., Li., Chang, Fortin, F., Harding, J. C. S., Dyck, M. K., Dekkers, J. C. M., Field, C. J., PigGen Canada, and Plastow, G. S. (2020). Investigating the genetic architecture of disease resilience in pigs by genome-wide association studies of complete blood count traits collected from a natural disease challenge model. doi:10.21203/rs.3.rs-50174/v1.

of these circulating blood cells, which may help to uncover the layers of immune system complexity (George-Gay and Parker, 2013). Our previous study (Bai et al., 2020) found that CBC traits collected from blood samples of pigs in both healthy and challenged conditions at 2-weeks before, and 2 and 6-weeks after exposure to a polymicrobial challenge were moderately to highly heritable (0.08 ± 0.04 to 0.53 ± 0.05). Changes of each CBC trait between blood samples collected at different time points (e.g. the change of a CBC level from 2-weeks before to 2-weeks after exposure to the challenge) were also found to be heritable, with estimates ranging from 0.06 ± 0.04 to 0.24 ± 0.04 (Bai et al., 2020). These heritability estimates indicate the importance of the genetic component of CBC traits. Moreover, significant genetic correlations (either positive or negative) were found for several CBC traits collected after exposure to the challenge with the economically important production traits of grow-to-finish growth rate (GFGR) and treatment rate (TR) in response to the polymicrobial challenge (-0.82 ± 0.47 to 0.89 ± 0.26) (Bai et al., 2020), which may further indicate the potential of developing those CBC traits as indicator traits of disease resilience. In addition to these significant genetic correlations for CBC with GFGR and TR, our previous study (Bai et al., 2020) also found high genetic correlations (≥ 0.40 ± 0.04) between the CBC traits. Changes in CBC traits between each time point were also found to be genetically correlated, with significant estimates ranging from -0.42 ± 0.21 to -0.92 ± 0.11 to 0.44 ± 0.22 to 0.98 ± 0.03 (Bai et al., 2020). This allows multivariate models to be used for joint analyses of these genetically correlated traits, which provides the potential to improve statistical power and explore pleiotropy (Park et al, 2011; Shriner, 2012; Lu et al., 2018; Fatumo et al., 2019).

To date, some quantitative trait loci (QTL) have been identified for some blood cell traits in pigs under either healthy or disease challenged status by linkage and association analyses (Edfors-Lijia et al., 1998; Edfors-Lijia et al., 2000; Wattrang et al., 2005; Reiner et al., 2007; Reiner et al., 2008; Zou et al., 2008; Gong et al., 2010; Ponsuksili et al., 2016). However, due to the use of a pathogen-specific challenge or a relatively low density of genetic markers, the genetic components of blood cell traits in pigs under typical commercial environments, where multiple disease-causing pathogens are present, remains largely unknown.

In this study, CBC traits were collected from pigs in a natural polymicrobial disease challenge model, as described by Bai et al. (2020). Standard univariate genome-wide association studies (GWAS) and multivariate GWAS based on a relatively high-density panel of 465,910 autosomal single-nucleotide polymorphisms (SNPs) were conducted for these CBC traits. The objectives were: (1) to reveal the genomic regions associated with the CBC traits and with their changes in response to the polymicrobial challenge; and (2) to explore the underlying genetic architecture for disease resilience of pigs in the face of a polymicrobial infectious challenge.

5.2. Material and Methods

5.2.1 Natural disease challenge model and phenotypic traits

Details of the natural disease challenge model (NDCM) and the collection of phenotypic traits are described in **Chapter 3**. Descriptions of phenotypic traits, including CBC traits before and after exposure to the challenge, grow-to-finish growth rate (GFGR), and treatment rate (TR) can also be found in **Chapter 3**.

5.2.2 SNP array genotyping and quality control

The genotyping using the 650K Affymetrix Axiom® Porcine Genotyping Array was performed at Delta Genomics (Edmonton AB, Canada). Raw Affymetrix SNP data were processed by Delta Genomics with the Axiom Analysis Suite, using all defaults (sample call rate ≥ 97%; SNP call rate ≥ 97%; number of minor alleles observed ≥ 2). Imputation of sporadic missing genotypes was completed using FImpute (Sargolzaei et al., 2014). The pedigree was utilized for imputation but only included the dam, since sire was typically unknown due to the use of pooled semen. The preGSf90 software in the BLUPF90 suite of programs was used to remove SNPs with minor allele frequency lower than 0.01 (Misztal et al., 2002).

5.2.3 Population stratification and linkage disequilibrium estimation

Population stratification among genotyped animals was investigated using PLINK 1.90 (Purcell et al., 2007) based on pairwise identity-by-state (IBS) distance, which was estimated using SNP genotypes. A multidimensional scaling (MDS) plot based on IBS pairwise distance was drawn by the 'ggplot2' package in R (R core team, 2019; Wickham, 2016) to show the first three dimensions of the population structure. The genomic inflation factor and quantile–quantile (Q–Q) plots were applied to assess genomic inflation of the test statistics using the R packages of 'GenABEL' and 'qqman' (Aulchenko et al., 2007; Turner, 2014; R core team, 2019). The linkage disequilibrium (LD) of pairwise SNPs was measured as the squared correlation (r^2) of allele counts for the two SNPs and haplotype blocks were built using the Haploview software (Barrett et al., 2005; VanLiere and Rosenberg, 2008).

5.2.4 Single-step GWAS and Models

Univariate and multivariate single-step GWAS (SSGWAS) for CBC traits were implemented in the BLUPF90 suite of programs (Misztal et al., 2002; Aguilar et al., 2019) with the joint pedigree-genomic relationship matrix (**H**) for single-marker associations, accommodating both genotyped (n = 2593) and non-genotyped (n = 150) animals. Details for algorithms employed for these analyses have been described by Aguilar et al. (2019). Briefly, BLUPF90 combines the algorithms for single-step GBLUP and for back-solving to obtain estimates and p-values for SNP associations from estimates of breeding values. The genomic relationship matrix (**G**) for genotyped animals was constructed as $\mathbf{ZZ}'/2\sum p_i(1-p_i)$, where the **Z** matrix contains centered SNP genotype codes and p_i is the minor allele frequency for SNP *i* (VanRaden, 2008). The p-values for SNP associations were adjusted for multiple testing by the Benjamini and Hochberg correction (false discovery rate, *FDR*) (Benjamini et al., 1995; R core team, 2019). An *FDR* threshold of 0.05 was used to control false positive results and to declare significant associations. The most significant SNP above the genome-wise *FDR* of 0.05 in each genomic region were referred to as the top significant SNP, which were further separated into top lead and top floating SNPs, which referred to top significant SNPs in a genomic region with or without a group of supportive SNPs, respectively.

The univariate mixed linear model used for GWAS can be described as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e}$$

where ***y*** is a vector of observations on a CBC trait for all individuals, **b** is a vector of fixed effects, including the effect of batch and the covariate of bleeding age, **X** is a design matrix relating observations to the fixed effects, **a** is a vector of breeding values, **Z** is a design matrix that relates observations to breeding values, including genotyped and ungenotyped animals, and

e is a vector of residual effects. Vector **c** represents a stack of vectors ($\mathbf{c}_{\mathbf{Litter}}$, $\mathbf{c}_{\mathbf{Pen1}}$, $\mathbf{c}_{\mathbf{Pen2}}$, and $\mathbf{c}_{\mathbf{Pen3}}$) of independent and uncorrelated random environmental effects, including litter ($\mathbf{c}_{\mathbf{Litter}}$) and pen effects in the quarantine unit ($\mathbf{c}_{\mathbf{Pen1}}$), in the challenge barn second-stage nursery ($\mathbf{c}_{\mathbf{Pen2}}$), and in the challenge barn grow-to-finish stage ($\mathbf{c}_{\mathbf{Pen3}}$). These random environmental effects were tested and fitted in the model for each CBC trait when they were significant (*p-value* < *0.05*). Matrix **W** ($\mathbf{W}_{\mathbf{Litter}}$, $\mathbf{W}_{\mathbf{Pen1}}$, $\mathbf{W}_{\mathbf{Pen2}}$, and $\mathbf{W}_{\mathbf{Pen3}}$) is a stack of incidence matrices that relate observations to the corresponding random environmental effects. The random effects fitted for each of CBC traits were the same as Bai et al. (2020).

Assuming the random effects **c** and **e** are uncorrelated and identically distributed, the (co-) variances of random effects for univariate models are:

$$\text{var}\begin{bmatrix}\mathbf{a}\\ \mathbf{c}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{H}\sigma_a^2 & 0 & 0\\ 0 & \mathbf{I}\boldsymbol{\sigma}_\mathbf{c}^\mathbf{2} & 0\\ 0 & 0 & \mathbf{I}\sigma_e^2\end{bmatrix}$$

where **H** is the joint pedigree-genomic relationship matrix for genotyped and non-genotyped animals as mentioned above, **I** is the identity matrix, σ_a^2 is the additive genetic variance, $\boldsymbol{\sigma}_\mathbf{c}^\mathbf{2}$ represents a stack of random effect variances (e.g. $\boldsymbol{\sigma}_\mathbf{c}^\mathbf{2} = \begin{bmatrix}\sigma_{c_{\text{Litter}}}^2 & 0\\ 0 & \sigma_{c_{\text{Pen1}}}^2\end{bmatrix}$, when the random effects $\mathbf{c}_{\mathbf{Litter}}$ and $\mathbf{c}_{\mathbf{Pen1}}$ are significant and fitted in the model for a trait), and σ_e^2 is the residual variance.

The model for multivariate analyses resembles a stack of univariate models for each of the traits that were found to be highly genetic correlated in (Bai et al., 2020), which can be written as (Mrode, 2013; Lu et al., 2018):

$$\begin{bmatrix} \mathbf{y_1} \\ \mathbf{y_2} \\ \mathbf{y_3} \end{bmatrix} = \begin{bmatrix} \mathbf{X_1 b_1 + Z_1 a_1 + W_1 c_1 + e_1} \\ \mathbf{X_2 b_2 + Z_2 a_2 + W_2 c_2 + e_2} \\ \mathbf{X_3 b_3 + Z_3 a_3 + W_3 c_3 + e_3} \end{bmatrix}$$

For each trait in the multivariate model, the same effects were fitted as in the univariate models. For multivariate models, assuming random effects $\mathbf{c_n}$ and residual effects $\mathbf{e_n}$ for the nth trait (n = 1, 2, 3) are uncorrelated and identically distributed, the (co-) variances of random effects are:

$$\text{Var}\begin{bmatrix} \mathbf{a_1} \\ \mathbf{a_2} \\ \mathbf{a_3} \\ \mathbf{c_1} \\ \mathbf{c_2} \\ \mathbf{c_3} \\ \mathbf{e_1} \\ \mathbf{e_2} \\ \mathbf{e_3} \end{bmatrix} = \begin{bmatrix} \mathbf{H}\sigma^2_{a_1} & \mathbf{H}\sigma_{a_{12}} & \mathbf{H}\sigma_{a_{13}} & 0 & 0 & 0 & 0 & 0 & 0 \\ \mathbf{H}\sigma_{a_{21}} & \mathbf{H}\sigma^2_{a_2} & \mathbf{H}\sigma_{a_{23}} & 0 & 0 & 0 & 0 & 0 & 0 \\ \mathbf{H}\sigma_{a_{31}} & \mathbf{H}\sigma_{a_{32}} & \mathbf{H}\sigma^2_{a_3} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \mathbf{I}\sigma^2_{c_1} & \mathbf{I}\sigma_{c_{12}} & \mathbf{I}\sigma_{c_{13}} & 0 & 0 & 0 \\ 0 & 0 & 0 & \mathbf{I}\sigma_{c_{21}} & \mathbf{I}\sigma^2_{c_2} & \mathbf{I}\sigma_{c_{23}} & 0 & 0 & 0 \\ 0 & 0 & 0 & \mathbf{I}\sigma_{c_{31}} & \mathbf{I}\sigma_{c_{32}} & \mathbf{I}\sigma^2_{c_3} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{e_1} & \mathbf{I}\sigma_{e_{12}} & \mathbf{I}\sigma_{e_{13}} \\ 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma_{e_{21}} & \mathbf{I}\sigma^2_{e_2} & \mathbf{I}\sigma_{e_{23}} \\ 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma_{e_{31}} & \mathbf{I}\sigma_{e_{32}} & \mathbf{I}\sigma^2_{e_3} \end{bmatrix}$$

where $\sigma_{a_{12}} = \sigma_{a_{21}}$, $\sigma_{a_{13}} = \sigma_{a_{31}}$, and $\sigma_{a_{23}} = \sigma_{a_{32}}$ are additive genetic covariances between traits, $\boldsymbol{\sigma}_{\mathbf{c_{12}}} = \boldsymbol{\sigma}_{\mathbf{c_{21}}}$, $\boldsymbol{\sigma}_{\mathbf{c_{13}}} = \boldsymbol{\sigma}_{\mathbf{c_{31}}}$, and $\boldsymbol{\sigma}_{\mathbf{c_{23}}} = \boldsymbol{\sigma}_{\mathbf{c_{32}}}$ are covariances for common random effects between two traits, $\sigma_{e_{12}} = \sigma_{e_{21}}$, $\sigma_{e_{13}} = \sigma_{e_{31}}$, and $\sigma_{e_{23}} = \sigma_{e_{32}}$ are covariances for residual effects between two traits.

5.2.5 Post-GWAS marker effect analyses

The percentage of additive genetic variance explained by a 1 Mb window (with a median of 224 adjacent SNPs) was estimated by conducting window-based inferences for additive genetic variance in the BLUPF90 suit of programs (Misztal et al., 2002). Each chromosome was evaluated by using a sliding (moving) 1 Mb window by using every SNP on the chromosome as a starting SNP for a window segment (Misztal et al., 2002). Therefore, a top significant SNP was contained within multiple windows and among them, the largest percentage of additive genetic

variance explained by a window that contained that top significant SNP was reported for each trait.

Additive and dominance effects of each top significant SNP were estimated using the BLUPF90 suite of programs (Misztal et al., 2002) based on the following model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{v}\alpha + \mathbf{d}\delta + \mathbf{e}$$

where **y**, **X**, **b**, **Z**, **a**, **W**, **c**, and **e** are the same as for the univariate model described above; **v** is a vector of the top significant SNP genotypes coded as -1, 0, and 1 for the AA, AB, and BB, respectively; α is the additive effect; **d** is a vector of dominance coded as 1 for heterozygous genotype (AB) and 0 for homozygous genotypes (AA and BB); δ is the dominance effect. Vectors **v** and **d** were fitted as covariates and the top significant SNPs were fitted one by one in the model. The likelihood ratio test was used to test the significance of the additive and dominance effects for each of the top significant SNPs by comparing full models to restricted models that constrained additive or dominance effects to zero using the REMLF90 program of BLUPF90 (Misztal et al., 2002). When the dominance effect was not significant (*p-value > 0.05*), the additive effect for a SNP was re-estimated by removing the dominance effect from the model.

5.2.6 Post-GWAS bioinformatics analyses

Ingenuity Pathway Analysis (IPA) (Ingenuity® Systems, United States; https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/, IPA Spring 2020 release) was used for functional enrichment analyses of candidate genes in significant genomic regions for the CBC traits. A maximum distance of 1 Mb on either side of the lead SNPs based on a genome-wise *FDR < 0.10* was used to search for candidate genes for white blood cell traits

of EOSB3 and MONOΔ13. The lead pleiotropic SNPs at genome-wise *FDR < 0.10* were used to search for common candidate genes for red blood cell (MCH, MCV, and RBC) and platelet (MPV and PLT) traits in different time points before and after exposure to the challenge. A relaxed *FDR < 0.10* threshold was used here to increase identification of true positives for the significance of biological and functional relevance of candidate genes (Waide et al., 2017). Identification of positional candidate genes was conducted using the UCSC Genome Browser for the Ensembl annotation of the Sscrofa11.1 build of the swine genome (https://genome.ucsc.edu). One collective gene list was created for each trait by combing all candidate genes in associated genomic regions for IPA (Wang et al., 2020; Zhang et al., 2020). Human, mouse, and rat genes in the IPA knowledge base database were used as background for biological function analyses in diseases, molecular and cellular functions, and physiological system development and function categories. A biological function was considered significantly enriched if the p-value for the overlap comparison test between the input list of candidate genes and the IPA database was less than 0.05 (Wysocki et al., 2012; Wang et al., 2020; Zhang et al., 2020).

5.3. Results

5.3.1 Descriptive statistics and genetic parameters

Descriptive statistics (mean, standard deviation, minimum, maximum, and distribution) for the CBC data of 2743 animals in six cycles after removing the outliers, including both genotyped (n = 2593) and non-genotype (n = 150) animals, are shown in **Figures 25** to **27**. Details about genetic parameters for the evaluated CBC traits, including heritabilities and genetic correlations, can be found in our previous study (Bai et al., 2020), which used the same 2593 genotyped animals. In addition to the genetic correlations with resilience already reported for

these data by Bai et al. (2020), we also found significant genetic correlations for platelet concentration in Blood 3 collected at 2-weeks after exposure to a polymicrobial infectious challenge with GFGR (0.40 ± 0.22) and TR (-0.46 ± 0.26), and for the change of monocyte concentration from Blood 1 to Blood 3 (MONOΔ13) collected at 2-weeks before and 2-weeks after exposure to the challenge with GFGR (0.63 ± 0.21).

5.3.2 Population structure

As false positive results can be introduced in GWAS by confounding effects due to population stratification, MDS plots (**Figure 28**) were generated to provide a visualization of the population structure in the first three dimensions (C1, C2, and C3). Animals tended to cluster by farm of origin, as they shared a similar genetic background when they came from the same farm. Since batches were nested within farms and coded uniquely, population stratification associated with the farm effect was accounted for in the association analysis model by fitting the fixed effect of batch. The genomic inflation factors of SSGWAS for the CBC traits ranged from 0.98 to 1.06, suggesting that there was no population stratification that confounded the GWAS results.

5.3.3 Association results and estimates for SNP effects

White blood cell traits

Five genomic regions were found to be significantly associated with white blood cell traits at a genome-wise *FDR* of 0.05 by univariate SSGWAS. Of note, SNPs located on *Sus scrofa* chromosome (SSC) 4, SSC10, and SSC12 were found to be associated with eosinophil concentration in Blood 3, which was collected 2 weeks after exposure to the challenge (EOSB3). Meanwhile, SNPs on SSC2 and two adjacent floating SNPs (significant SNPs without a group of supportive SNPs) on SSC9 were identified to be associated with MONOΔ13. The Manhattan and

Q-Q plots for EOSB3 and MONOΔ13 are shown in **Figures 29** and **30**. Top lead SNPs (the most significant SNP with a group of supportive SNPs) for significant associations (genome-wise *FDR < 0.05*) with EOSB3 and MONOΔ13 are shown in **Table 16**. For EOSB3, the additive genetic variances explained by the 1 Mb window of the top lead SNPs (SNP1, SNP2, SNP3) and their adjacent SNPs on SSC4, SSC10, and SSC12 were estimated to be 0.46, 0.35, and 0.53% of the additive genetic variance for EOSB3, respectively. SNP4 was a floating SNP on SSC2 and its 1 Mb window explained 0.12% of the additive genetic variance for MONOΔ13. The 1 Mb window for SNP5, the top lead SNP on SSC9, was estimated to explain about 1.23% of the additive genetic variance for MONOΔ13.

Estimates of additive and dominance effects for the top significant SNPs (genome-wise *FDR < 0.05*, including both top lead and top floating SNPs) associated with EOSB3 and MONOΔ13 are summarized in **Table 16**. A significant dominance effect (*p-value < 0.05*) was only identified for SNP2, which was associated with EOSB3. Estimates of additive effects were found to be significant (*p-value < 0.05*) for all SNPs that were associated with EOSB3 and MONOΔ13. For EOSB3, estimates of additive effects were -0.05 ± 0.01, 0.14 ± 0.04, and -0.06 ± 0.01 for SNP1, 2 and 3, respectively. Estimates of additive effects for MONOΔ13 were 0.08 ± 0.02 and -0.08 ± 0.02 for SNP 4 and 5, respectively.

Due the relatively low genetic correlations and large standard errors between white blood cell traits (Bai et al., 2020), no genomic region was found to be significantly associated with white blood cell traits at *FDR < 0.05* from multivariate SSGWAS.

Red blood cell and platelet traits

Nine genomic regions were found to be significantly associated with red blood cell and platelet traits at the genome-wise *FDR* of 0.05 by univariate and or multivariate SSGWAS. The Manhattan plots and Q-Q plots are shown in **Figures 31** to **36**. The four top lead SNPs for significant associations (genome-wise $FDR < 0.05$) and estimates of additive genetic variances explained by these top lead SNPs and their adjacent SNPs in a 1 Mb window are summarized in **Table 17**. Five floating SNPs (genome-wise $FDR < 0.05$) that explained small amounts of the additive genetic variance (0.05% to 0.21%) for associated red blood cell and platelet traits were found and are summarized in **Table 18**. Of note, several pleiotropic SNPs associated with red blood cell or platelet traits were identified by multivariate SSGWAS of CBC traits in Blood 1, 3, and 4 (collected at 2-weeks before, 2- and 6-weeks after the challenge, respectively). High genetic correlations were found between mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and red blood cell concentration (RBC) traits (**Table 19**), and also between all three sampling time points for each of these traits ($\geq 0.77 \pm 0.08$) (Bai et al., 2020). Therefore, pleiotropic SNP7 on SSC6 was identified as the top lead and pleiotropic SNP for MCH in Blood 1 and for both MCV and RBC traits in all three blood samples (**Table 17**). The percentage of additive genetic variance explained by the 1 Mb window of SNP7 and its adjacent SNPs ranged from 0.29 to 0.57% for its associated traits. Moreover, SNP8 was the top lead and pleiotropic SNP on SSC8, which was associated with MCH, MCV, and RBC traits in all three blood samples. The percentages of additive genetic variance explained by SNP8 and its adjacent SNPs in a 1 Mb window were estimated to range from 0.28 to 0.35% for its associated traits. SNP9 on SSC17 was the top lead and pleiotropic SNP for mean platelet volume (MPV) in Blood 1 and 4. Together with adjacent SNPs in a 1 Mb window, SNP7 was estimated to explain about

0.49 and 0.40% of the additive genetic variances for MPV in Blood 1 and 4, respectively. Significant associations (genome-wise *FDR* < *0.05*) for SNP7 with MCV in Blood 1 (genome-wise *FDR* = 0.003) and for SNP8 with MCV in Blood 4 (genome-wise *FDR* = 0.04) were also found by univariate SSGWAS but at a lower significance level compared to the multivariate SSGWAS. Meanwhile, univariate SSGWAS only indicated suggestive associations (genome-wise *FDR* of 0.10) for SNP8 with RBC in Blood 1 (genome-wise *FDR = 0.09*) and with MCV in Blood 1 (genome-wise *FDR = 0.08*).

For red blood cell and platelet traits, the estimates of additive and dominance effects for the top lead SNPs are summarized in **Table 17** and for the top floating SNPs in **Table 18**. Of note, the additive effects for pleiotropic SNPs showed a tendency of affecting each CBC trait in the three blood samples in the same way, including SNP7 for MCV, SNP8 for MCH and MCV, SNP9 for MPV, SNP10 for MCV, SNP11 for PLT, and SNP12 for MCH. For pleiotropic SNP8, no significant additive effect was found for RBC traits.

5.3.4 Candidate genes and functional enrichment results

Browsing regions for candidate genes located within a maximum distance of 1 Mb on either side of the lead SNPs based on a genome-wise *FDR* < *0.1* for the associated CBC traits are summarized in **Table 21**. Enriched functions such as inflammatory responses, cell-to-cell signaling and interaction, cellular development, cell morphology, cellular growth and proliferation, and hematological system development and function were commonly identified for the candidate gene lists for white blood cell traits collected after exposure to the challenge, and or the pleiotropic candidate gene lists for red blood cell and platelet traits collected before and after exposure to the challenge.

Candidate genes that have been reported by previous studies of pigs, human, mice, or rats to be functionally and biologically related to the same category of blood cells, as explored here, are summarized in **Table 21**. A group of immunity genes on SSC2 has been reported to be functionally and biologically related to monocytes, including *TICAM2 (toll-like receptor adaptor molecule 2)*, *TMED7(transmembrane emp24 domain-containing protein 7 precursor)*, and *CDO1 (cysteine dioxygenase type 1)*, which were located proximal to SNP4, and *COMMD10 (COMM domain containing 10)*, which harbored SNP4 (**Table 21**). An overview of the location of these candidate genes and the distribution of all the SNPs in this region on SSC2 is shown in the LD haplotype map in **Figure 37**. SNP6 on SSC4 is intronic within candidate gene *SPTA1 (spectrin alpha, erythrocytic 1)* and the LD haplotype map for this region is shown in **Figure 38**. In **Table 21**, a group of candidate genes, including *THAP11 (THAP domain containing protein 11)*, *PSMB10 (proteasome subunit beta type 10)*, *LCAT (lecithin-cholesterol acyltransferase)*, and *SLC12A4 (Potassium/Chloride Cotransporter 1)*, was reported to be functionally related to red blood cells and were located close to SNP7 in the same haplotype block on SSC6 (**Figure 39**). SNP8 on SSC8 was found to be in LD ($r^2 > 0.30$) with SNPs in the *PDGFRA (platelet derived growth factor receptor alpha)* gene (**Figure 40**).

5.4. Discussion

5.4.1 Potential roles of candidate genes

Functional enrichment analyses for the candidate gene lists for CBC traits indicated multiple enriched functions that can be considered as functionally and biologically relevant to white blood cell traits in response to a polymicrobial infectious challenge, and red blood cell and platelet traits that were collected before and after exposure to the challenge, such as

inflammatory response, cell growth and proliferation, cell-to-cell signaling and interaction, and hematological system development and function.

The candidate genes in **Table 21** have been reported to be relevant to particular types of CBC traits by studies in pigs, human, mice, and rat, which may help us to further understand the functions of these candidate genes related to CBC traits in response to the polymicrobial challenge. Of note, candidate genes *ARHGEF2* (*Rho/Rac guanine nucleotide exchange factor*), *TGFB2* (*transforming growth factor beta 2*), and *MIR21* (*microRNA miR-21*) were identified to be functionally and biologically relevant to eosinophils. The product of *ARHGEF2* regulates the activity of GTPases and has been identified to be highly expressed in eosinophils. GTPases are known to be involved in mediator release from granulocytes, which is a crucial event in the activation of eosinophils and neutrophils during inflammation (Lacy, 2005; Turton, 2018). *TGFB2* has also been found to be expressed mainly in eosinophils, and greater expression of *TGFB2* has been identified to be associated with persistent eosinophilic inflammation (severe asthma) in human (Balzar et al., 2005). However, in the polymicrobial challenge, an increase in the number of eosinophils may be associated with parasitic infection (e.g. *Ascaris suum*) rather than respiratory disease. Eosinophils play an important role of killing larvae by releasing the toxic content of their granules as part of the immune response (Masure et al., 2013). Thus, further investigations are warranted to investigate the functional relationships between the expression of *TGFB2* and response to the challenge. Expression of *MIR21* has not been identified in eosinophils but in other white blood cells, including lymphocytes, monocytes, macrophages, and dendritic cells, which work collaboratively with eosinophils in the immune response (Cobb et al., 2006; Wu et al., 2007; Sheedy, 2015). Although the mRNA targets for

MIR21 are complex and remain an area of active investigation, it has been demonstrated that *MIR21* acts as a key signal mediating the balance of the inflammatory reaction to promote healing, resolution, and a return to homeostasis (Sheedy, 2015).

For the candidate genes on SSC2, the product of *COMMD10* has been found to be related to the function of phagosomes in murine macrophages, which promotes phagolysosome maturation and facilitates the timely killing of pathogens (Dill et al., 2015; Shlomo et al., 2019). The product of *ATG12* (*autophagy related 12*) is involved in autophagy of circulating monocytes for degradation and recycling of cellular components, which prevents apoptosis (programmed cell death) of monocytes and is essential for monocyte-macrophage differentiation and cytokine production in the innate immune response (Geng and Klionsky, 2008; Zhang et al., 2012). The product of *CDO1*, cysteine dioxygenase type 1, catalyzes taurine synthesis and it is commonly accepted that taurine plays an important role in the immune system as an antioxidant to protect phagocytes, including macrophages, from oxidative stress caused by the generation of reactive oxygen species at the site of inflammation (Booken et al., 2008; Schaffer et al., 2009; Wang et al., 2009; Marcinkiewicz and Kontny, 2012). Both *TMED7* and *TICAM2* are immunity genes and their products are involved in the function of toll-like receptors (TLRs), which are expressed on macrophages and monocytes and are responsible for the sensing of pathogen-associated-molecular-patterns in the extracellular environment and in endosomes (Oshiumi et al., 2003; Doyle et al., 2012; Mekonnen et al., 2018). Of note, overexpression of *TMED7* has been found to be associated with inhibition of MyD88-independent TLR4 signaling and the protein encoded by *TICAM2* has been identified as a bridge adaptor recruiting TLRs to mediate innate immune responses (Oshiumi et al., 2003; Doyle et al., 2012; Mekonnen et al., 2018). In addition, *NAMPT*

(*nicotinamide phosphoribosyl transferase*) on SSC9 has been found to be functionally and biologically related to monocytes, and its gene product has been found to play an important role in governing monocyte recruitment and in monocyte-macrophage differentiation (Schilling et al., 2011; Travelli et al., 2018).

For red blood cells, the majority of candidate genes reported here have been identified as key components involved in hematopoiesis and erythropoiesis responsible for the differentiation and development of red blood cells, including *MNDA* (*myeloid cell nuclear differentiation antigen*) on SSC4, *CBFB* (*core-binding factor subunit beta*) and *THAP11* on SSC6, *PDGFRA* and *KIT* (*KIT proto-oncogene, receptor tyrosine kinase*) on SSC8, and *RARA* (*retinoic receptor alpha*) and *THRA* (*thyroid hormone receptor alpha*) on SSC12 (Moller et al., 1996; Xie et al., 1998; Kastner and Chan, 2001; Kastner et al., 2001; Zhu et al., 2001; Cools et al., 2003; Kendrick et al., 2008; Thorén et al., 2008; Kong et al., 2014; Zhai et al., 2014). In addition, *SPTA1* on SSC4 encodes a protein in the red blood cell membrane, the products of *LCAT* and *SLC12A4* on SSC6 regulate the lipid composition in the red blood cell membrane and cell swelling, respectively, and all these gene products work together to maintain the normal volume and biconcave shape of red blood cells, which helps to ensure the biological and biomechanical functions of the cells (Godin et al., 1978; Karai et al., 1982; Bize et al., 2000; Rust et al., 2007; Diez-Silva et al., 2010). *ACKR1* (*atypical chemokine receptor 1*) on SSC4 and *PSMB10* on SSC6 are candidate genes that have been shown to be involved in the immune response of red blood cells. The receptor ACKR1 expressed in red blood cells was found to regulate immune responses by interacting with chemokines, and which works as a blood-based chemokine buffer involved with the uptake and degradation of chemokines (Permanyer et al., 2018). Meanwhile, ACKR1

has also been identified as an essential regulator of hematopoiesis and erythropoiesis promoting interactions between nuclear progenitor red blood cells and hematopoietic stem cells in the bone marrow (Bonavita et al., 2018; Permanyer et al., 2018). PSMB10 is found to be responsible for intracellular protein degradation and generation of peptides that bind to class I major histocompatibility complex (MHC) molecules (Wu et al., 2006). The MHC molecules display these peptides to cytotoxic $CD8^+$ T cells to support their activity of immune surveillance (Leone et al., 2013). Further, through a study of anemia caused by congenital red blood cell aplasia in human, PSMB10 has been suggested to be functional in the MHC class I machinery in mature red blood cells in response to inflammatory signaling (Pesciotta et al., 2015).

Candidate genes for platelet traits were annotated into two major functions, platelet aggregation and megakaryopoiesis. Platelet aggregation involves platelet-to-platelet adhesion, which is essential for effective hemostasis following injury and bleeding, and megakaryopoiesis is the process of differentiation and development of platelets (Rumbaut et al., 2010). Among them, CD9 (CD9 antigen) on SSC5 encodes a major platelet cell surface glycoprotein and plays dual roles in megakarypoiesis and platelet aggregation (Worthington et al., 1990; Boucheix et al., 1991; Kaprielian et al., 1995; Clay et al., 2001). The products of VWF (von Willebrand factor), PHB2 (prohibitin 2), and GNB3 (G protein subunit beta 3) on SSC5 and GNAS (guanine nucleotide binding protein) on SSC17 were found to be involved in platelet aggregation (Frey et al., 2003; Freson et al., 2008; Dusse et al., 2012; Freson et al., 2012; Kanaji et al., 2012; Zhang et al., 2012). In addition to megakaryopoiesis, tubulin beta class VI coded by TUBB1 (tubulin beta 1 class VI) on SSC17 has been reported to play a role in maintaining platelet morphology (Schwer et al., 2001; Kunishima et al., 2014; Burley et al., 2018).

5.4.2 Overlap with previously discovered QTL

In addition to the novel QTL for CBC traits identified in this study, some of the QTL identified have been previously reported. QTL on SSC8 located nearby the *KIT* gene were found to be associated with MCH, MCV, and RBC in this study. In addition, this region has also been identified to show a significant effect on the levels of NEU and HCT in the crossbreds of European Wild Boar × Yorkshire and Landrace × Yorkshire subsequent to stress and disease challenges (Edfors-Lijia et al., 2000; Wattrang et al., 2005). For QTL on SSC5 that associated with PLT traits here, Reiner et al. (Reiner et al., 2007) found them to be associated with red blood cell traits in Pietrain × Meishan pigs including HCT, HGB, and RBC traits. These results may be caused by the common myeloid progenitors for all cells mentioned above. Moreover, it may also further indicate the pleiotropic roles of QTL involved in the functions of different blood cells. Apart from studies in pigs, the candidate gene *SPTA1* associated with MCHC has also been identified by GWAS for red blood cell traits in human, which also functions in maintaining the shape and deformability of human red blood cells (Ganesh et al., 2009).

5.4.3 Potential links with disease resilience

Although the QTL uncovered for blood cell traits have small effects in this study, which has also been found in previous GWAS for blood cell traits of pigs and human (Zou et al., 2008; Ganesh et al., 2009), the genes involved in these QTL are suggested to be involved in hematopoiesis and immune responses in the face of a polymicrobial infectious challenge. In turn, they may contribute to disease resilience, as hematopoiesis and immune response are collaborative mechanisms that play essential roles in defending against pathogens, maintaining homeostasis, and preventing death from the infection (Baldridge et al., 2011; Calder, 2013;

Boettcher and Manz, 2017). None of the QTL identified for the CBC traits were pleiotropic with GFGR or TR in response to the challenge. However, some candidate genes are known to have pleiotropic effects among different CBC traits and play roles in both hematopoiesis and immune response. For example, *KIT* may be a pleiotropic gene for multiple blood cell populations in response to stress and disease challenge, and *ACKR1* exhibits pleiotropic effects on hematopoiesis and immune responses, as discussed above (Edfors-Lijia et al., 2000; Wattrang et al., 2005; Bonavita et al., 2018; Permanyer et al., 2018). Accordingly, these results highlight the importance of further investigating and validating the function of such pleiotropic genes in disease resilience.

5.5. Conclusion

In this study, we identified fourteen genomic regions that were significantly associated (genome-wise $FDR < 0.05$) with CBC traits collected from the natural polymicrobial challenge model, including five for white blood cell traits and nine for red blood cell and platelet traits. Candidate genes or regions located nearby significant SNPs were found to have potential roles in immune response pathways, red blood cell morphology, platelet aggregation, and hematopoiesis, including granulopoiesis and granulocytic differentiation, erythropoiesis, and megakarypoiesis. These results complement previous GWAS for blood cell traits in pigs and contribute to improving our understanding of the genetic basis of blood cell composition before and after exposure to a polymicrobial infectious challenge. This study also advances understanding of the genetic control of disease resilience, as blood cells are key players in an animal's immune response and are recruited by hematopoiesis. Validation and identification of the candidate genes

and causal mutations are necessary to further investigate and develop the use of CBC traits to enhance genetic improvement of disease resilience for the pig industry.

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Table 16. Top significant SNPs identified by univariate SSGWAS for significant associations with white blood cell traits at a genome-wise false discovery rate (FDR) of 0.05.

Trait[1]	Blood[2]	SNP ID	SNP status[3]	SSC[4]	SNP position (bp)	MAF[5]	FDR	GVar (%)[6]	1-Mb window start SNP position[7] (bp)	Dominance effect ± standard error	Additive effect[8] ± standard error
EOS	Blood 3	SNP1: rs336560074	Top lead	4	93,647,202	0.31	0.006	0.46	93,331,316	0.001 ± 0.01	-0.05 ± 0.01[9]
EOS	Blood 3	SNP2: rs346258273	Top lead	10	8,186,695	0.08	0.03	0.35	7,396,201	-0.09 ± 0.04	0.14 ± 0.04
EOS	Blood 3	SNP3: rs339860061	Top lead	12	36,308,994	0.13	0.003	0.53	35,450,868	0.002 ± 0.02	-0.06 ± 0.01
MONO	Δ13	SNP4: rs321357560	Top floating	2	120,341,201	0.47	0.049	0.12	120,219,793	-0.03 ± 0.02	0.08 ± 0.02
MONO	Δ13	SNP5: rs327963623	Top lead	9	105,461,701	0.43	0.049	1.23	105,461,701	-0.02 ± 0.02	-0.08 ± 0.02

[1]EOS: eosinophil concentration; MONO: monocyte concentration.
[2]Blood 3: the CBC measures in Blood 3 collected at 2-weeks after exposure to the challenge; Δ13: the change of CBC measures from Blood 1 collected at 2-weeks before the challenge to Blood 3 collected at 2-weeks after exposure to the challenge.
[3]Top lead: the most significant SNP with a group of supportive SNPs; Top floating: the most significant SNP without a group of supportive SNPs.
[4]*Sus scrofa* chromosome.
[5]Minor allele frequency.
[6]The largest percentage of additive genetic variance explained by the top significant SNP and its adjacent SNPs in a 1 Mb window.
[7]Positions of the start SNP for the 1 Mb window segment with the largest amount of additive genetic variance.
[8]Estimates of additive effects per additional copy of the "B" allele. When the dominance effect was not significant (*p-value > 0.05*) the estimate of the additive effect was based on a model without the dominance effect.
[9]Significant estimates of additive and dominance effects are highlighted in bold (*p-value < 0.05*).

Table 17. Top lead SNPs[1] identified by univariate and multivariate SSGWAS for significant associations with red blood cell and platelet traits at a genome-wise false discovery rate (*FDR*) of 0.05.

SNP ID	SSC[2]	SNP position (bp)	MAF[3]	Trait[4]	Blood[5]	FDR	GVar (%)[6]	1-Mb window start SNP[7] position (bp)	Dominance effect ± standard error	Additive effect[8] ± standard error
SNP6: rs336055186	4	91,591,493	0.38	MCHC	Blood 3	0.04	1.15	91,291,800	**1.08 ± 0.02**[9]	**-2.13 ± 0.02**
SNP7[10]: rs325274805	6	28,511,423	0.41	MCH	Blood 1	0.04	0.29	28,110,554	0.10 ± 0.07	**-0.25 ± 0.06**
				MCV	Blood 1	0.001	0.57	28,110,554	**0.29 ± 0.07**	**-0.73 ± 0.06**
					Blood 3	0.002	0.49	28,096,004	0.05 ± 0.04	**-0.45 ± 0.04**
					Blood 4	0.002	0.48	28,096,004	**-0.12 ± 0.04**	**-0.53 ± 0.04**
				RBC	Blood 1	0.01	0.44	28,110,554	-0.02 ± 0.07	0.08 ± 0.06
					Blood 3	0.01	0.44	28,110,554	0.001 ± 0.04	**0.08 ± 0.04**
					Blood 4	0.03	0.40	28,110,554	-0.04 ± 0.05	0.06 ± 0.04
SNP8[10]: rs344612650	8	41,156,538	0.45	MCH	Blood 1	0.01	0.36	40,257,441	**0.15 ± 0.06**	**0.20 ± 0.05**
					Blood 3	0.04	0.36	40,257,441	**0.11 ± 0.04**	**0.16 ± 0.04**
					Blood 4	0.04	0.35	40,257,441	-0.006 ± 0.04	**0.18 ± 0.04**
				MCV	Blood 1	0.03	0.33	40,219,864	**0.35 ± 0.06**	**0.59 ± 0.05**
					Blood 3	0.006	0.27	40,219,864	**0.19 ± 0.04**	**0.38 ± 0.04**
					Blood 4	0.002	0.33	40,219,864	0.04 ± 0.04	**0.51 ± 0.04**
				RBC	Blood 1	0.007	0.31	40,219,864	0.01 ± 0.06	-0.08 ± 0.05
					Blood 3	0.02	0.31	40,219,864	-0.02 ± 0.04	-0.06 ± 0.04
					Blood 4	0.03	0.30	40,219,864	0.01 ± 0.04	-0.06 ± 0.04

SNP9[10]: rs323125939	17	59,739,745	0.34	MPV	Blood 1	0.02	0.49	59,053,639	0.002 ± 0.10	**0.28 ± 0.08**
					Blood 4	0.04	0.40	59,053,639	-0.09 ± 0.07	**0.25 ± 0.05**

[1]The most significant SNP with a group of supportive SNPs.
[2]*Sus scrofa* chromosome.
[3]Minor allele frequency.
[4]MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin; MCV: mean corpuscular volume; RBC: red blood cell concentration; MPV: mean platelet volume.
[5]Blood 1, Blood 3, and Blood 4: CBC measures in blood samples collected at 2-weeks before, and 2- and 6-weeks after a polymicrobial infectious challenge.
[6]The largest percentage of additive genetic variance explained by the top lead SNP and its adjacent SNPs in a 1 Mb window.
[7]Positions of the start SNP for the 1 Mb window segment with the largest amount of additive genetic variance.
[8]Estimates of additive effects per additional copy of the "B" allele. When the dominance effect was not significant ($p > 0.05$) the estimate of the additive effect was based on a model without the dominance effect.
[9]Significant estimates of additive and dominance effects are highlighted in bold ($p < 0.05$).
[10]SNPs identified and results estimated by multivariate SSGWAS.

Table 18. Top floating SNPs1 identified by univariate and multivariate SSGWAS for significant associations with red blood cell and platelet traits at a genome-wise false discovery rate (FDR) of 0.05.

SNP ID	SSC[2]	SNP Position (bp)	MAF[3]	Trait[4]	Blood[5]	FDR	GVar (%)[6]	1-Mb window start SNP[7] position (bp)	Dominance effect ± standard error	Additive effect[8] ± standard error
SNP10[10]: rs319452131	1	18,792,764	0.37	MCV	Blood 1	0.003	0.18	18,536,535	-0.16 ± 0.18	**0.52 ± 0.14**[9]
					Blood 3	0.004	0.21	18,546,024	0.03 ± 0.13	**0.52 ± 0.10**
					Blood 4	0.02	0.15	18,536,535	-0.02 ± 0.14	**0.37 ± 0.11**
SNP11[10]: rs1109789977	5	64,520,638	0.31	PLT	Blood 1	0.001	0.09	63,861,170	-3.80 ± 6.83	**26.78 ± 5.18**
					Blood 3	0.001	0.09	63,861,170	-9.28 ± 7.44	**23.92 ± 5.39**
					Blood 4	0.03	0.05	63,861,170	-10.57 ± 7.40	**18.33 ± 5.46**
SNP12[10]: rs320615395	9	40,919,049	0.45	MCH	Blood 1	0.03	0.05	39,919,771	0.07 ± 0.08	**0.21 ± 0.07**
					Blood 3	0.04	0.07	40,490,005	-0.04 ± 0.05	**0.19 ± 0.05**
					Blood 4	0.04	0.06	40,490,005	0.03 ± 0.05	**0.21 ± 0.05**
SNP13: rs80784550	11	13,749,336	0.12	MCHC	Δ14	0.02	0.07	13,011,748	1.89 ± 2.42	2.38 ± 2.33
SNP14[10]: rs323585109	12	22,234,265	0.3	MCV	Blood 3	0.04	0.08	21,749,390	-0.06 ± 0.14	**-0.40 ± 0.10**

[1]The most significant SNP without a group of supportive SNPs.
[2]*Sus scrofa* chromosome.
[3]Minor allele frequency.
[4]MCV: mean corpuscular volume; PLT: platelet concentration; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

[5]Blood 1, Blood 3, and Blood4: CBC measured in blood samples collected at 2-weeks before, and 2- and 6-weeks after a polymicrobial infectious challenge; Δ14: the change of CBC measures from Blood 1 collected at 2-weeks before the challenge to Blood 4 collected at 6-weeks after the challenge.
[6]The largest percentage of additive genetic variance explained by the top significant SNP and its adjacent SNPs in a 1 Mb window.
[7]Positions of the start SNP for the 1 Mb window segment with the largest amount of additive genetic variance.
[8]Estimates of additive effects per additional copy of the "B" allele. When the dominance effect was not significant ($p > 0.05$) the estimate of the additive effect was based on a model without the dominance effect.
[9]Significant estimates of additive and dominance effects are highlighted in bold ($p < 0.05$).
[10]SNPs identified and results estimated by multivariate SSGWAS.

Table 19. Genetic correlations between red blood cell traits of mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and red blood cell concentration (RBC).

Traits		MCH		
		Blood 1[1]	**Blood 3**[2]	**Blood 4**[3]
RBC	**Blood 1**	-0.71 ± 0.10	-0.83 ± 0.07	-0.60 ± 0.09
	Blood 3	-0.62 ± 0.10	-0.74 ± 0.06	-0.43 ± 0.09
	Blood 4	-0.69 ± 0.10	-0.66 ± 0.08	-0.55 ± 0.08
MCV	**Blood 1**	0.90 ± 0.03	0.81 ± 0.07	0.77 ± 0.08
	Blood 3	0.75 ± 0.07	0.86 ± 0.03	0.77 ± 0.05
	Blood 4	0.95 ± 0.02	0.86 ± 0.04	0.95 ± 0.02
Traits		**MCV**		
		Blood 1	**Blood 3**	**Blood 4**
RBC	**Blood 1**	-0.72 ± 0.09	-0.68 ± 0.08	-0.54 ± 0.09
	Blood 3	-0.65 ± 0.09	-0.59 ± 0.08	-0.44 ± 0.10
	Blood 4	-0.71 ± 0.10	-0.56 ± 0.08	-0.55 ± 0.08

[1]Blood 1: Blood samples collected at 2-weeks before exposure to the challenge.
[2]Blood 3: Blood samples collected at 2-weeks after exposure to the challenge.
[3]Blood 4: Blood samples collected at 6-weeks after exposure to the challenge.

Table 20. Browsing regions for candidate genes located within 1-Mb on either side of lead SNPs (*FDR < 0.*10) associated with complete blood count traits.

Traits[1]	Blood[2]	Browsing region *Sus scrofa* chromosome (SSC): position (bp)
EOS	Blood 3	SSC1: 110,498,112bp – 112,498,112bp; SSC4: 92,647,202bp – 94,647,202bp; SSC10: 7,186,695bp – 9,186,695bp; SSC12: 35,308,994bp – 37,308,994bp.
MONO	Δ13	SSC2: 119,341,201bp – 121,341,201bp; SSC9: 104,461,701bp – 106,461,701bp
MCH	Blood 1, 3, 4	SSC5: 9,683,166bp – 11,683,166bp; SSC6: 27,511,423bp – 29,511,423bp; SSC6: 164,588,523bp – 166,588,523bp; SSC7: 22,056,369bp – 24,056,369bp; SSC8: 40,156,538bp – 42,156,538bp; SSC9: 39,919,049bp – 41,919,049bp; SSC12: 23,001,577bp – 25,001,577bp; SSC13: 199,855,463bp – 201855463bp.
MCV	Blood 1, 3, 4	SSC1: 17,792,764bp – 19,792,764bp; SSC4: 76,486,634bp – 78,486,634bp; SSC6: 27,511,423bp – 29,511,423bp; SSC8: 40,156,538bp – 42,156,538bp; SSC12: 21,234,265bp – 23,234,265bp.
RBC	Blood 1, 3, 4	SSC2: 59,174,089bp – 61,174,089bp; SSC2: 104,736,448bp – 124,736,448bp; SSC3: 96,212,688bp – 98,212,688bp; SSC3: 120,086,804bp – 122,086,804bp; SSC6: 27,511,423bp – 29,511,423bp; SSC8: 40,156,538bp – 42,156,538bp.
MPV	Blood 1, 4	SSC4: 110,541,124bp – 112,541,124bp; SSC17: 58,739,745bp – 60,739,745bp.
PLT	Blood 1, 3, 4	SSC1: 1,655,014bp – 3,655,014bp; SSC5: 63,520,638bp – 65,520,638bp.

[1]The category of traits that associated with candidate genes. EOS: eosinophil concentration; MONO: monocyte concentration; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; RBC: red blood cell concentration; MCH: mean corpuscular hemoglobin; MPV: mean platelet volume; PLT: platelet concentration.
[2] Blood 1, Blood 3, and Blood4: CBC measured in blood samples collected at 2-weeks before, and 2- and 6-weeks after exposure to the challenge; Δ13: the change of CBC measures from Blood 1 collected at 2-weeks before the challenge to Blood 3 collected at 2-weeks after exposure to the challenge; Δ14: the change of CBC measures from Blood 1 collected at 2-weeks before the challenge to Blood 4 collected at 6-weeks after exposure to the challenge.

Table 21. Candidate genes located within 1 Mb on either side of the top significant SNPs[1] that have been reported by previous studies of pigs, human, mice, and rats to be functionally and biologically related to CBC traits.

SNPID	Traits[2]	Browsing region	Candidate genes and locations
SNP1	Eosinophils	SSC4: 92,647,202bp – 94,647,202bp	*ARHGEF2* (94,026,391bp – 94,082,206bp)
SNP2	Eosinophils	SSC10: 7,186,695bp – 9,186,695bp	*TGFB2* (8,327,779bp – 8,435,306bp)
SNP3	Eosinophils	SSC12: 35,308,994bp – 37,308,994bp	*MIR21* (36,065,267bp – 36,065,358bp)
SNP4	Monocytes	SSC2: 119,341,201bp – 121,341,201bp	*COMMD10* (120,238,623bp – 120,429,913bp), *ATG12* (119,948,443bp – 119,965,702bp), *CDO1* (119,928,476bp – 119,940,425bp), *TMED7* (119,794,608bp – 119,804,406bp), *TICAM2* (119,758,636bp – 119,760,759bp)
SNP5	Monocytes	SSC9: 104,461,701bp – 106,461,701bp	*NAMPT* (106,121,909bp – 106,161,841bp)
SNP6	Red blood cells	SSC4: 90,591,493bp – 92,591,493bp	*SPTA1* (91,485,067bp – 91,640,063bp), *MNDA* (91,416,410bp – 91,433,243bp), *ACKR1* (91,221,889bp – 91,225,651bp)
SNP7	Red blood cells	SSC6: 27,511,423bp – 29,511,423bp	*CBFB* (27,684,030bp – 27,776,751bp), *THAP11* (28,465458bp – 28,466,387bp), *PSMB10* (28,544,910bp – 28,547,609bp), *LCAT* (28,550,363bp – 28,553,512bp), *SLC12A4* (28,554,162bp – 28,576,458bp)
SNP8	Red blood cells	SSC8: 40,156,538bp – 42,156,538bp	*PDGFRA* (40,967,493bp – 41,021,442bp), *KIT* (41,402,334bp – 41,492,306bp)
SNP9	Platelets	SSC17: 58,739,745bp – 60,739,745bp	*GNAS* (59,031,820bp – 59,053,022bp), *TUBB1* (59,161,420bp – 59,168,385bp)
SNP10	Red blood cells	SSC1: 17,792,764bp – 19,792,764bp	*STXBP5* (18,345,363bp – 18,513,252bp), *RAB32* (18,870,875bp – 19,097,892bp)
SNP11	Platelets	SSC5: 63,520,638bp – 65,520,638bp	*VWF* (64,517,593bp – 64,655,938bp), *CD9* (64,420,177bp – 64,459,776bp), *GNB3* (63,863,656bp – 63,870,396bp), *PHB2* (63,751,566bp – 63,756,480bp)
SNP12	Red blood cells	SSC9: 39,919,049bp – 41,919,049bp	*ZBTB16* (41,639,701bp – 41,836,742bp)
SNP13	Red blood cells	SSC11: 12,749,336bp – 14,749,336bp	*TRPC4* (13,300,556bp – 13,517,568bp), *FREM2* (13,959,865bp – 14,154,246bp)
SNP14	Red blood cells	SSC12: 21,234,265bp – 23,234,265bp	*RARA* (22,047,442bp – 22,085,674bp), *THRA* (22,270,062bp – 22,296,618bp)

[1]The most significant SNP above the genome-wise *FDR* of 0.05 in each genomic region.
[2]The category of traits that associated with candidate genes.
[3]The gene location from the top significant SNP, described as “within” or to the left (L) or right (R) side of the gene as found on the reference genome sequence.

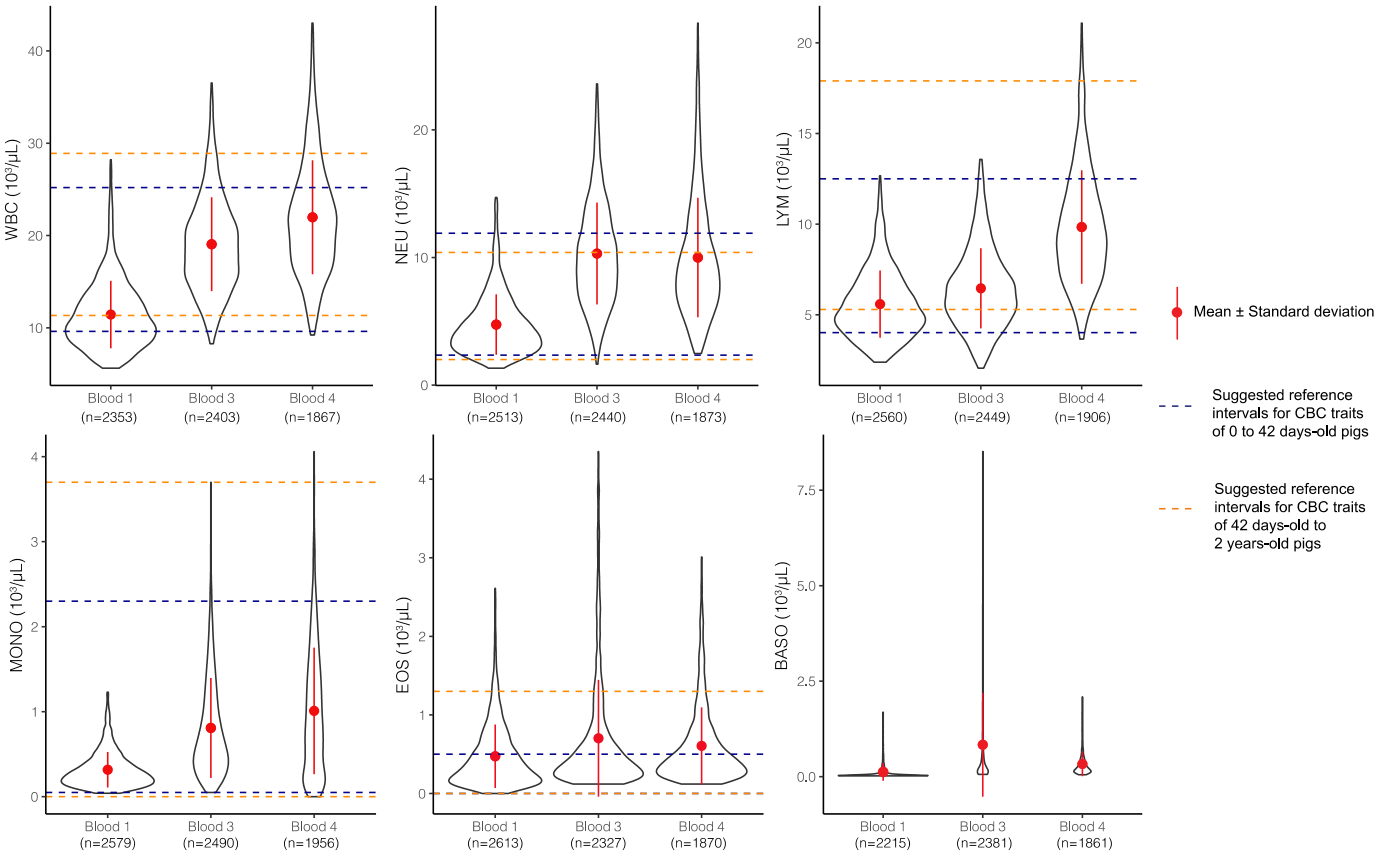

Figure 25. Violin plots for descriptive statistics for white blood cell traits of first six cycles in Blood 1, Blood 3, and Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

WBC: total white blood cell concentration; NEU: neutrophil concentration; LYM: lymphocyte concentration; MONO: monocyte concentration; EOS: eosinophil concentration; BASO: basophil concentration.

Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability. Suggested reference intervals for CBC traits were derived from Iowa State University's Clinical Pathology Laboratory (2011). The suggested reference intervals for BASO traits are not applicable.

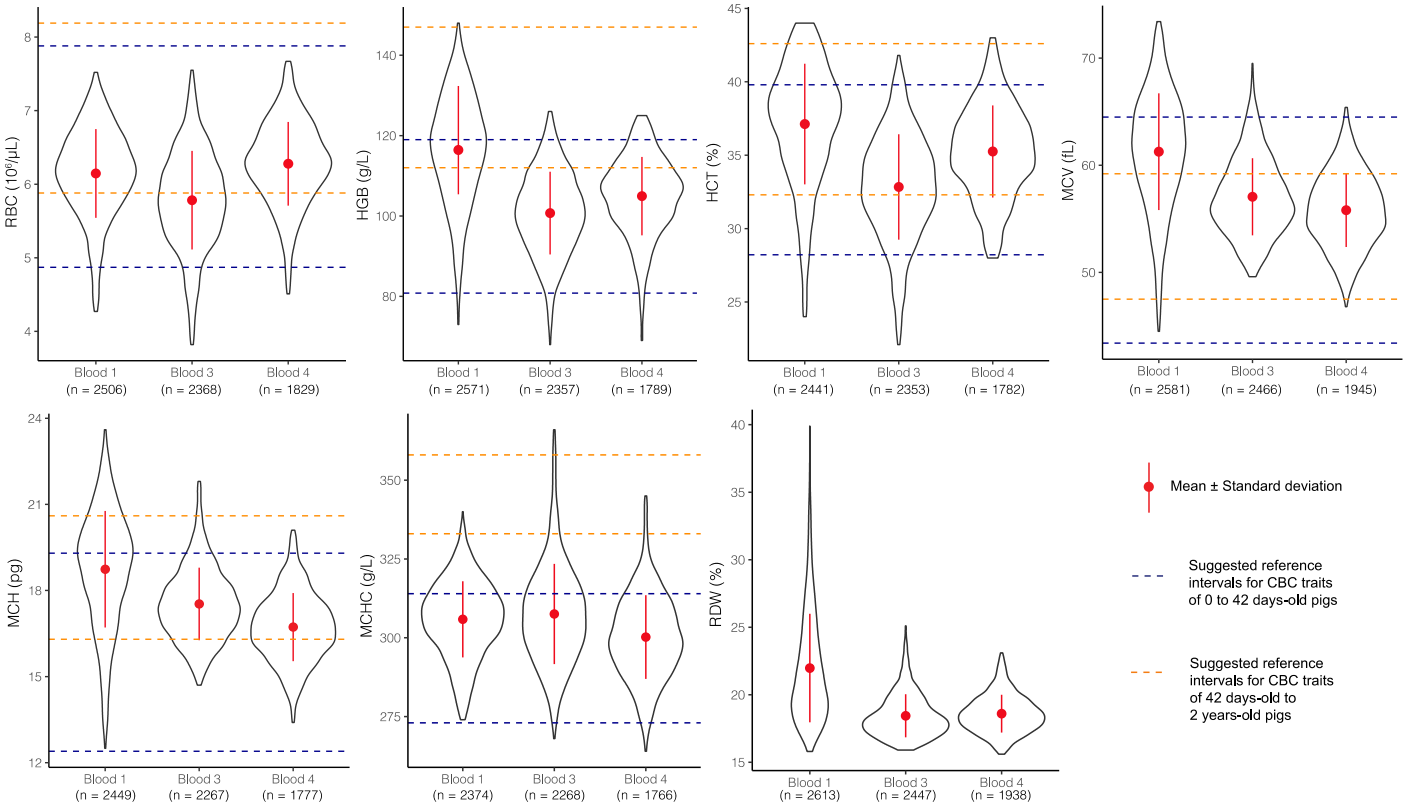

Figure 26. Violin plots for descriptive statistics for red blood cell traits of first six cycles in Blood 1, Blood 3, and Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively. RBC: red blood cell concentration; HGB: hemoglobin concentration; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width.

Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability.

Suggested reference intervals for CBC traits were derived from Iowa State University's Clinical Pathology Laboratory (2011). The suggested reference intervals for RDW traits are not applicable.

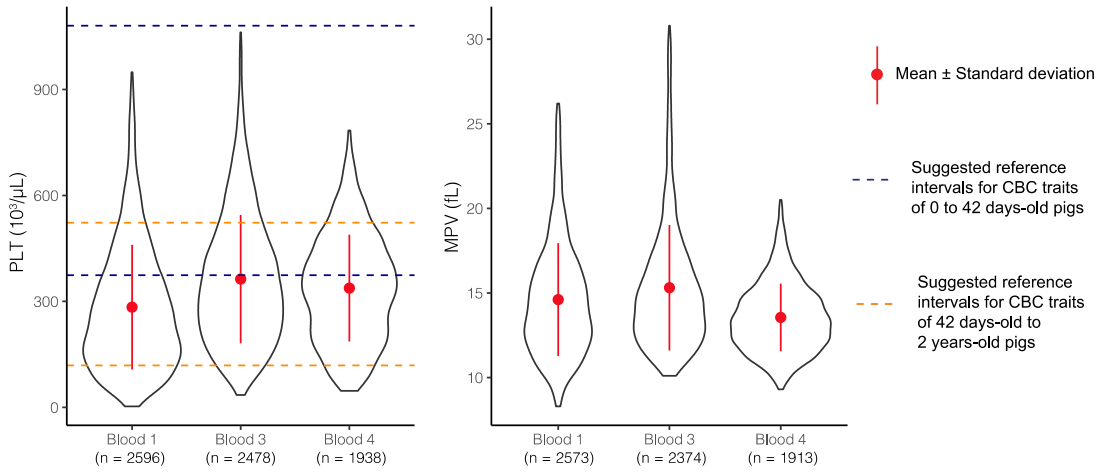

Figure 27. Violin plots for descriptive statistics for platelet traits of first six cycles in Blood 1, Blood 3, and Blood 4

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

PLT: platelet concentration; MPV: mean platelet volume. Wider sections of the violin plots represent a higher probability density of the data at the given value and the skinnier sections represent a lower probability.

Suggested reference intervals for CBC traits were derived from Iowa State University's Clinical Pathology Laboratory (2011). The suggested reference intervals for MPV traits are not applicable.

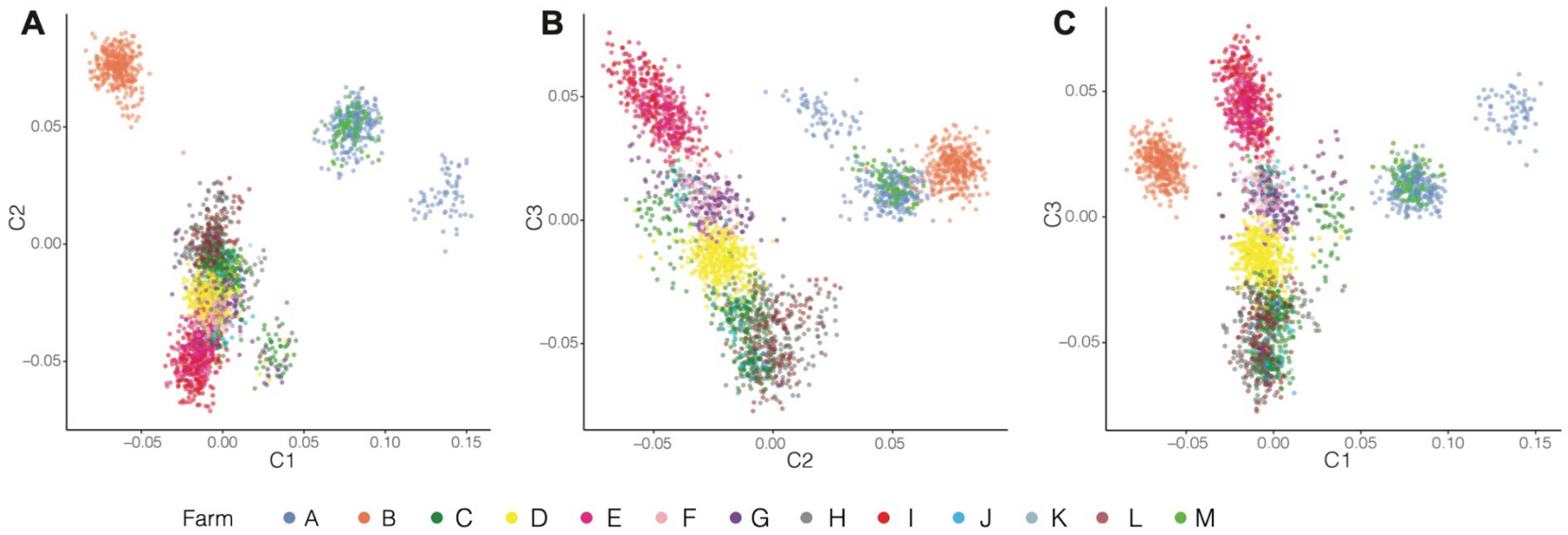

Figure 28. Multidimensional scaling (MDS) plots for the population structure of genotyped animals in the first six cycles.

The MDS plot showing the first three dimensions (C1, C2, and C3) of the population structure for genotyped animals based on pairwise identity-by-state distance. Each point represents a genotyped animal and the colors of the points represent the origin farms for animals. The MDS plot showing in the C1 and C2 dimensions **(A)**. The MDS plot showing in the C2 and C3 dimensions **(B)**. The MDS plot showing in the C1 and C3 dimensions **(C)**.

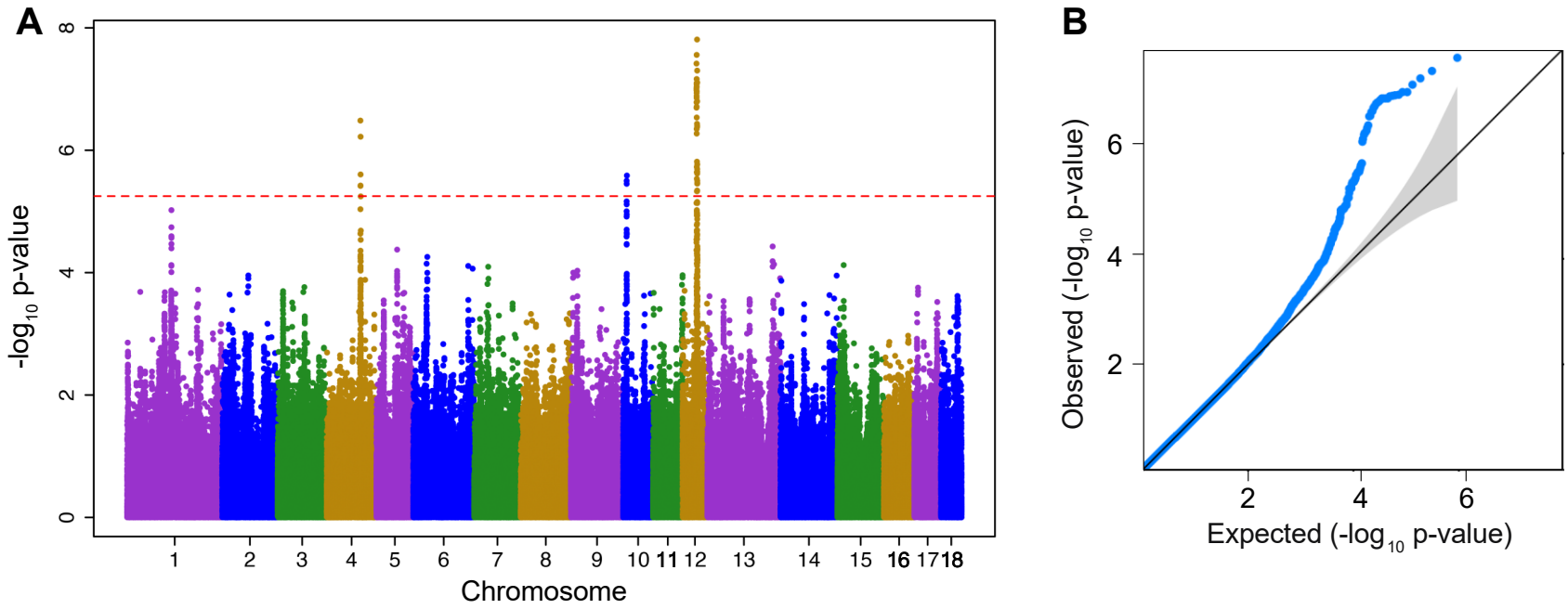

Figure 29. Manhattan plot for the eosinophil concentration in Blood 3 (EOSB3) (A) and Quantile-Quantile plot for EOSB3 (B).

Blood 3 was collected at 2-weeks after exposure to the challenge.

Significant SNPs were determined with the genome-wise false discovery rate at 0.05 (red dashed line) in the Manhattan plot, the grey region represents a 95% confidence interval in the Quantile-Quantile plot. Genomic inflation factor (λ) = 1.01.

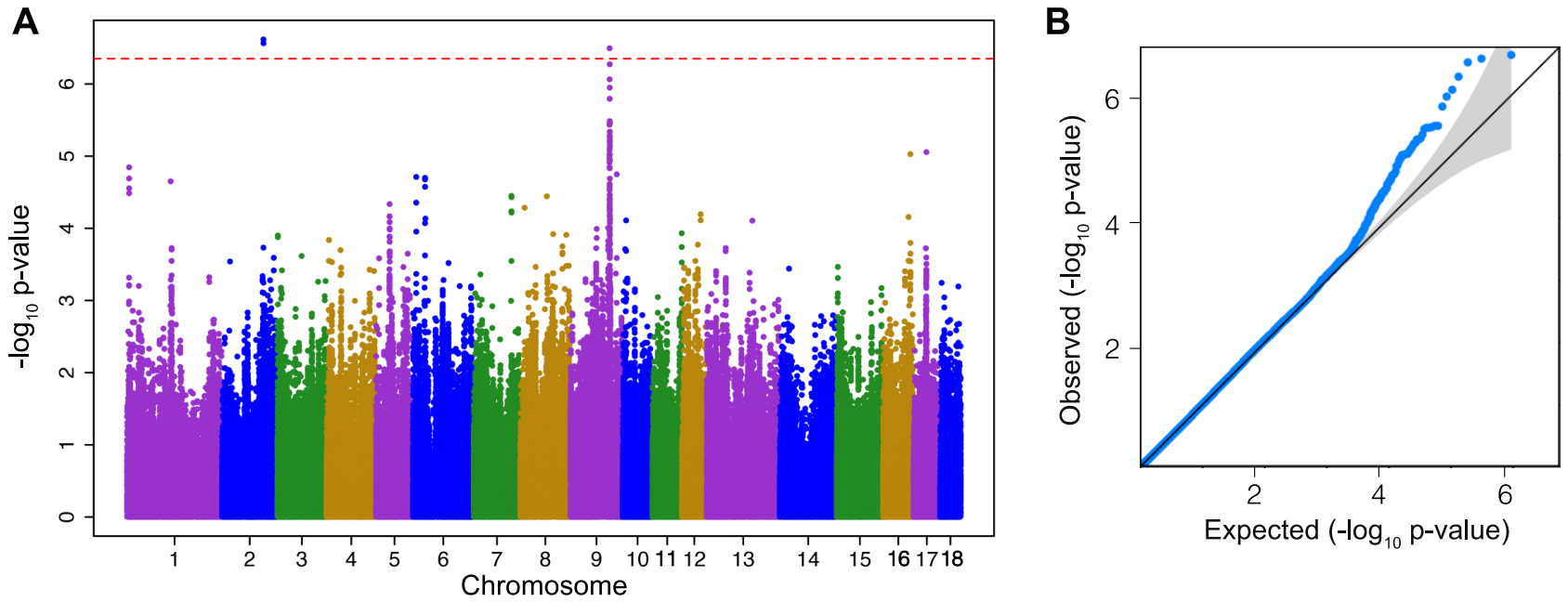

Figure 30. Manhattan plot for the change of monocyte concentration from Blood 1 to Blood 3 (MONOΔ13) (A) and **Quantile-Quantile plot for MONOΔ13 (B).**

Blood 1 and Blood 3 were collected at 2-weeks before and at 2-weeks after exposure to the challenge, respectively.

Significant SNPs were determined with the genome-wise false discovery rate at 0.05 (red dashed line) in the Manhattan plot, the grey region represents a 95% confidence interval in the Quantile-Quantile plot. Genomic inflation factor (λ) = 0.98.

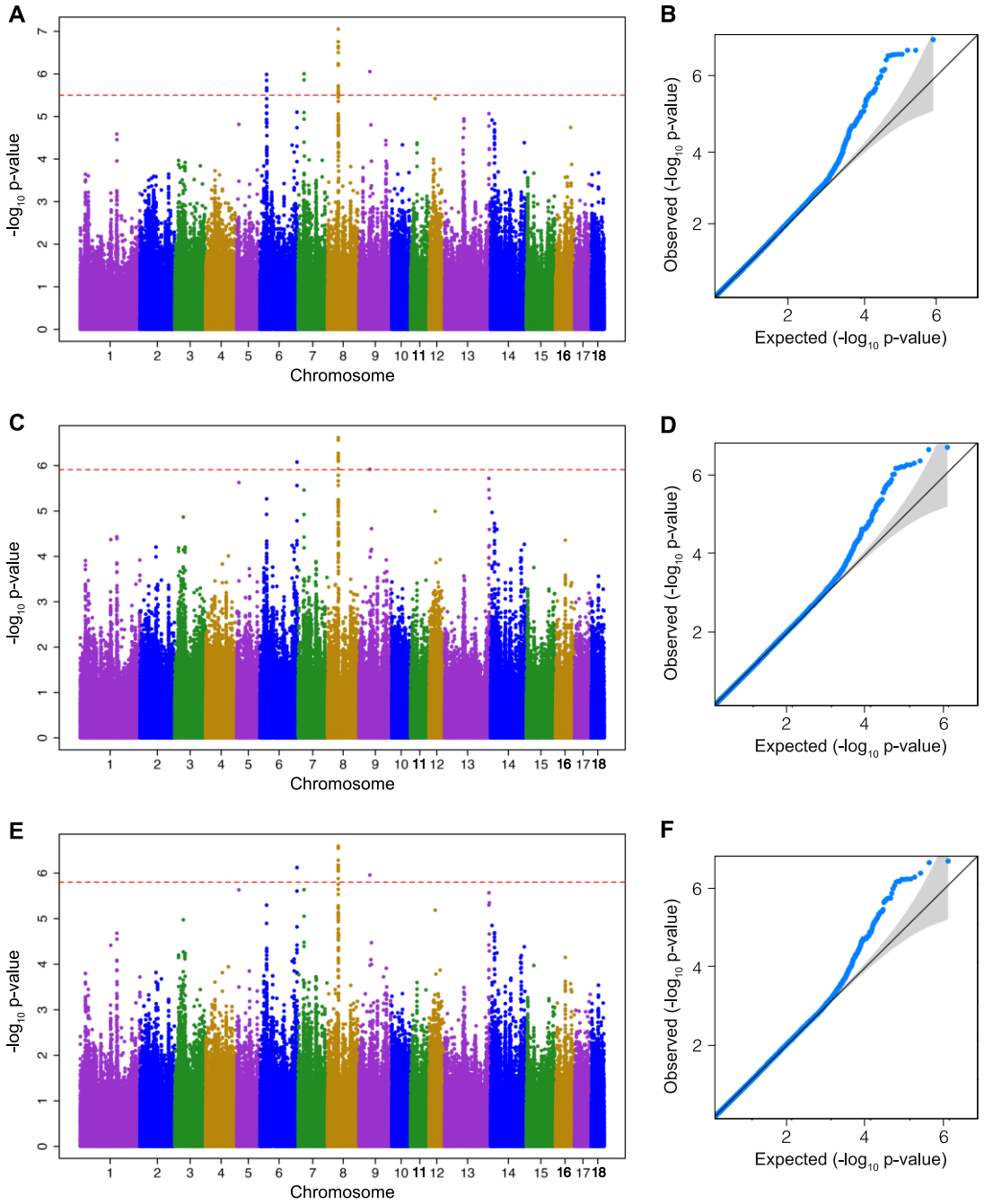

Figure 31. Manhattan plots (A, C, E) and Quantile-Quantile plots (B, D, F) for the mean corpuscular hemoglobin (MCH) in Blood 1, Blood 3, and Blood 4, respectively.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

Significant SNPs were determined with the genome-wise false discovery rate at 0.05 (the red dashed line in the Manhattan plot). The grey region in the Quantile-Quantile plot represents a 95% confidence interval. Genomic inflation factors (λ) were 1.01, 0.99, and 1 for MCH in Blood 1, Blood 3, and Blood 4, respectively.

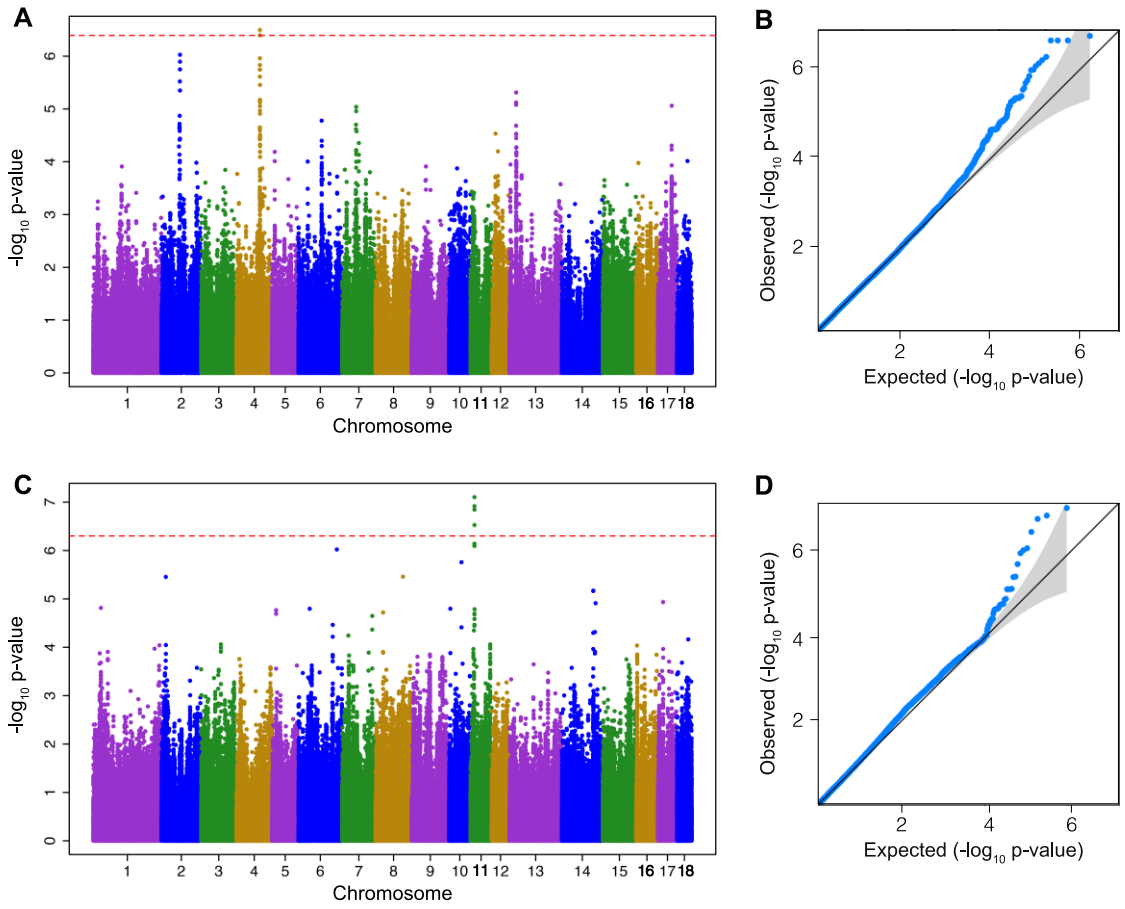

Figure 32. Manhattan plots (A, C) and Quantile-Quantile plots (B, D) for the mean corpuscular hemoglobin concentration (MCHC) in Blood 3 and for the change of MCHC from Blood 1 to Blood 4 (Δ14), respectively.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

Significant SNPs were determined with the genome-wise false discovery rate at 0.05 (the red dashed line in the Manhattan plot). The grey region represents a 95% confidence interval in the Quantile-Quantile plot. Genomic inflation factors (λ) were 1 for MCHC in Blood 3 and 1.06 for MCHC in Δ14.

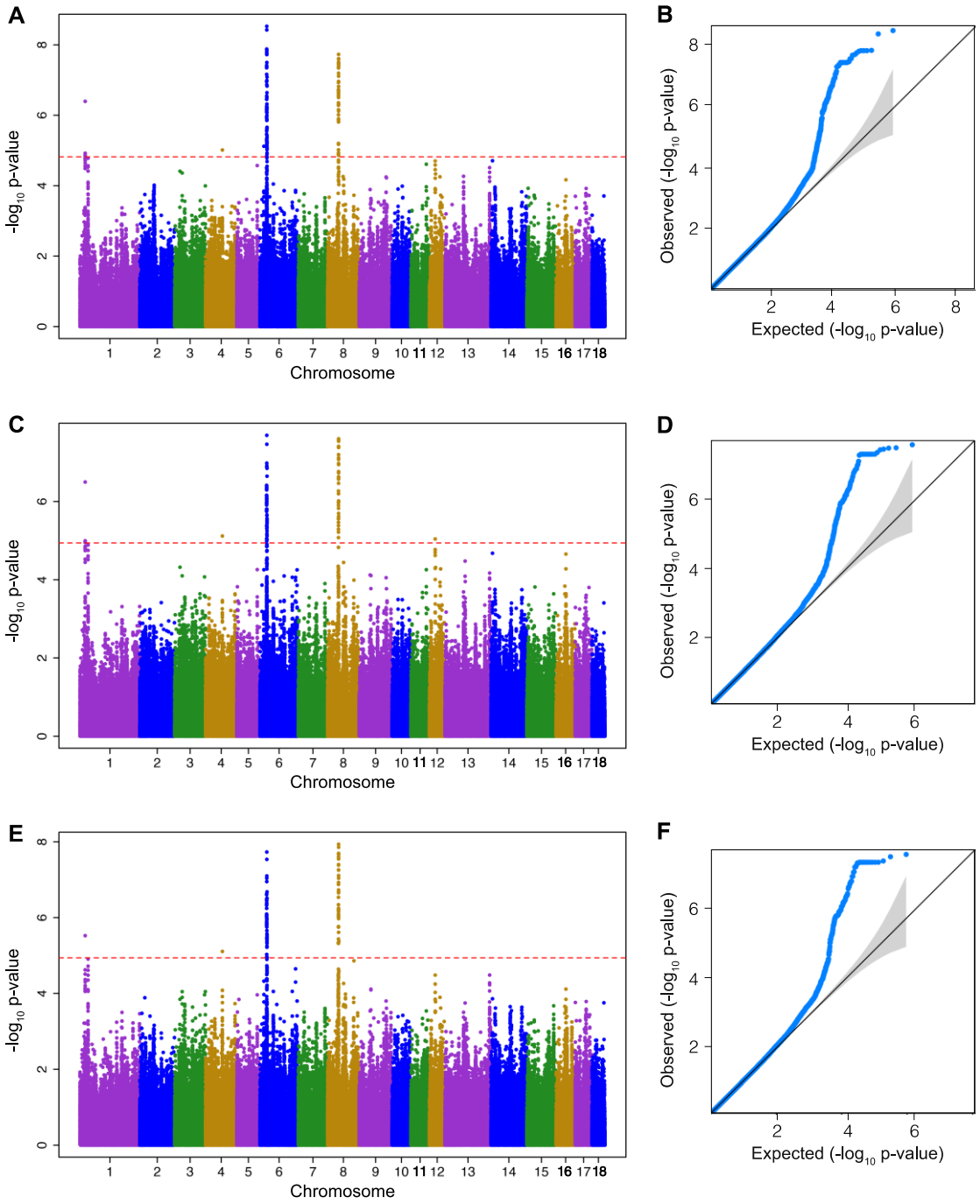

Figure 33. Manhattan plots (A, C, E) and Quantile-Quantile plots (B, D, F) for the mean corpuscular volume (MCV) in Blood 1, Blood 3, and Blood 4, respectively.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

Significant SNPs were determined with the genome-wise false discovery rate at 0.05 (the red dashed line in the Manhattan plot). The grey region represents a 95% confidence interval in the Quantile-Quantile plot. Genomic inflation factors (λ) were 0.99, 1, and 0.99 for MCV in Blood 1, Blood 3, and Blood 4, respectively.

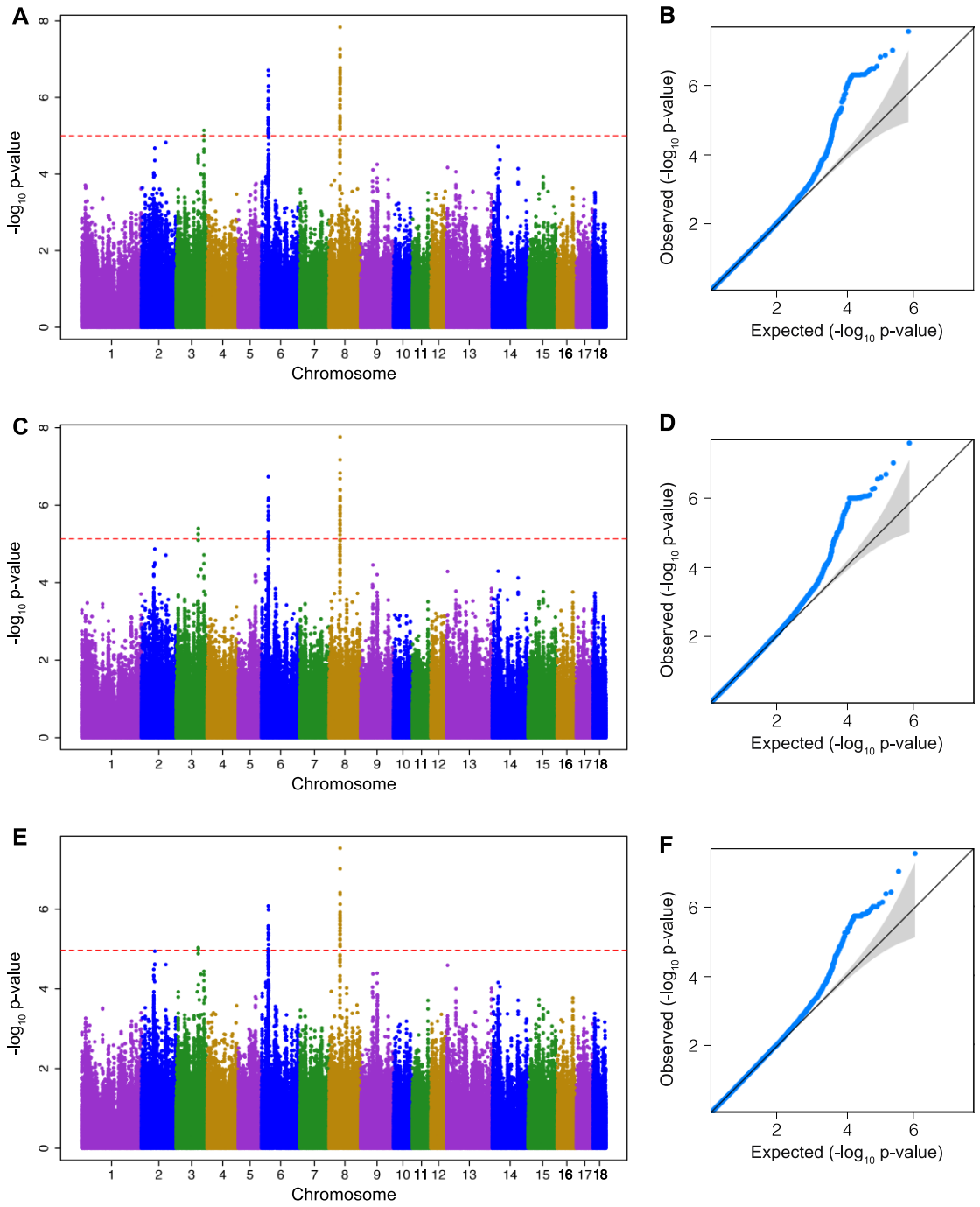

Figure 34. Manhattan plots (A, C, E) and Quantile-Quantile plots (B, D, F) for the red blood cell concentration (RBC) in Blood 1, Blood 3, and Blood 4, respectively.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

Significant SNPs were determined with the genome-wise false discovery rate at 0.05 (the red dashed line in the Manhattan plot). The grey region represents a 95% confidence interval in the Quantile-Quantile plot. Genomic inflation factors (λ) were 1, 1, and 0.99 for RBC in Blood 1, Blood 3, and Blood 4, respectively.

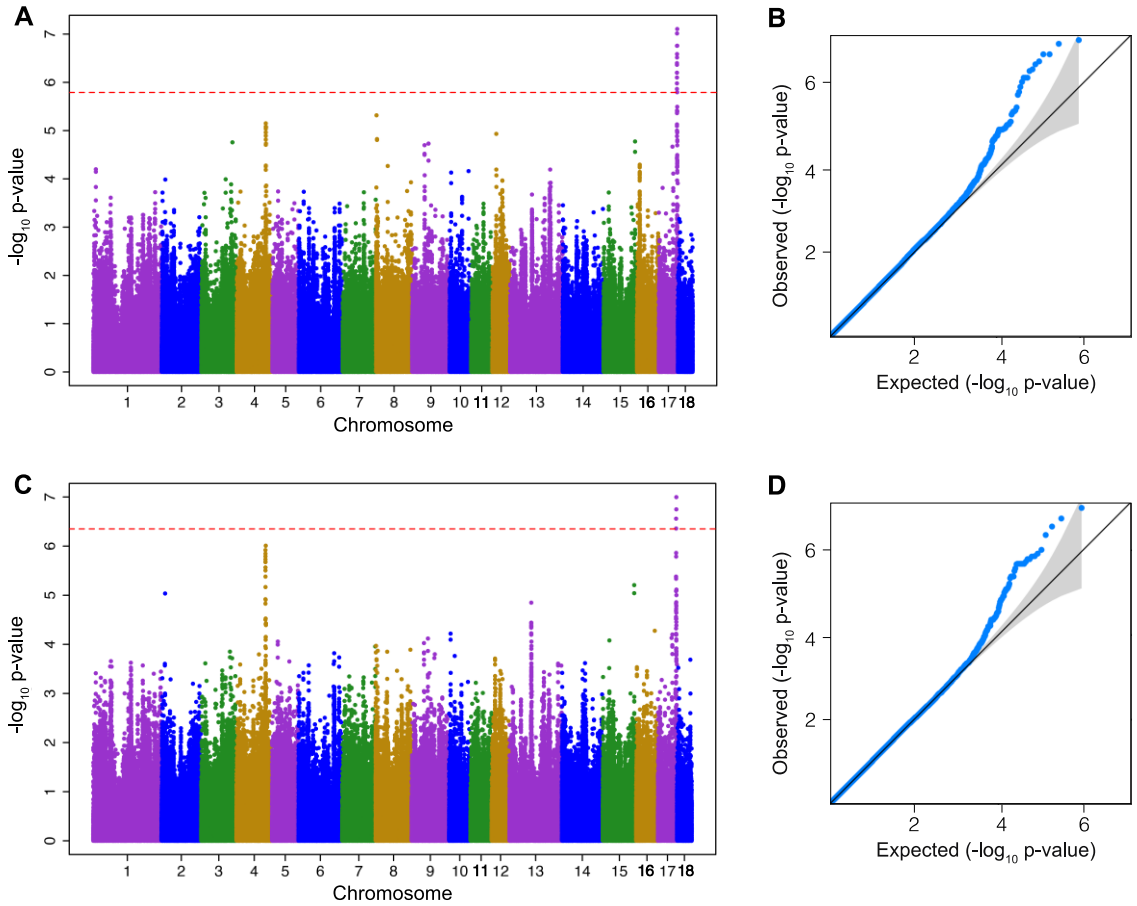

Figure 35. Manhattan plots (A, C) and Quantile-Quantile plots (B, D) for the mean platelet volume (MPV) in Blood 1 and Blood 4, respectively.

Blood 1 and Blood 4 were collected at 2-weeks before, and at 6-weeks after exposure to the challenge, respectively.

Significant SNPs were determined with the genome-wise false discovery rate at 0.05 (the red dashed line in the Manhattan plot). The grey region represents a 95% confidence interval in the Quantile-Quantile plot. Genomic inflation factors (λ) were 1.02 and 1.01 for MPV in Blood 1 and Blood 4, respectively.

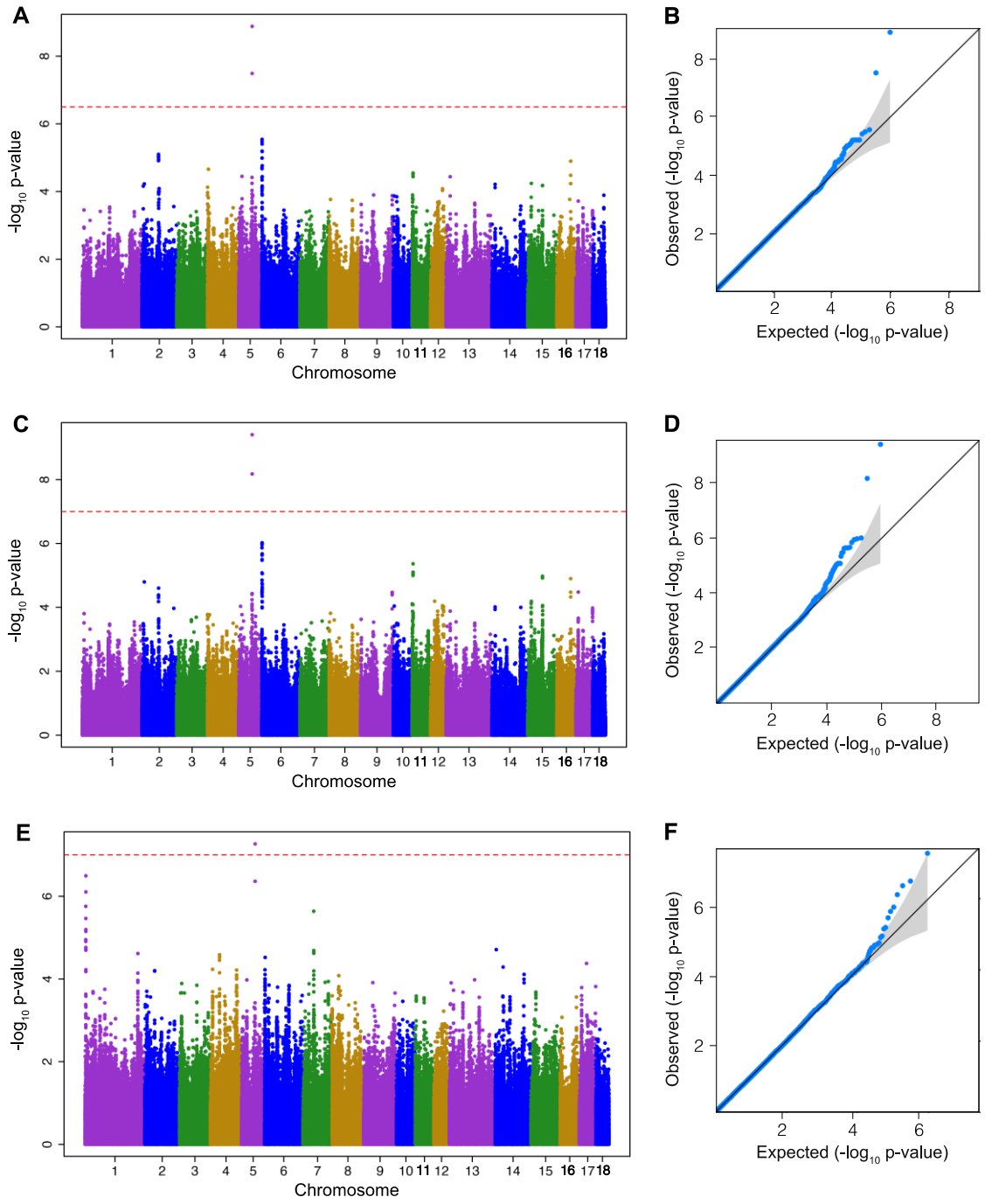

Figure 36. Manhattan plots (A, C, E) and Quantile-Quantile plots (B, D, F) for the platelet concentration (PLT) in Blood 1, Blood 3, and Blood 4, respectively.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

Significant SNPs were determined with the genome-wise false discovery rate at 0.05 (the red dashed line in the Manhattan plot). The grey region represents a 95% confidence interval in the Quantile-Quantile plot. Genomic inflation factors (λ) were 0.99, 1, and 1 for PLT in Blood 1, Blood 3, and Blood 4.

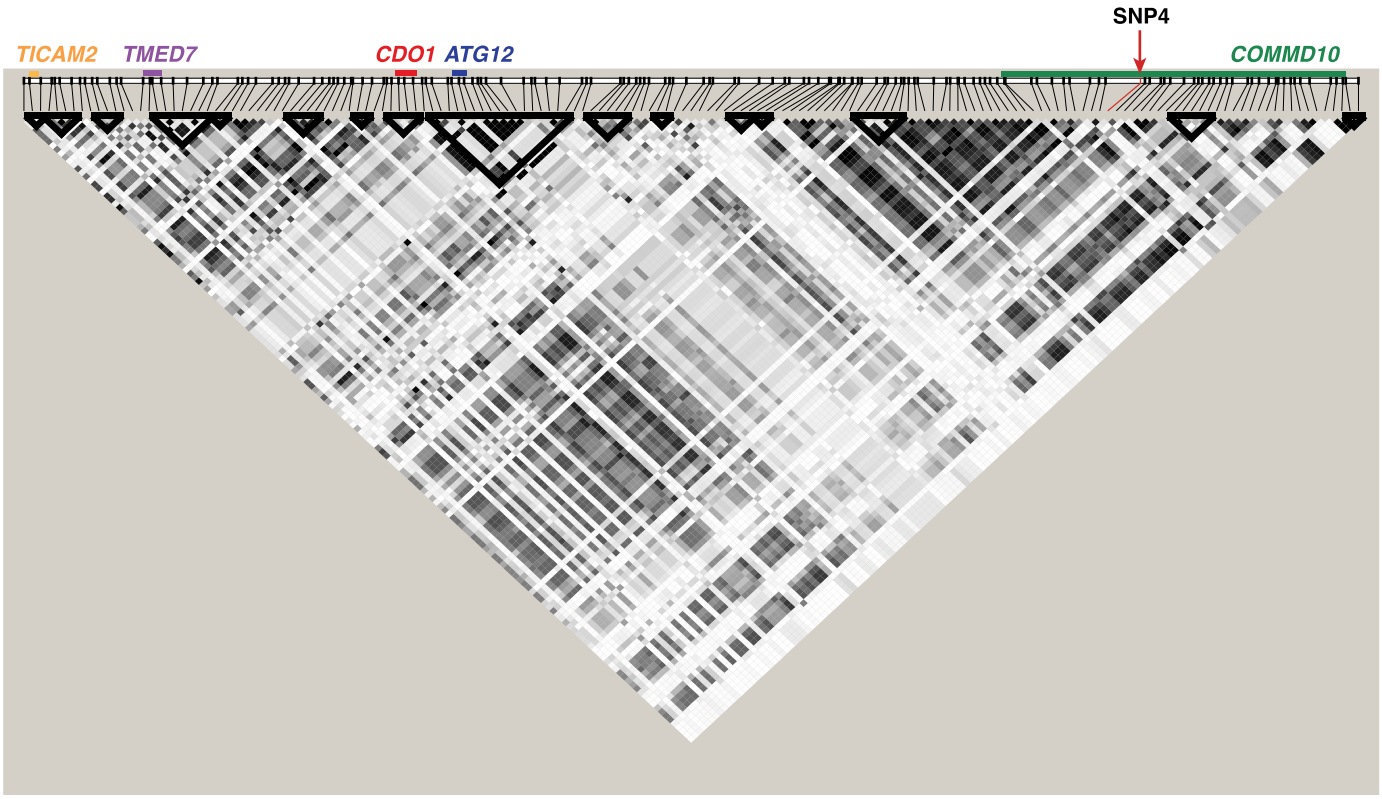

Figure 37. Haplotype block pattern (r^2-scheme) for the region of candidate genes on SSC2.

The region of candidate genes located within the maximum distance of 1-Mb on either side of the top lead SNP4 (SSC2, 120,341,201bp, the most significant SNP at genome-wise false discovery rate < 0.05 with a group of supportive SNPs in this region), based on the linkage disequilibrium (LD, measured as r^2) among the SNPs within this region. The SNPs in each triangle box are suggested to be grouped in one haplotype block based on LD information.

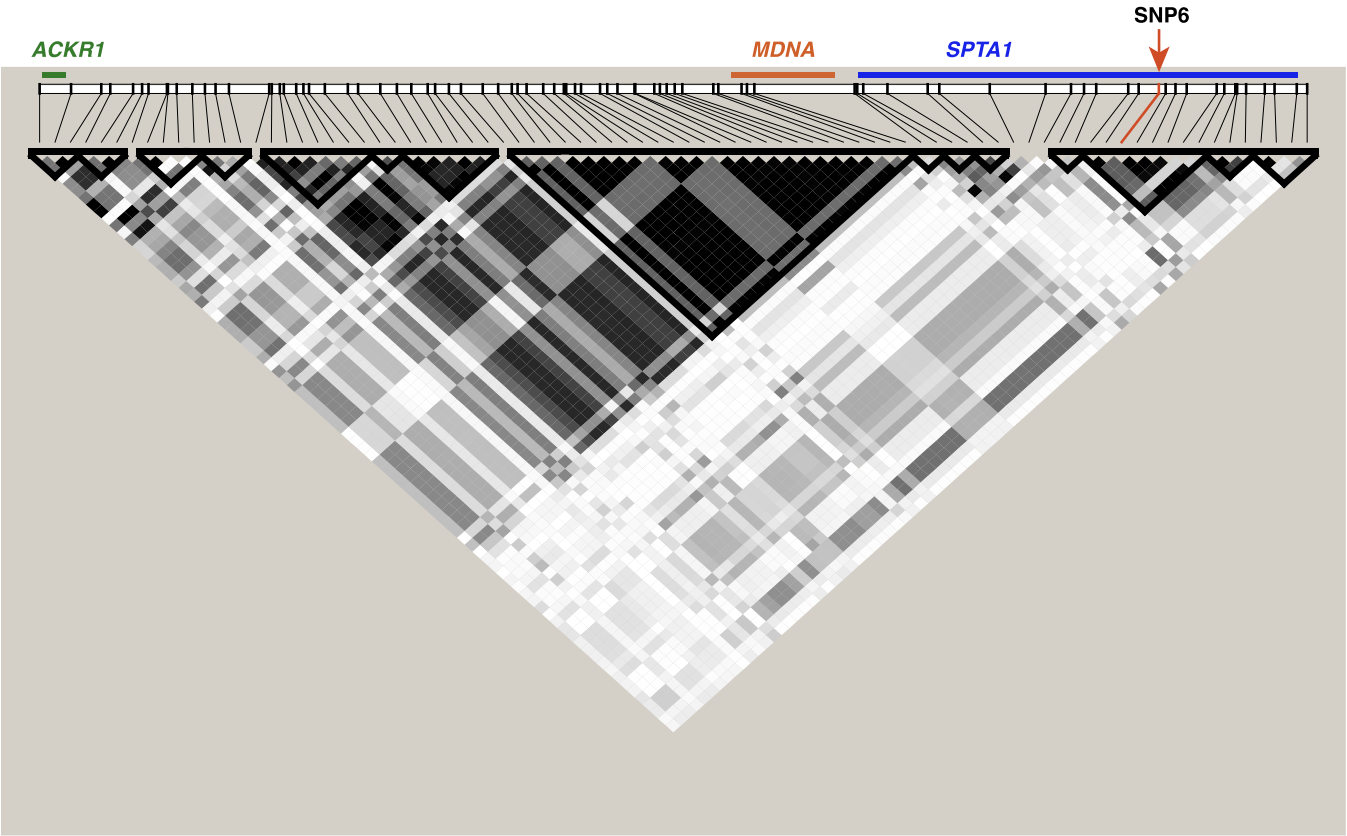

Figure 38. Haplotype block pattern (r^2-scheme) for the region of candidate genes on SSC4.

The region of candidate genes located within the maximum distance of 1-Mb on either side of the top lead SNP6 (SSC4, 91,591,493bp, the most significant SNP at genome-wise false discovery rate < 0.05 with a group of supportive SNPs in this region), based on the linkage disequilibrium (LD, measured as r^2) among the SNPs within this region. The SNPs in each triangle box are suggested to be grouped in one haplotype block based on LD information.

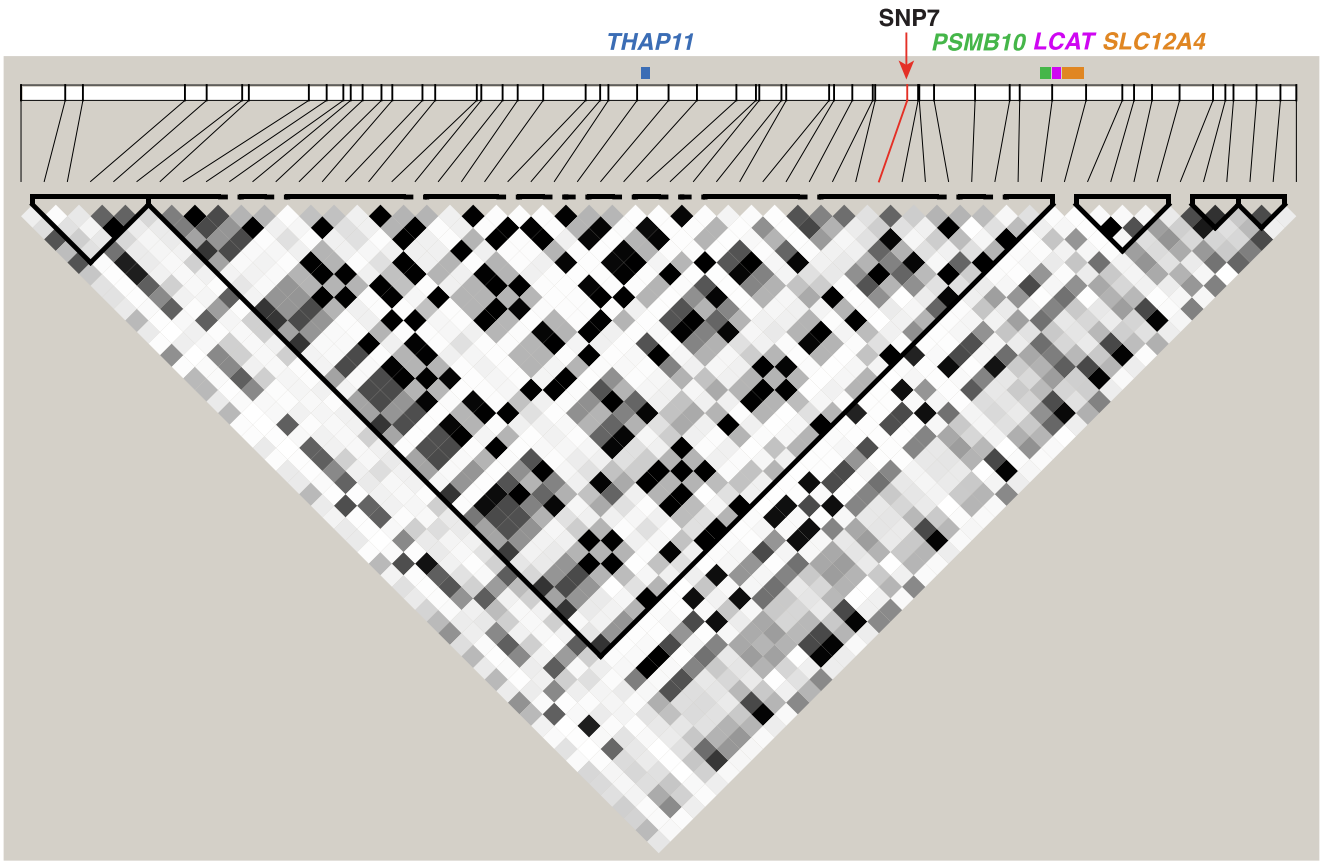

Figure 39. Haplotype block pattern (r^2-scheme) for the region of candidate genes on SSC6.

The region of candidate genes located within the maximum distance of 1-Mb on either side of the top lead SNP7 (SSC6, 28,511,423bp, the most significant SNP at genome-wise false discovery rate < 0.05 with a group of supportive SNPs in this region), based on the linkage disequilibrium (LD, measured as r^2) among the SNPs within this region. The SNPs in each triangle box are suggested to be grouped in one haplotype block based on LD information.

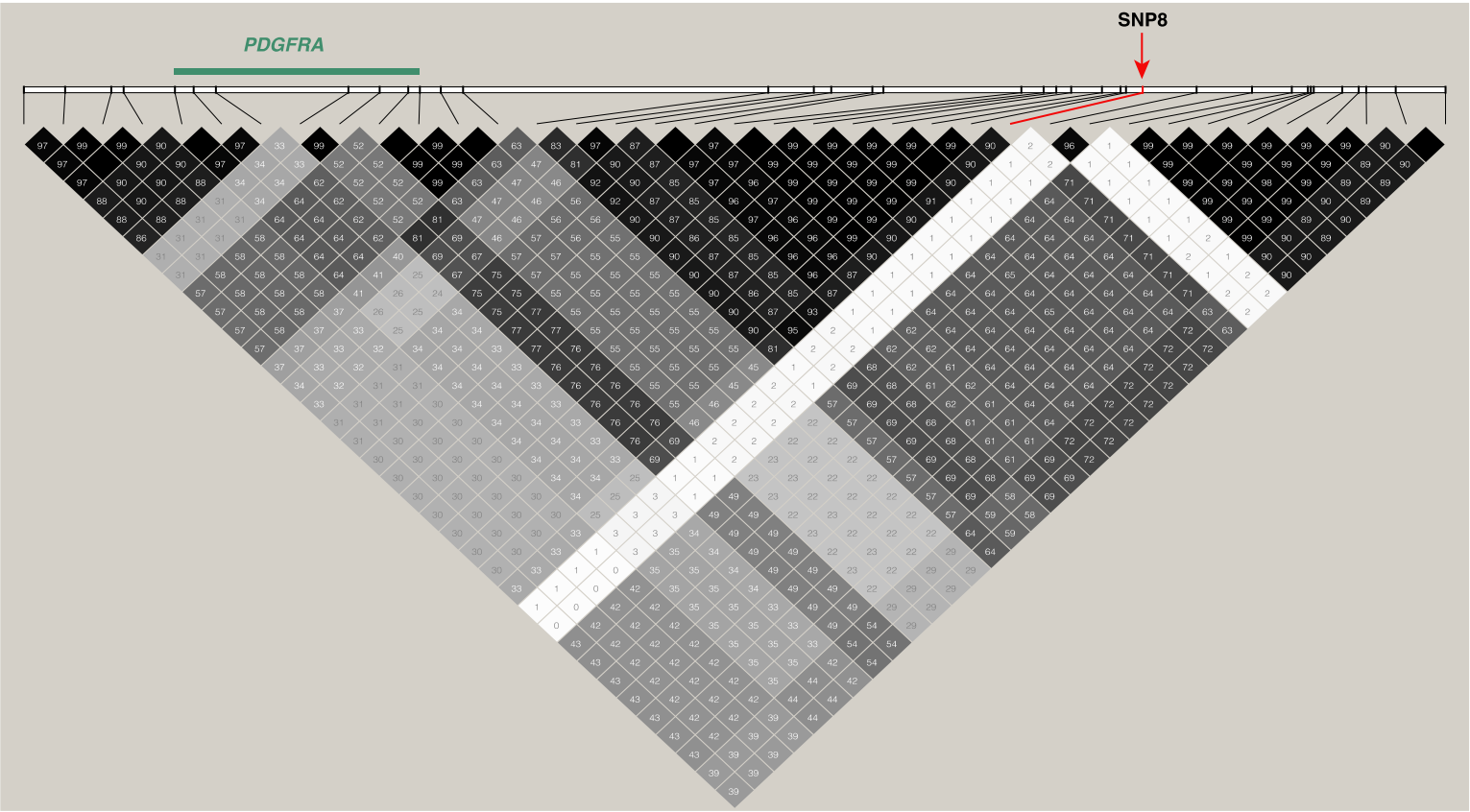

Figure 40. Haplotype block pattern (r^2-scheme) for SNPs (40,946,144bp to 41,198,574bp) on SSC8.

The region of candidate genes located within the maximum distance of 1-Mb on either side of the top lead SNP8 (SSC8, 41,156,538bp, the most significant SNP at genome-wise false discovery rate < 0.05 with a group of supportive SNPs in this region), based on the linkage disequilibrium (LD, measured as r2) among the SNPs within this region.

Chapter 6. Exploration of genes associated with the changes in lymphocyte and neutrophil concentration in response to the polymicrobial infectious challenge

6.1. Introduction

The concentrations of circulating lymphocytes (LYM) and neutrophils (NEU) in blood samples collected at 2-weeks before (Blood 1), and 2- (Blood 3) and 6-weeks (Blood 4) after exposure to the polymicrobial challenge in the natural disease challenge model (NDCM) were quantified using complete blood count (CBC) as described in **Chapter 3**. The changes of LYM from Blood 1 to Blood 3 (LYMΔ13) and NEU from Blood 3 to Blood 4 (NEUΔ34) were found to be heritable (0.11 ± 0.04 for both) and genetically correlated with TR (-0.46 ± 0.24) and GFGR (-0.45 ± 0.21), respectively (**Chapter 4**). Therefore, higher LYMΔ13 but lower NEUΔ34 may favour disease resilience due to the association with the decreased TR and increased GFGR, respectively, in response to the polymicrobial challenge. Accordingly, we hypothesized that resilient animals were primed to initiate a faster immune response with higher LYMΔ13 during the acute stage of infection because lymphocytes are of fundamental importance in the adaptive immune system with specificity and memory to defend against pathogens (LaRosa and Orange, 2008). Due to the dual roles of neutrophils in inflammation, the lower NEUΔ34 associated with disease resilience was hypothesized to be caused by having more neutrophils transported to infected tissues to defend against pathogens or inflammation resolution to re-establish tissue homeostasis and functionality at the later stage of the disease (Jones et al., 2016; Rosales, 2018).

Further investigation of the LYMΔ13 and NEUΔ34 traits may provide a more in-depth insight into the immune mechanisms employed by disease resilient animals in response to polymicrobial infectious challenge. Disappointingly we did not find any major genes associated

with LYMΔ13 and NEUΔ34 in the genome-wide association studies (GWAS) reported in **Chapter 5** and concluded that these traits are likely to be controlled by multiple genes with small effects. RNA-sequencing (RNA-seq) is a widely used method for quantification of gene expression levels in blood samples and has been applied successfully to identify genes involved with pig response to important pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), African swine fever virus, *Salmonella enterica*, and *Mycoplasma suis* (Kommadath et al., 2014, 2017; Schroyen et al., 2016; Wilkinson et al., 2016; Nascimento et al., 2018). As we collected samples for RNA analysis at each timepoint (Blood 1, 3 and 4) of the natural disease challenge model (NDCM), we can use this approach to investigate the genes associated with LYMΔ13 and NEUΔ34 in relation to disease resilience.

Therefore, we selected a subset of samples based on LYMΔ13 and NEUΔ34 for RNA-seq to quantify the gene expression levels at 2- and 6-weeks after exposure to a polymicrobial challenge, with the objectives of 1) exploring genes and their expression patterns associated with the LYMΔ13 and NEUΔ34 in response to the polymicrobial challenge, and 2) investigating the immune mechanisms associated with disease resilience.

6.2. Material and Methods

6.2.1 NDCM and whole blood sample collection

Details of NDCM and whole blood sample collection can be found in **Chapter 3**. Briefly, three sets of whole blood samples (Blood 1, Blood 3, and Blood 4) were drawn from the jugular vein of each pig at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge. Blood 1, Blood 3, and Blood 4 samples were collected into K2 ethylenediaminetetraacetic acid (EDTA) tubes (Applied Biosystems™, United States) and Tempus™ Blood RNA tubes (Applied Biosystems™, United States). Blood samples in EDTA tubes were used for CBC using the

ADVIA®2120i Hematology System (Siemens Healthineers, Germany). A detailed description of CBC traits can be found in **Chapter 3, section 3.3.1**. The samples in Tempus™ Blood RNA tubes were stored at -20 °C for future RNA extraction.

6.2.2 Selection of animals for RNA-Seq analysis

RNA analysis requires that the blood samples were collected and processed correctly to ensure thorough mixing of the sample with the stabilizing reagent to inactivate cellular RNases and precipitate RNA. We found that this was not always the case for the NCDM as indicated by the presence of clots on thawing the tubes. As this is a prerequisite to obtain RNA with sufficient concentration and integrity for RNA-Seq analysis, animals (n = 30) used for this study were selected from three more recent batches (Batch 38, 39, and 42) which were taken after the problem of insufficient mixing was addressed at the barn. Pigs were selected from each batch and classified into two groups (Group 1 and Group 2) based on LYMΔ13 and NEUΔ34. To enlarge the difference between the two groups, animals in Group 1 were required to have first increased LYM level from Blood 1 to Blood 3 then decreased NEU level from Blood 3 to Blood 4, shown as positive LYMΔ13 but negative NEUΔ34. Five animals with the highest LYMΔ13 within each batch were selected for Group 1 (n = 15) among the candidates who met the requirement. Conversely, animals in Group 2 had first decreased LYM from Blood 1 to Blood 3 then increased NEU from Blood 3 to Blood 4, shown as negative LYMΔ13 but positive NEUΔ34. Five animals with the highest NEUΔ34 were selected from each batch for Group 2 (n = 15) among candidates.

6.2.3 RNA preparation, library construction and Illumina sequencing

Blood samples (n = 60) in Tempus™ Blood RNA tubes collected at 2- and 6-weeks after exposure to the challenge from the selected pigs were used for total RNA extraction. Total RNA

from each sample was extracted using the Preserved Blood RNA Purification Kit I (Norgen Biotek Corp, Canada, Cat. 43400) following the manufacturer's instructions. The DNA was removed using the RNase-Free DNase I kit (Norgen Biotek Corp, Canada, Cat. 25710, 25720) following the manufacturer's instructions. Globin reduction of total RNA samples was conducted following the protocol developed in-house using porcine-specific oligonucleotides (Choi et al., 2014). Globin depleted RNA was immediately purified with the RNeasy MinElute Cleanup Kit (Qiagen, Canada, Cat. No.: 74204). The RNA integrity number (RIN) and concentration were measured using the Agilent 2100 Bioanalyzer (Agilent Technologies, United States) and Nanodrop 2000 (Thermo Scientific, United States), respectively. The RIN values ranged from 5.8 to 8.7, and 56 out of 60 samples had RIN values higher than 6.5. The concentrations of RNA samples ranged from 50.82 to 538.20 ng/µl with a volume of 13 µl, but were standardized and diluted with nuclease-free water (Thermo Scientific, United States) to approximately 500 ng in 30 µl. The mRNA libraries were constructed using the NEBNext® Multiplex Oligos for Illumina (New England BioLabs, United States) and pair-end sequenced using the Illumina NovaSeq 6000 S4 PE100 system (Illumina, United States) at Génome Québec (Canada).

6.2.4 RNA-Seq reads processing

The raw RNA-Seq reads were processed using BBDuk (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/) to trim the adaptor sequences and to filter out reads with a length less than 50 bp after trimming. FASTQC 0.11.9 (Andrews, 2010) was used first to check RNA-Seq reads quality before and after trimming. Trimmed reads were aligned to the *Sus scrofa* reference genome sequence (Scrofa 11.1; Warr et al., 2020) using STAR 2.7.6a (Dobin et al., 2013). For gene annotation, we used the GTF file for Scrofa 11.1 from Ensembl version 101 (Yates et al., 2019). The number of reads uniquely mapped to each gene was

determined using STAR 2.7.6a, which coincided with the HTSeq-count with default parameters (Dobin et al., 2013; Dobin, 2020). The Bioconductor (Gentleman et al., 2004) package edgeR (version 3.28.1) (Robinson et al., 2010) was used for further processing of the read counts in R (R core team, 2020). The read counts were normalized to counts per million (CPM), and genes expressed at low levels were removed by only keeping genes that achieve CPM above one in at least 50% of samples. Reads mapped to HBA and HBB were also filtered out in this way as they were expressed in low levels after globin reduction. The remaining read counts were normalized by the trimmed mean of M-values (TMM) to account for compositional differences between libraries. The normalized read counts were adjusted for batch effect using edgeR and transformed using $\log_2(n + 1)$ to obtain scaled expression values for gene co-expression network analysis.

6.2.5 Differential expression analysis

Differential expression (DE) analysis was performed using edgeR by fitting a negative binomial generalized linear model (Robinson et al., 2010). The batch effect was corrected, and DE analysis was conducted for multiple comparisons within each batch. Comparisons between Group 1 and Group 2 within Blood 3 and Blood 4 were used to identify DE genes related to the changes of CBC traits in response to infection. Comparisons between Blood 3 and Blood 4 across and within Group 1 and Group 2 were conducted to explore DE genes associated with the time course of infection. Genes that had at least a 2-fold change ($\log_2FC > 1$) in expression between the groups for comparison, $\log_2CPM > 2$, and a Benjamini-Hochberg correction (Benjamini et al., 1995) of false discovery rate (*FDR*) < 0.05 were recognized as DE genes.

6.2.6 Co-expression network analysis

Gene co-expression network analysis was performed using the R package Weighted Correlation Network Analysis (WGCNA, version 1.69) (Langfelder and Horvath, 2008) to explore the gene co-expression network in Blood 3 and Blood 4 associated with the CBC traits in response to infection. The analysis was conducted on Blood 3 and Blood 4 expression data to explore consensus modules between Blood 3 and Blood 4 using the "automatic module detection (function *blockwiseConsensusModules*)" in WGCNA with the following options: maxBlockSize of 5000, minModuleSize of 30, networkType=" signed", corType= "bicor", and mergeCutHeight of 0.25. The lowest soft threshold power ($\beta = 20$) that resulted in approximate scale-free topology as measured by the scale-free topology fitting index was chosen for the analysis (Langfelder and Horvath, 2008). The modules were tested for their associations by correlation module eigengenes with the quantitative traits of LYMΔ13 and NEUΔ34. LYMΔ13 and NEUΔ34 traits were adjusted for the batch effect using linear regression for association with module eigengenes.

6.2.7 Functional enrichment analysis

Gene Ontology (GO) analysis was performed on DE genes and co-expressed gene modules to explore the biological processes that the genes are involved in. The human ortholog Entrez gene IDs corresponding to the pig Ensembl gene IDs were used in the enrichment test to take advantage of the more complete annotation available for human genes. The Bioconductor package clusterProfiler (version 3.14.3) (Yu et al., 2012) and org.Hs.eg.db (version 3.10.0) (Carlson, 2019) were used for over-representation tests of GO terms in R. The set of all expressed genes expressed in porcine whole blood was selected to be the universal set of genes for GO term enrichment test. The GO terms were ranked based on p-value to explore the candidate genes and immune mechanisms that may be associated with LYMΔ13 and NEUΔ34 in

response to the polymicrobial infectious challenge. The Benjamini-Hochberg correction of *FDR* on p-value was also conducted for the GO terms (Benjamini et al., 1995; Yu et al., 2012).

6.3. Results

6.3.1 Group classification

The group classification of animals based on LYMΔ13 and NEUΔ34 is shown in **Figure 41**. After correcting for the batch effect, the least-square mean (LSM) of LYMΔ13 was significantly higher (*p-value < 0.0001*) in Group 1 (3.28 ± 0.45) compared to Group 2 (-3.32 ± 0.45), and the LSM of NEUΔ34 was significantly lower (*p-value < 0.0001*) in Group 1 (-4.35 ± 0.54) compared to Group 2 (3.41 ± 0.54). The least-square mean of GFGR trended higher in Group 1 than Group 2 (Group1: 0.93 ± 0.03; Group 2: 0.90 ± 0.03, *p-value = 0.51*), and TR trended lower in Group 1 compared to Group 2 (Group1: 0.26 ± 0.13; Group 2: 0.50 ± 0.13, *p-value = 0.20*). Most animals were sourced from different pens and litters (**Table 22**) to avoid any confounding effects for comparing results. Within each batch, the bleeding ages were not found to be significantly different (*p-value > 0.05*) between the two groups based on the t-test results (Batch 38: *p-value = 1*; Batch 39: *p-value = 0.17*; Batch 42: *p-value = 0.47*) as a batch of animals were managed as "all-in-all-out" in the NDCM (detail in **Chapter 3**). Overall, only batch effect was fitted as the fixed effect in the model for DE and gene co-expression analyses since the disease pressure likely varied by batch and pigs from different batches were not exposed to all the same pathogens, which would be the case on a commercial farm. Therefore, the focus was on differences in response between pigs within a batch, which all had the same exposure, rather than on comparison across batches.

6.3.2 DE genes in response to infection

DE analysis between Group 1 and Group 2 was performed separately for Blood 3 and Blood 4 samples. The numbers of DE genes are summarized in **Table 23**. Among 11691 expressed genes, 68 DE genes in Blood 3 samples with 61 up-regulated and 7 down-regulated genes, were found in Group 2 compared to Group 1. However, no DE genes were identified between Group 1 and Group 2 in Blood 4. The GO terms enriched in the DE genes set of each contrast were ranked based on the p-value. The top 10 GO terms enriched in the set of up-regulated DE genes in Group 2 compared to Group 1 within Blood 3 samples are shown in **Table 24**. Genes involved in immune-related GO terms, including negative regulation of NF-kappaB (NF- κB) transcription factor activity and tumor necrosis factor (TNF) -mediated signaling pathway, were up-regulated in Blood 3 samples of Group 2 compared to Group 1.

DE analysis between Blood 3 and Blood 4 was performed separately for Group 1 and Group 2 samples to explore DE genes associated with the time course of infection. In total, 267 DE genes were identified between Blood 3 and Blood 4 samples in Group 1 with 153 up-regulated genes and 114 down-regulated genes in Blood 4 compared to Blood 3 (**Table 23**). For Group 2, 492 DE genes, including 197 up-regulated and 295 down-regulated genes, were identified in Blood 4 compared to Blood 3 (**Table 23**). There were 193 overlapped DE genes (95 up-regulated and 98 down-regulated in Blood 4) identified between the contrast in Group 1 and Group 2. The top 10 GO terms enriched in the set of up-regulated DE genes in Blood 4 compared to Blood 3 in both groups are shown in **Table 25**. Several up-regulated genes in Blood 4 compared to Blood 3 of Group 1 were involved in immune-related GO terms, including regulation of lymphocyte migration, cytoskeleton organization, T cell migration, and cellular response chemokine. The top 10 enriched GO terms in the set of down-regulated DE genes in Blood 4 compared to Blood 3 in both groups are shown in **Table 26**. For the comparison of

Blood 4 to Blood 3 in Group 1, down-regulated genes in Blood 4 were found to be involved in immune-related GO terms of regulation of transforming growth factor-beta (TGF-β) production. For Group 2, genes involved in the GO terms of immune response regulation, negative regulation of immune factors (NF- κB, Interleukin 6) production, and Interleukin-1-mediated signaling pathway were downregulated in Blood 4 compared to Blood 3.

6.3.3 Gene co-expression network associated with LYMΔ13 and NEUΔ34 in response to infection

Gene co-expression modules (clusters of highly positively correlated genes) were labelled in colours by WGCNA. Nine Blood 3-Blood 4 consensus modules were identified between Blood 3 and Blood 4 gene expression profiles, except the grey gene module of all background genes that do not clearly belong to any other modules. The relationships for consensus module eigengenes with LYMΔ13 and NEUΔ34 in gene expression profiles of Blood 3 and Blood 4 are shown in **Figure 42A** and **42B**, respectively. Positive correlations in **Figure 42** indicated that increased and decreased expression levels of module eigengenes associated with the higher and lower levels of the trait, respectively. In contrast, the negative correlations indicated the increased and decreased expression levels of module eigengenes associated with the lower and higher levels of the trait, respectively. Multiple consensus modules, including magenta, turquoise, brown, red, and black consensus modules showed significant positive correlations with LYMΔ13 in the expression profile of Blood 3. Meanwhile, the turquoise, brown, black, and blue consensus modules shown negative correlations with NEUΔ34 in Blood 3, which corresponded to the negative genetic correlation (-0.93 ± 0.36) between LYMΔ13 and NEUΔ34. In addition, the blue consensus module had a negative correlation with LYMΔ13 but a positive correlation with NEUΔ34 in Blood 3. The yellow consensus module was the only one that had a

positive correlation with LYMΔ13 in the expression profile of Blood 4. The green consensus module was the only one that showed significant results in both Blood 3 and Blood 4 expression profiles, including a negative correlation with LYMΔ13 in Blood 3 and a positive correlation with NEUΔ34 in Blood 4.

Several immune-related GO terms were found among the top 10 enriched (p-value ranged from 0.001 to 0.01, FDR ranged from 0.39 to 0.88) GO terms of magenta, red, blue, and green consensus modules (**Figures 43** to **46**). Immune-related GO terms of cytokine production, regulation of T cell mediated immunity, and JUN kinase activity were enriched in the magenta module (**Figure 43**). The GO terms of innate immune response signaling pathways (nucleotide-binding oligomerization domain-like receptors, also known as nucleotide-binding leucine repeat receptors, are types of pattern recognition receptors) and cellular iron homeostasis were enriched in the red module (**Figure 44**). The positive correlations for the magenta and red modules with LYMΔ13 in **Figure 42A** suggested that decreased expression levels of eigengenes in both modules were associated with the lower LYMΔ13 in Blood 3. However, not all genes involved in the top 10 GO terms of the magenta and red modules were found to have lower expression levels in Blood 3 of Group 2 (lower LYMΔ13) than Group 1 (**Figure 43** and **44**). For the blue module, immune-related GO terms of I-kappaB kinase/NF- κB signaling, negative regulation of protein kinase B signaling, and TNF-mediated signaling pathway were enriched (**Figure 45**). The negative correlation for the blue module with LYMΔ13 but positive correlation with NEUΔ34 in **Figure 42A** indicated that higher expression levels of blue module eigengenes in Blood 3 associated with the lower level of LYMΔ13 but higher NEUΔ34. Accordingly, all genes involved in the top 10 enriched GO terms in the blue module were found to have higher expression levels in Blood 3 of Group 2 (lower LYMΔ13 but higher NEUΔ34) than Group 1

(**Figure 45**). Multiple top 10 GO terms enriched in the green module were found to be related to inflammatory and immune responses, including the negative regulation of inflammatory response, regulation of apoptosis (oxidative stress-induced cell death), cellular response to hypoxia (decreased oxygen levels), and myeloid cell (progenitor cell for granulocytes, monocytes, erythrocytes, and platelet) homeostasis (**Figure 46**). For Blood 3, all genes involved in the top 10 GO terms of the green module showed higher expression levels in Group 2 (lower LYMΔ13) than Group 1, except the *TSC2* (Tuberous Sclerosis Complex 2) gene (**Figure 46A**), which corresponded to the negative correlation in **Figure 42A** for the green module with LYMΔ13. And then, all the genes continue to show higher expression levels in Group 2 (higher NEUΔ34) than Group 1 in Blood 4 (**Figure 46B**), corresponding to the positive correlation in **Figure 42B** between the green module and NEUΔ34 as it also suggested higher expression levels to be associated with higher NEUΔ34. The differential gene expression levels measured as $\log_2$(fold change) in Group 2 compared to Group 1 were found to be further enlarged in Blood 4 than Blood 3 for multiple genes (*TSC2, TMEFF2, ENDOG, SVIL, MPL, MIR140, CDK10, EXOSC10, PPM1D, ATF7IP, PGK1, STK25, TGFBR3, STAT5B, FEM1A*) involved in the top 10 GO terms of the green modules. Of note, the above genes in parentheses for the green module were ranked based on the difference of $\log_2$(fold change) in Blood 4 compared to Blood 3, from higher to lower levels ranged from 0.58 to 0.05. In addition to the top 10 enriched GO terms, multiple immune-related GO terms were enriched at the *p-value* of 0.05 in the turquoise, brown, black, and yellow modules (**Table 27**), which also showed significant correlations with LYMΔ13 and or NEUΔ34 in **Figure 42**.

The adjacency and network for module eigengenes are shown in **Figure 47,** where the trait of LYMΔ13 or NEUΔ34 is also included as an eigengene in corresponding plots. The

module eigengenes were only considered to be in high adjacencies (0.6 to 1) and labeled by intense red when they had high positive correlations in WGCNA, indicating the genes were either up-regulated or down-regulated in the same direction. Therefore, the significant but negative correlations for LYMΔ13 with blue and green modules in Blood 3, and NEUΔ34 with turquoise, brown, red and black modules in Blood 3 (**Figure 42A**) were shown as low to zero adjacencies in **Figure 47A** and **C** when the trait was added as an eigengene in the plot. The positive correlations for modules with LYMΔ13 and or NEUΔ34 in **Figure 42** can also be observed in **Figure 47**, shown as high adjacencies and colored by intense red in heatmap plots. The adjacencies between modules were highly conserved between Blood 3 and Blood 4 as the heatmap plots for Blood 3 and Blood 4 were indeed very similar. Of note, the turquoise, brown, black, and red modules were found to have high adjacencies with each other. Also, a high adjacency was identified between the blue and green modules.

6.4. Discussion

Our previous study (**Chapter 4**) has identified negative genetic correlations for LYMΔ13 with GFGR and NEUΔ34 with TR, indicating the higher LYMΔ13 and lower NEUΔ34 may favour disease resilience and result in higher GFGR and lower TR in response to the polymicrobial infection (Bai et al., 2020). Thus, two groups of animals with divergent phenotypes of LYMΔ13 and NEUΔ34 were selected for transcriptome analysis to improve our understanding of disease resilience. Group 1 animals with positive LYMΔ13 but negative NEUΔ34 were expected to be more resilient, shown as higher GFGR and lower TR. Group 2 animals with negative LYMΔ13 but positive NEUΔ34 were expected to be more susceptible to disease challenge, shown as lower GFGR and higher TR. LYM and NEU in Blood 1 (collected at 2-weeks before the challenge) were not found to be correlated with GFGR or TR, and also did

not show any significant differences (*FDR > 0.05*) between animals in different resilient groups (results in **Chapter 4**). Therefore, this study focused on the Blood 3 and Blood 4 samples that were collected after the animals were exposed to the challenge. Blood 3 and Blood 4 samples were collected at 2- and 6-weeks after exposure to the challenge, respectively. Therefore, the Blood 3 timepoint was expected to represent the relatively acute stage of the disease and the Blood 4 timepoint to represent the later stage of infection (Díaz et al., 2005; Reiner et al., 2007; Lunney et al., 2014).

6.4.1 Candidate genes and immune mechanisms associated with LYMΔ13

Through the DE and co-expression network analyses on mRNA-seq data in Blood 3, the up-regulation of *NFKBIA* (NF-kappa-B Inhibitor Alpha) gene was consistently found to be associate with the lower LYM Δ13 (**Table 24** and **Figure 45**). NF- κB inhibitor protein (IκB) encoded by *NFKBIA* is a major down-regulator of NF- κB by retaining inactive NF- κB dimers in the cytosol in unstimulated cells (Baeuerle and Baltimore, 1988). NF- κB is a transcription factor expressed in almost all cell types and tissues (Oeckinghaus and Ghosh, 2009). Many external stimuli lead to activation of NF- κB through signal-induced degradation of IκB proteins (Baldwin, 2001). NF- κB acts as a central mediator of immune and inflammation responses, stress responses, cell proliferation, and apoptosis (programmed cell death) regulation by binding specific promoter elements of DNA and inducing gene transcription (Barkett and Gilmore, 1999; Lentsch and Ward, 1999; Oeckinghaus and Ghosh, 2009). The increased level of IκB associated with the up-regulation of pro-apoptotic signaling has been found in gilts at two days post infected (2 dpi) with PRRSV (Wilkinson et al., 2016), which is also one of the major target pathogens in the NDCM. Accordingly, apoptosis has already been observed mainly in lymphocytes in a number of tissues from PRRSV-infected pigs, including lung, lymph node,

thymus, and reproductive tissues, and the 14 dpi has been indicated as the peak of apoptosis in multiple tissues (Labarque et al., 2003; Karniychuk et al., 2011; Gómez-Laguna et al., 2013; Wang et al., 2014). Besides, acute decrease of total leukocytes and lymphocyte subpopulations, such as B cells, NK cells, T cells, and T helper cells, have also been observed in blood samples collected from PRRSV-infected gilts at 2 dpi, some leukocytes rebounded slowly from 6 dpi but still tended to be lower than in uninfected gilts till 19 dpi (Ladinig et al., 2014). In addition to PRRSV, a significant drop in lymphocytes between 3 and 7 dpi probably due to apoptosis was found in pigs infected with swine influenza virus (Pomorska-Mól et al., 2012), which is also a target pathogen in the NDCM. Although classical swine fever virus (CSFV) is not present in the NDCM, the up-regulation of *NFKBIA* was also observed in pigs during CSFV infection corresponding with severe lymphopenia and apoptosis in thymus, spleen, lymph nodes and bone marrow (Sato et al., 2000; Summerfield et al., 2000, 2001; Shi et al., 2009).

Overall, the lower and even negative LYMΔ13 Group 2 animals in response to the polymicrobial infectious challenge may be associated with pro-apoptosis induced by the up-regulation of *NFKBIA* during the acute phase of infection. In addition to *NFKBIA* gene, the up-regulation of the genes involved in TNF-mediated signaling pathways (*PSMC1, CPNE1, CD70, TRAF1*) and negative regulation of protein kinase B signaling (*BANK1, SFRP5, PLEKHA1*) in Blood 3 were also found to be associated with the lower value for LYMΔ13 (**Table 24** and **Figures 45**). This may further suggest that apoptosis is the cause of low and even negative LYMΔ13 because TNF is known as one of the most potent inducers of apoptosis (Rath and Aggarwal, 1999). In addition, the important role of protein kinase B (PKB/AKT) in the inhibition of apoptosis has been discovered by many studies (Datta et al., 1997; Peso et al., 1997; Erhardt et al., 1999; Majewski et al., 1999; Cross et al., 2000). NF- κB has been known to be a

target for PKB in anti-apoptosis signaling as PKB can activate IκB kinase (IKK- α) that regulates IκB and leads to activation of NF- κB to behave as an antagonist of apoptosis (Romashkova and Makarov, 1999). The suppression of PKB activation has been found to induce cell cycle arrest and apoptosis (Persad et al., 2000). Therefore, the negative regulation of PKB signaling may further indicate the association between apoptosis and decreased LYMΔ13 during the acute phase of the polymicrobial infection. Meanwhile, genes involved in negative regulation of inflammatory response (*RORA, NCOA7, KLF4*) in the green module were also up-regulated in animals with negative LYMΔ13 during the acute phase of infection (**Figure 46A**) when animals were supposed to promote inflammation and initiate immune responses to defend against pathogens.

In contrast, the lower expression level of *NFKBIA* may not induce significant apoptosis in Group 1 animals that had high and increased LYMΔ13. Alternatively, the animals may be able to overcome the apoptosis rapidly. Thus, the immune responses were initiated and activated during the acute stage of infection, shown as positive correlations between the higher LYMΔ13 and the up-regulation of genes involved in the activation of immune responses, such as complement cascade, cell population proliferation, and humoral immune response (**Table 27** and **Figure 42**). The negative genetic correlation (-0.46 ± 0.24) between LYMΔ13 and resilience trait of TR (Bai et al., 2020), suggested that animals with higher LYMΔ13 may overcome apoptosis and initiate proper immune responses during the acute phase of infection and tended to be more resilient with lower TR.

6.4.2 Candidate genes and immune mechanisms associated with NEUΔ34

According to the negative genetic correlation (-0.45 ± 0.21) between NEUΔ34 and the disease resilience trait GFGR (Bai et al., 2020), different immune mechanisms have been

proposed to be associated with the negative NEUΔ34 and disease resilience. On the one hand, negative NEUΔ34 was expected to indicate the resolution of inflammation and convalescence during the later stage of infectious disease in resilient animals when pathogens have been brought under control by high LYMΔ13. On the other hand, negative NEUΔ34 may also reflect that circulating neutrophils have already been transported to the infected tissues to defend against pathogens rather than inflammation resolution.

The up-regulation of genes involved in T lymphocyte migration, cellular response to chemokine, and cytoskeleton organization in Blood 4 collected at the later stage of infection was associated with the negative NEUΔ34 (**Table 25, Blood 4 vs Blood 3 in Group 1**). Of note, the expression of *CCR8* in peripheral blood has been suggested to participate in the induction and amplification phase of inflammatory responses to pathogens (Soler et al., 2006). The CCR8 receptor encoded by *CCR8* is a chemokine receptor involved in recruiting leukocytes to inflamed tissues (Qu et al., 2004; Soler et al., 2006; Sokol et al., 2018). However, the effect of CCR8 on neutrophil migration was only found in a study of murine allergic enteritis (Blanco-Pérez et al., 2019). Contrary to our results, Blanco-Pérez et al. (2019) suggested that CCR8 deficiency may promote neutrophil recruitment and accumulation in the inflamed intestinal tissues. Since the activation, polarization, migration, and phagocytosis of neutrophils are all driven by cytoskeletal remodeling (Fenteany and Glogauer, 2004), the up-regulation of genes involved in cytoskeleton organization in Blood 4 of animals with negative NEUΔ34 (Group 1) compared to Blood 3 may support the hypothesis that negative NEUΔ34 may be caused by the migration of neutrophils to the infected tissue.

The DE analysis found that down-regulation of *CD200* (Cluster of Differentiation 200) and *SERPINF2* (Alpha 2-antiplasmin) were associated with negative NEUΔ34 (**Table 26, Blood**

4 vs Blood 3 in Group 1). The down-regulation of *SERPINF2* may be of particular interest here and support the hypothesis that neutrophils are being transported to the infected tissue to defend against pathogens. This is because the α2-antiplasmin encoded by *SERPINF2* is known as the primary physiological inhibitor of elastase (Kolev et al., 1994; Reyes-García et al., 2019). Neutrophils secrete elastase during inflammation, which is an important component in forming neutrophil extracellular traps to kill extracellular pathogens at the infection site (Brinkmann et al., 2004; Thomas et al., 2014). The CD200 encoded by *CD200* gene is a membrane protein expressed by a broad range of cell types, however, the CD200 receptor (CD200R) is expressed almost exclusively by myeloid cells, including neutrophils, macrophages, dendritic cells, basophils and mast cells (Barclay et al., 2002; Snelgrove et al., 2008). The CD200 is thought to deliver a strong "off" signal to myeloid cells to inhibit myeloid cell pro-inflammatory activation through the CD200-CD200R interaction (Nathan and Muller, 2001; Barclay et al., 2002; Minas and Liversidge, 2006). The down-regulation of *CD200* may indicate the pro-inflammation is still taking place. The biological function of the CD200-CD200R interaction has also been proposed to modulate myeloid cell functions during inflammation, such as proliferation, survival, trafficking, migration and secretion (Nathan and Muller, 2001), but no substantial evidence has been identified yet. Therefore, the association between down-regulation of *CD200* and negative NEUΔ34 needs to be further explored.

In contrast to the negative NEUΔ34 in Group 1 following the positive LYMΔ13, animals in Group 2 had a positive NEUΔ34 following the negative LYMΔ13. A rebound neutrophil concentration from 19 dpi was also observed in the previous study of PRRSV-infected gilts that showed a significant drop of leukocyte levels from 2 to 19 dpi (Ladinig et al., 2014). The up-regulation of multiple genes in the green module (*STAT5B, TGFBR3, MPL, EPAS1*) involved in

myeloid cell homeostasis, the process of regulating the proliferation and elimination of myeloid cells, was found to be positively correlated with the higher level of NEUΔ34 (**Figure 46B**). Meanwhile, the expression levels of these genes in Group 2 were further up-regulated in Blood 4 compared to Group 1. Genes involved in the negative regulations of NF- κB transcription factor activity and interleukin-6 (IL-6) production were found to be down-regulated in Blood 4 compared to Blood 3 of Group 2 (**Table 26, Blood 4 vs Blood 3 in Group 2**), indicating the activation of NF- κB and increased production of IL-6 may be associated with the positive NEUΔ34. As discussed above, NF- κB can be activated in response to infection and acts as a central regulator of multiple aspects of immune functions and a mediator of inflammation responses (Liu et al., 2017). The activation of NF- κB was also associated with the degradation of IκB proteins, which can regulate the apoptosis induced by the up-regulation of the *NFKBIA* gene (encodes IκB) in Blood 3 (Liu et al., 2017). In addition to the role of pro-inflammatory cytokine, IL-6 has also been found to be responsible for delaying neutrophil and apoptosis and resulting in a large population of surviving neutrophils (Biffl et al., 1996). Overall, it suggests that polymicrobial challenge-induced apoptosis may be mitigated in Group 2 and immune responses were finally evoked during the later stage of infection to defend against pathogens. However, a negative genetic correlation was observed between GFGR and NEUΔ34 (-0.45 ± 0.21) (Bai et al., 2020), indicating decreased disease resilience associated with higher NEUΔ34, probably due to the late induction of immune responses following the apoptosis induced by the polymicrobial challenge.

6.5. Conclusion

In conclusion, this study integrates differential expression analysis and gene co-expression network analysis to improve our understanding of LYMΔ13 and NEUΔ34 in

response to the polymicrobial challenge. It also reveals candidate genes and immune mechanisms that may be associated with disease resilience. The results suggest that apoptosis was responsible for negative LYMΔ13, which may be associated with PRRSV and swine influenza virus infection. Apoptosis during the acute stage of infection would reduce the concentrations of leukocytes in multiple tissues and blood, especially lymphocytes, resulting in impaired immune responses and increasing disease susceptibility. Although the apoptosis may be controlled and immune responses were evoked at the later stage of infection, shown as positive NEUΔ34, animal health and disease resilience may have already been impaired in these animals. In contrast, animals with positive LYMΔ13 were expected to overcome apoptosis and initiate immune responses successfully during the acute stage of infection to support disease resilience. The results here do not help determine whether the negative NEUΔ34 in blood samples (following positive LYMΔ13) were caused by inflammation resolution or migration of neutrophils to the infected tissues to further support disease resilience. Our interpretation of the results will need to be investigated further using more robust methods, such as single-cell sequencing, which could provide a higher resolution of cellular differences and a better understanding of the function of each individual cell in response to the polymicrobial challenge.

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Table 22. The levels of pen and litter effects for the selected animals.

Animals	Levels[1]			
	Pen1[2]	**Pen2**[3]	**Pen3**[4]	**Litter**
All selected animals (n = 30)	21	21	13	24
Animals in Group 1[5] (n = 15)	12	12	11	13
Animals in Group 2[6] (n = 15)	11	11	9	15

[1]The number of unique levels for each factor.

[2]The pen arrangement in the healthy quarantine unit.

[3]The pen arrangement in the challenge late nursery.

[4]The pen arrangement in the challenge grow-to-finish stage.

[5]A group of animals with top five levels of the change of lymphocyte concentration from Blood 1 to Blood 3 (LYMΔ13) among animals with positive LYMΔ13 but the negative change of neutrophil concentration from Blood 3 to Blood 4 (NEUΔ34) of each batch.

[6]A group of animals with top five levels of NEUΔ34 among animals with negative LYMΔ13 but positive NEUΔ34 of each batch.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

Table 23. Numbers of differentially expressed genes[1].

Comparison	Total[2]	Up[3]	Down[4]
Group 2 (n = 15) vs Group 1[5] (n = 15) in Blood 3[7]	68	61	7
Group 2[6] (n = 15) vs Group 1 (n = 15) in Blood 4[8]	0	0	0
Blood 4 (n = 15) vs Blood 3 (n = 15) in Group 1	267	153	114
Blood 4 (n = 15) vs Blood 3 (n = 15) in Group 2	492	197	295

[1]Differentially expressed genes that had at least a 2-fold change ($log_2FC > 1$) in expression between the groups for comparison, $log_2CPM > 2$, and false discovery rate using the Benjamini-Hochberg correction (*FDR*) < 0.05.

[2]The count of differentially expressed genes.

[3]The count of differentially expressed genes that were being up-regulated.

[4]The count of differentially expressed genes that were being down-regulated.

[5]A group of animals with top five levels of the change of lymphocyte concentration from Blood 1 to Blood 3 (LYMΔ13) among animals with positive LYMΔ13 but the negative change of neutrophil concentration from Blood 3 to Blood 4 (NEUΔ34) of each batch.

[6]A group of animals with top five levels of NEUΔ34 among animals with negative LYMΔ13 but positive NEUΔ34 of each batch.

[7]Blood samples collected at 2-weeks after exposure to the challenge.

[8]Blood samples collected at 6-weeks after exposure to the challenge.

Table 24. List of top 10 enriched Gene Ontology (GO) terms in biological processes in the set of up-regulated differentially expressed (DE) genes in Group 2 compared to Group 1 within Blood 3 samples. The GO terms were ranked based on p-value.

GO ID	Description	p-value	FDR[1]	Up-regulated DE genes
GO:0044772	Mitotic cell cycle phase transition	0.0002	0.14	*LSM10/ACVR1/PSMC1/MIR16-1/CHMP7/GFI1*
GO:0044770	Cell cycle phase transition	0.0004	0.14	*LSM10/ACVR1/PSMC1/MIR16-1/CHMP7/GFI1*
GO:1903047	Mitotic cell cycle process	0.0004	0.14	*LSM10/INTS13/ACVR1/PSMC1/MIR16-1/CHMP7/GFI1*
GO:0000082	G1/S transition of mitotic cell cycle	0.0006	0.14	*LSM10/ACVR1/MIR16-1/GFI1*
GO:0032088	Negative regulation of NF-kappaB transcription factor activity	0.0006	0.14	*NFKBIA/MIR16-1/GFI1*
GO:0044843	Cell cycle G1/S phase transition	0.0007	0.14	*LSM10/ACVR1/MIR16-1/GFI1*
GO:0001667	Ameboidal-type cell migration	0.0008	0.14	*GNA12/ACVR1C/EFNA1/ACVR1/MIR16-1*
GO:0000278	Mitotic cell cycle	0.001	0.16	*LSM10/INTS13/ACVR1/PSMC1/MIR16-1/CHMP7/GFI1*
GO:0060317	Cardiac epithelial to mesenchymal transition	0.001	0.16	*EFNA1/ACVR1*
GO:0033209	Tumor necrosis factor-mediated signaling pathway	0.002	0.18	*NFKBIA/PSMC1/CPNE1*

[1]Benjamini-Hochberg correction of false discovery rate (FDR).

Table 25. List of top 10 enriched GO terms in biological processes in the set of up-regulated differentially expressed (DE) genes in Blood 4 compared to Blood 3 within Group 1 and Group 2. The GO terms were ranked based on p-value.

GO ID	Description	p-value	FDR[1]	Up-regulated DE genes
Blood 4 vs Blood 3 in Group 1				
GO:2000401	Regulation of lymphocyte migration	0.002	0.66	*CRK/AIRE/CCL3*
GO:0007010	Cytoskeleton organization	0.003	0.66	*DOCK2/CEP57/ATP8A2/MYLK3/LARP4/CRK/AKAP9/SPAG16/ARHGAP6/CDK1/CORO1C/CCL3/CAPN10/MIR138-1*
GO:0072678	T cell migration	0.004	0.66	*CRK/AIRE/CCL3*
GO:1990868	Response to chemokine	0.005	0.66	*DUSP1/CCR8/CCL3*
GO:1990869	Cellular response to chemokine	0.005	0.66	*DUSP1/CCR8/CCL3*
GO:0042446	Hormone biosynthetic process	0.006	0.66	*CACNA1H/CHST8/HSD11B1*
GO:0043087	Regulation of GTPase activity	0.006	0.66	*DOCK2/ADPRH/FICD/CRK/ARHGAP6/CORO1C/CCL3*
GO:0044782	Cilium organization	0.007	0.66	*CEP57/BBS7/AKAP9/SPAG16/CDK1/LCA5L*
GO:0042775	Mitochondrial ATP synthesis coupled electron transport	0.008	0.66	*NDUFB1/CDK1/UQCRFS1*
GO:0043547	Positive regulation of GTPase activity	0.008	0.66	*DOCK2/ADPRH/CRK/ARHGAP6/CORO1C/CCL3*
Blood 4 vs Blood 3 in Group 2				
GO:0042775	Mitochondrial ATP synthesis coupled electron transport	0.002	0.75	*UQCRH/UQCRFS1/NDUFB1/CDK1*
GO:0044782	Cilium organization	0.002	0.75	*WDR11/BBS7/IFT57/CEP57/AKAP9/SPAG16/LCA5L/CDK1*
GO:0042773	ATP synthesis coupled electron transport	0.002	0.75	*UQCRH/UQCRFS1/NDUFB1/CDK1*
GO:0030010	Establishment of cell polarity	0.003	0.75	*DOCK2/KIF20B/FSCN1/WWC1/CRK*
GO:0007389	Pattern specification process	0.003	0.75	*HES7/EMX2/PGAP1/GDF3/AP1B1/LRP4/BBS7/IFT57/NEUROG1*
GO:0006119	Oxidative phosphorylation	0.003	0.75	*UQCRH/ATP5ME/UQCRFS1/NDUFB1/CDK1*
GO:0043113	Receptor clustering	0.003	0.75	*LRP4/ITGAL/PTN*
GO:0022904	Respiratory electron transport chain	0.004	0.75	*UQCRH/UQCRFS1/NDUFB1/CDK1*
GO:0015980	Energy derivation by oxidation of organic compounds	0.004	0.75	*UQCRH/ENPP1/UQCRFS1/NDUFB1/MDH1/GCGR/CDK1*

[1]Benjamini-Hochberg correction of false discovery rate (FDR).

Table 26. List of top 10 enriched GO terms in biological processes in the set of down-regulated differentially expressed (DE) genes in Blood 4 compared to Blood 3 within Group 1 and Group 2. The GO terms were ranked based on p-value.

GO ID	Description	*p-value*	*FDR*[1]	Down-regulated DE genes
Blood 4 vs Blood 3 in Group 1				
GO:0006305	DNA alkylation	0.002	0.73	*FOS/GNAS/ATF7IP*
GO:0006306	DNA methylation	0.002	0.73	*FOS/GNAS/ATF7IP*
GO:0044728	DNA methylation or demethylation	0.003	0.73	*FOS/GNAS/ATF7IP*
GO:0035137	Hindlimb morphogenesis	0.006	0.73	*SHH/GNAS*
GO:0042572	Retinol metabolic process	0.008	0.73	*ALDH1A3/TTR*
GO:0071604	Transforming growth factor beta production[2]	0.008	0.73	*CD200/SERPINF2*
GO:0071634	Regulation of transforming growth factor beta production	0.008	0.73	*CD200/SERPINF2*
GO:0006304	DNA modification	0.008	0.73	*FOS/GNAS/ATF7IP*
GO:0060349	Bone morphogenesis	0.008	0.73	*CDX1/PHOSPHO1/GNAS*
GO:0034754	Cellular hormone metabolic process	0.009	0.73	*SHH/ALDH1A3/TTR*
Blood 4 vs Blood 3 in Group 2				
GO:0032088	Negative regulation of NF-kappaB transcription factor activity	0.002	0.80	*CD200/NFKBIA/IRAK2/GFI1/MIR16-1*
GO:0003143	Embryonic heart tube morphogenesis	0.003	0.80	*SHH/ACVR1/IFT172/SETDB2*
GO:0035050	Embryonic heart tube development	0.004	0.80	*SHH/ACVR1/IFT172/SETDB2*
GO:0050776	Regulation of immune response	0.005	0.80	*CD200/FOS/NFKBIA/SPON2/HLA-DMB/IRAK2/PSMC1/IL12RB1/CD300LD/EIF2B5/RBM14/TRAFD1/IFNGR2/PLEKHA1/GFI1/PSMA7*
GO:0050709	Negative regulation of protein secretion	0.005	0.80	*CD200/RAB11FIP5/ACVR1C/BANK1/RHBDF1*
GO:0032924	Activin receptor signaling pathway	0.005	0.80	*SHH/ACVR1C/ACVR1*
GO:0046661	Male sex differentiation	0.005	0.80	*SHH/BRIP1/ING2/PLEKHA1/NCOA4*
GO:0032259	Methylation	0.005	0.80	*FOS/GNAS/ATPSCKMT/PRDM13/SPOUT1/GFI1/SETDB2/ATF7IP*
GO:0070498	Interleukin-1-mediated signaling pathway	0.006	0.80	*NFKBIA/IRAK2/PSMC1/PSMA7*
GO:0032715	Negative regulation of interleukin-6 production	0.006	0.80	*CD200/BANK1/SLAMF1*

[1] Benjamini-Hochberg correction of false discovery rate (*FDR*) on *p-values*.

Table 27. List of immune-related GO terms enriched at a *p-value < 0.05* in the turquoise, brown, black and yellow consensus modules of clusters of highly positively correlated genes.

Module	GO ID	Description	*p-value*	*FDR*[1]
Turquoise	GO:0006956	Complement activation	0.009	1
	GO:1904892	Regulation of receptor signaling pathway via STAT	0.02	1
	GO:0050854	Regulation of antigen receptor-mediated signaling pathway	0.02	1
	GO:0002684	Positive regulation of immune system process	0.03	1
	GO:0002455	Humoral immune response mediated by circulating immunoglobulin	0.03	1
	GO:0046635	Positive regulation of alpha-beta T cell activation	0.03	1
	GO:0046425	Regulation of receptor signaling pathway via JAK-STAT	0.04	1
	GO:0002253	Activation of immune response	0.04	1
Brown	GO:2000377	Regulation of reactive oxygen species metabolic process	0.01	0.84
	GO:0008284	Positive regulation of cell population proliferation	0.01	0.84
	GO:0045739	Positive regulation of DNA repair	0.01	0.84
	GO:0097190	Apoptotic signaling pathway	0.03	0.84
Black	GO:0007249	I-kappaB kinase/NF-kappaB signaling	0.01	0.91
	GO:0033209	Tumor necrosis factor-mediated signaling pathway	0.01	0.91
	GO:0016236	Macroautophagy	0.02	0.91
	GO:0046330	Positive regulation of JNK cascade	0.02	0.91
	GO:0046006	Regulation of activated T cell proliferation	0.04	0.91
	GO:2000107	Negative regulation of leukocyte apoptotic process	0.04	0.91
Yellow	GO:0051897	Positive regulation of protein kinase B signaling	0.02	0.82

[1]Benjamini-Hochberg correction of false discovery rate (*FDR*) on *p-values*.

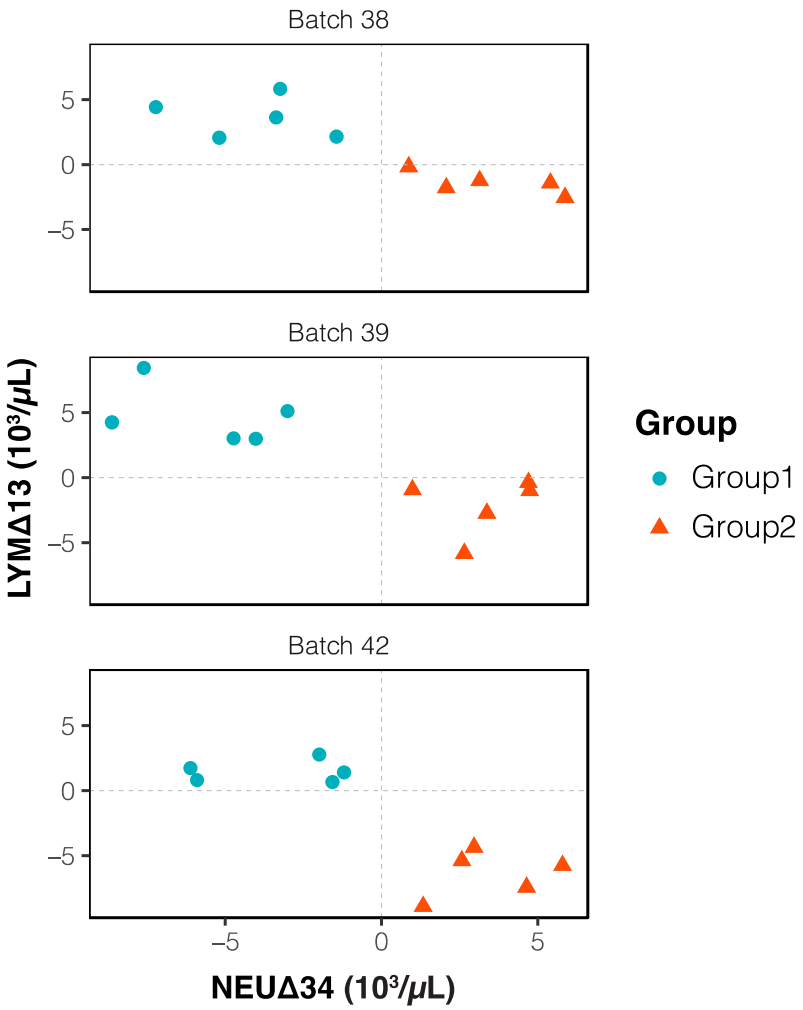

Figure 41. The group classification of animals selected from Batch 38, 39, and 42 based on LYMΔ13 and NEUΔ34.

LYMΔ13 represents for the change of lymphocyte concentration from Blood 1 to Blood 3.

NEUΔ34 represents for the change of neutrophil concentration from Blood 3 to Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

Group 1: a group of animals with top five levels of LYMΔ13 among animals with positive LYMΔ13 but negative NEUΔ34 of each batch.

Group 2: a group of animals with top five levels of NEUΔ34 among animals with negative LYMΔ13 but positive NEUΔ34 of each batch.

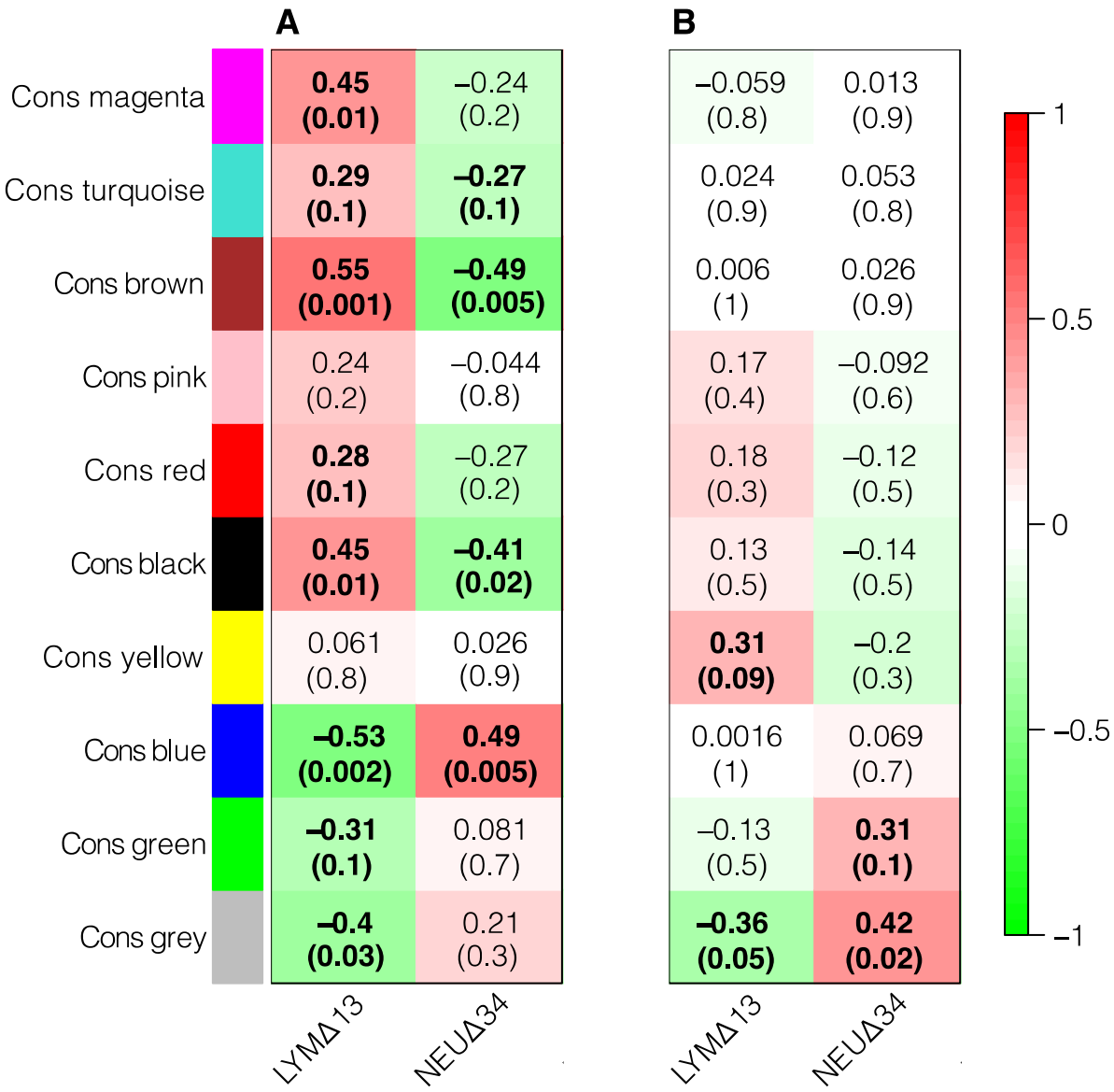

Figure 42. Relationships of consensus module eigengenes and traits in gene expression profiles of Blood 3 (A) and Blood 4 (B).

LYMΔ13 represents the change of lymphocyte concentration from Blood 1 to Blood 3. NEUΔ34 represents the change of neutrophil concentration from Blood 3 to Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively.

Numbers in each block report the correlations of the corresponding module eigengenes and traits, with the p-values printed below the correlations in parentheses. The block is color-coded by correlation according to the color legend.

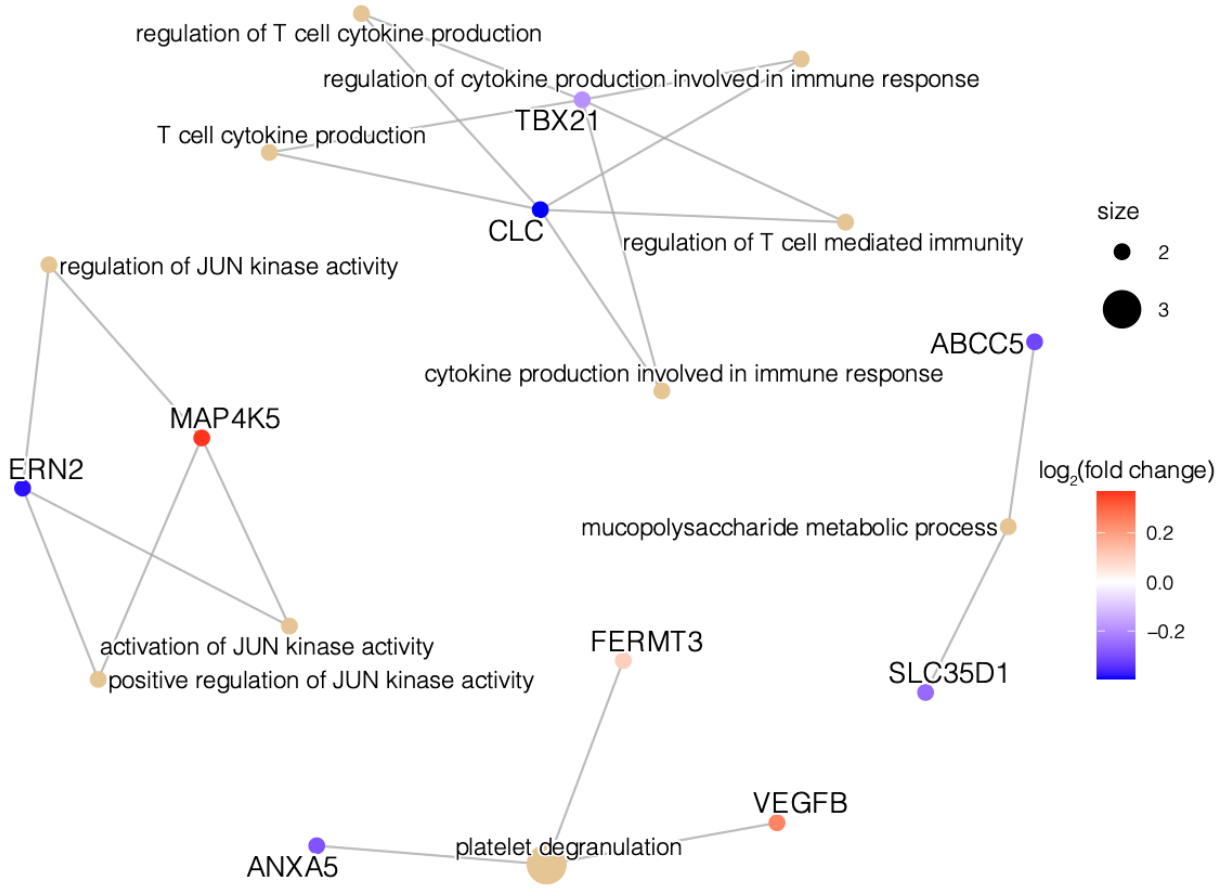

Figure 43. Network plot of top 10 enriched GO terms in the magenta module.

The p-value ranged from 0.001 to 0.01 and the FDR (Benjamini and Hochberg correction) was 0.39 for the top 10 enriched GO terms. The $\log_2$(fold change) indicates the different expression level of the gene in Group 2 compared to Group 1 in Blood 3. The size indicated the number of genes in the module associated with the GO term.

Group 1: a group of animals with top five levels of LYMΔ13 among animals with positive LYMΔ13 but negative NEUΔ34 of each batch.

Group 2: a group of animals with top five levels of NEUΔ34 among animals with negative LYMΔ13 but positive NEUΔ34 of each batch.

Blood 3 samples were collected at 2-weeks after exposure to the challenge.

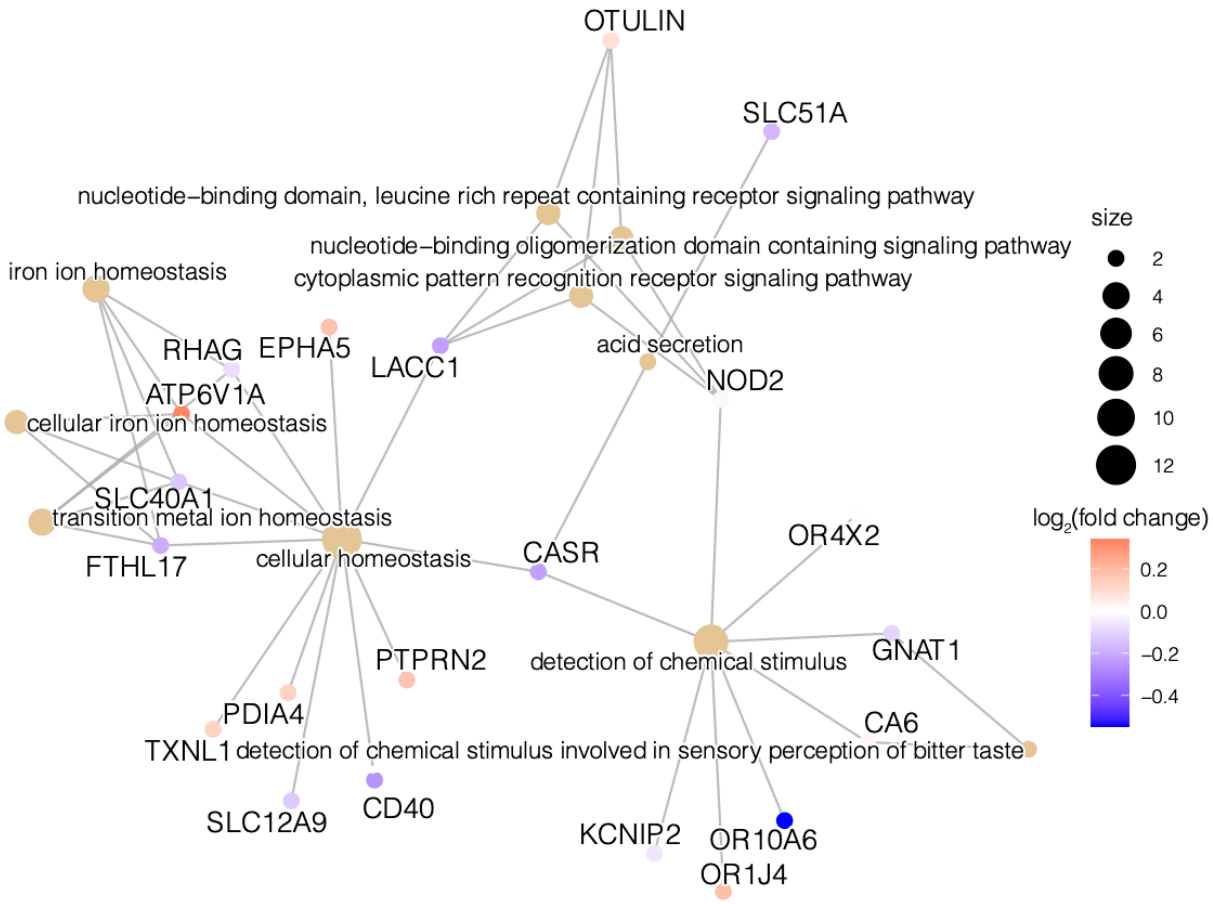

Figure 44. Network plot of top 10 enriched GO terms in the red module.

The p-value ranged from 0.002 to 0.01 and the FDR (Benjamini and Hochberg correction) was 0.87 for the top 10 enriched GO terms. The $\log_2$(fold change) indicates the different expression level of the gene in Group 2 compared to Group 1 in Blood 3. The size indicated the number of genes in the module associated with the GO term.

Group 1: a group of animals with top five levels of LYMΔ13 among animals with positive LYMΔ13 but negative NEUΔ34 of each batch.

Group 2: a group of animals with top five levels of NEUΔ34 among animals with negative LYMΔ13 but positive NEUΔ34 of each batch.

Blood 3 samples were collected at 2-weeks after exposure to the challenge.

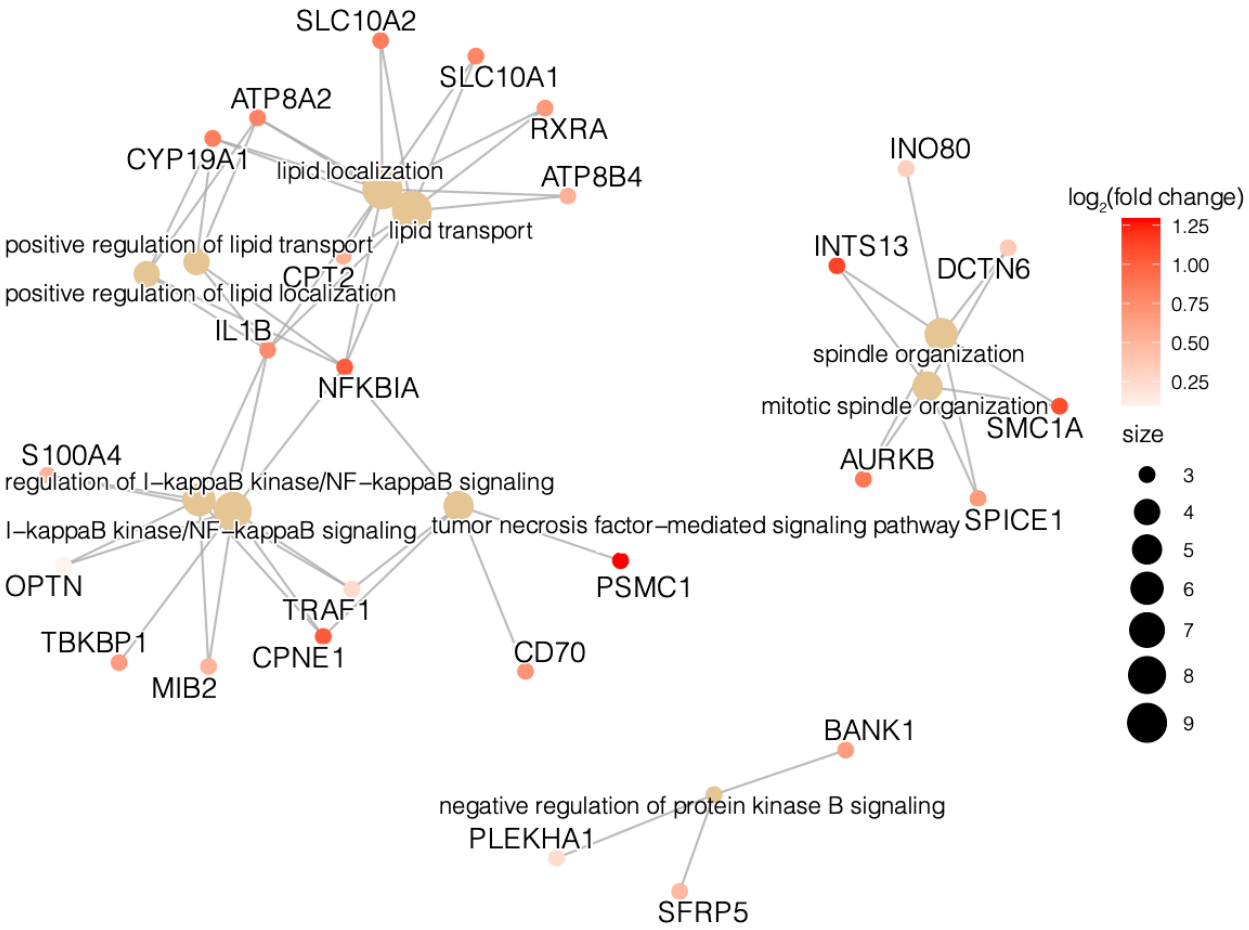

Figure 45. Network plot of top 10 enriched GO terms in the blue module.

The p-value ranged from 0.001 to 0.01 and the FDR (Benjamini and Hochberg correction) was 0.88 for the top 10 enriched GO terms. The $\log_2$(fold change) indicates the different expression level of the gene in Group 2 compared to Group 1 in Blood 3. The size indicated the number of genes in the module associated with the GO term.

Group 1: a group of animals with top five levels of LYMΔ13 among animals with positive LYMΔ13 but negative NEUΔ34 of each batch.

Group 2: a group of animals with top five levels of NEUΔ34 among animals with negative LYMΔ13 but positive NEUΔ34 of each batch.

Blood 3 samples were collected at 2-weeks after exposure to the challenge.

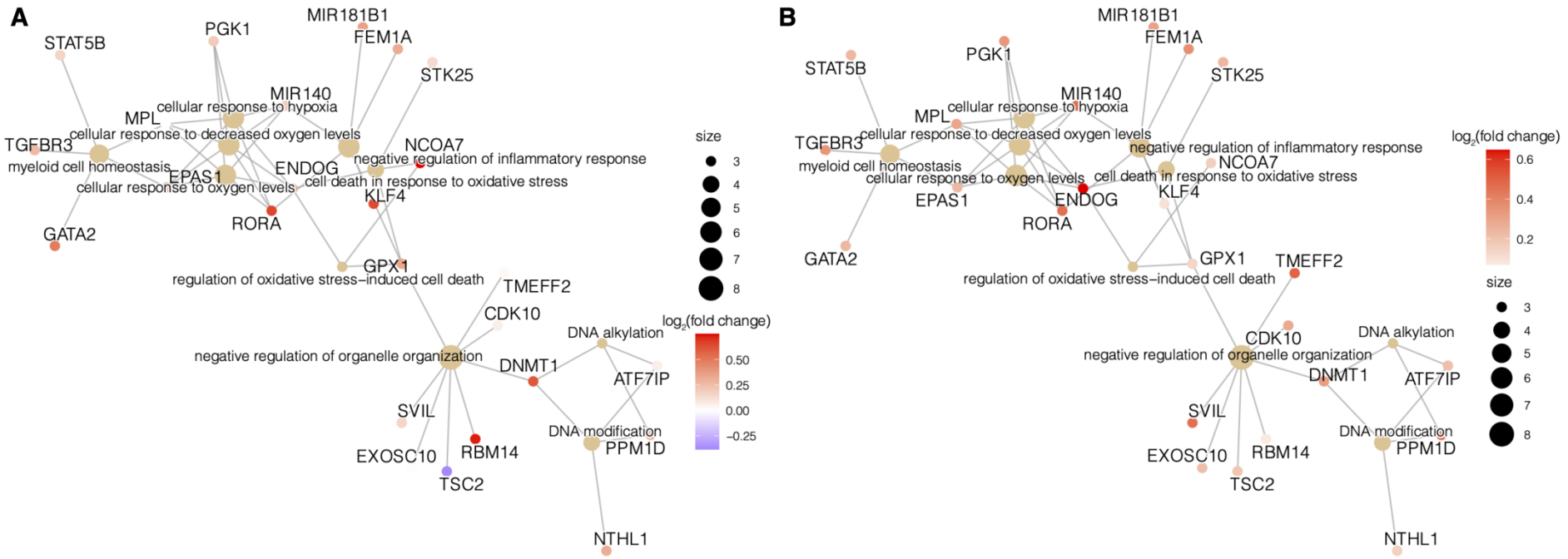

Figure 46. Network plot of top 10 enriched GO terms in the green module.

The p-value ranged from 0.001 to 0.008 and the FDR (Benjamini and Hochberg correction) was 0.78 for the top 10 enriched GO terms. The $\log_2$(fold change) indicates the different expression level of the gene in Group 2 compared to Group 1 in Blood 3 (**A**) and Blood 4 (**B**). The size indicated the number of genes in the module associated with the GO term.

Group 1: a group of animals with top five levels of LYMΔ13 among animals with positive LYMΔ13 but negative NEUΔ34 of each batch.

Group 2: a group of animals with top five levels of NEUΔ34 among animals with negative LYMΔ13 but positive NEUΔ34 of each batch.

Blood 3 and Blood 4 were collected at 2- and 6-weeks after exposure to the challenge, respectively.

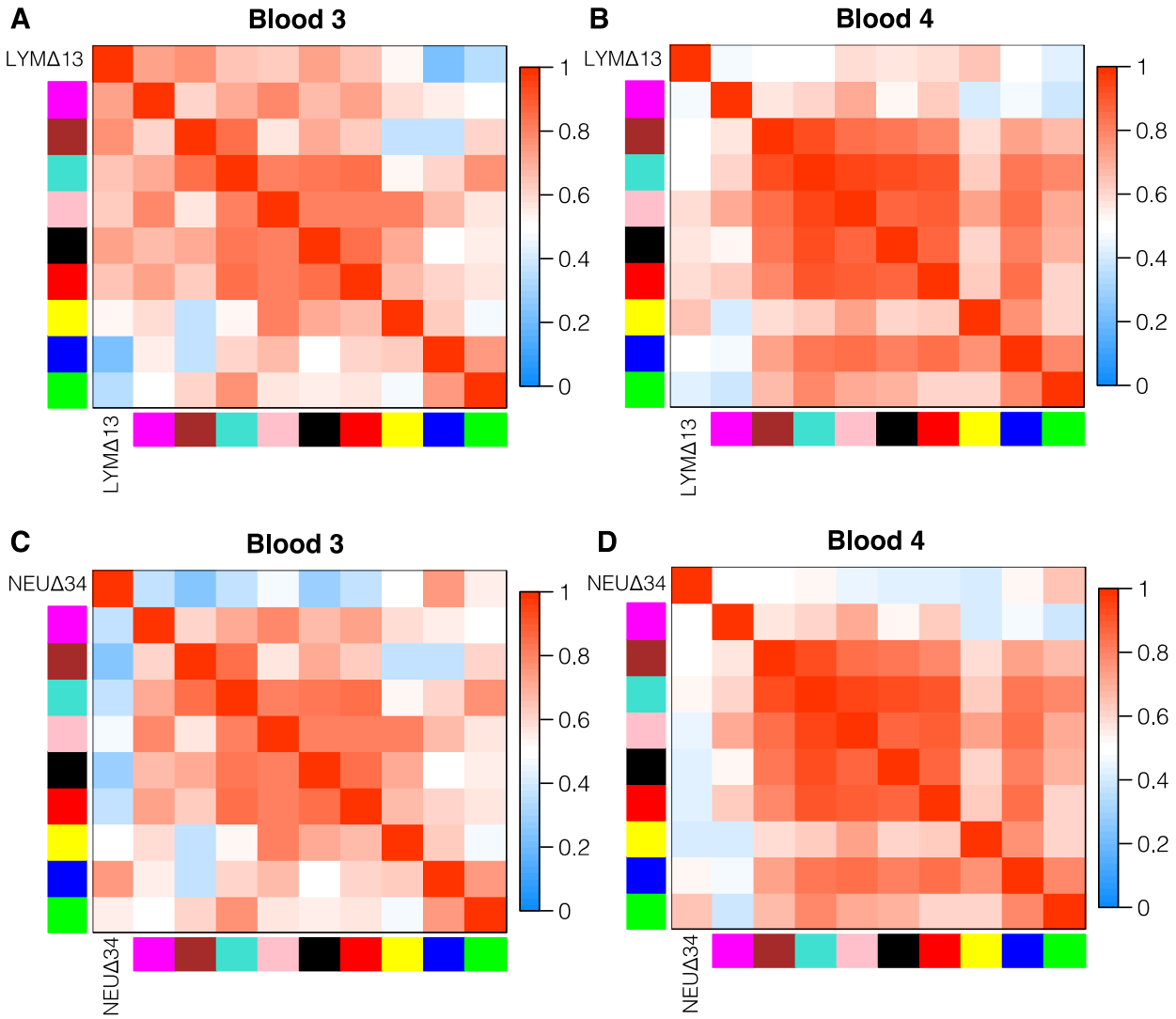

Figure 47. Heatmap plots of the adjacencies in the module eigengene network including the trait LYMΔ13 for gene expression profiles of Blood 3 (A) and Blood 4 (B), and the trait NEUΔ34 for gene expression profiles of Blood 3 (C) and Blood 4 (D).

The LYMΔ13 represents the change of lymphocyte concentration from Blood 1 to Blood 3, and the NEUΔ34 represents the change of neutrophil concentration from Blood 3 to Blood 4. Each row or column in the heatmap corresponds to one module (labeled by color) or the trait. In the heatmaps, red indicates high adjacency (positive correlation), and blue indicates low adjacency (negative correlation).

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before and at 2- and 6-weeks after exposure to the challenge.

Chapter 7. Estimating genomic prediction accuracy of disease resilience based on complete blood count traits

7.1. Introduction

Disease resilience is anticipated to be an important contributor to the productivity and profitability of the pig industry because resilient animals maintain relatively undepressed performance in the face of infectious disease challenges regardless of pathogen burden (Albers et al., 1987). Although disease resilience is of great interest to the pig industry, direct selection for disease resilience is hard to achieve because: (1) disease resilience to the challenge of multiple pathogens that exist in commercial pig production is a complex trait, which can be hard and expensive to measure; and (2) disease traits are generally not expressed in the purebred nucleus herds housed in high-health environments where the selection of elite breeding animals takes place (Wilkie and Mallard, 1999; Friggens et al., 2017). Regarding these challenges, the first study of this thesis (**Chapter 4**) indicated the potential for using complete blood count (CBC) traits under disease or commercial conditions as phenotypes to quantity disease resilience and developing them in the selection index for nucleus animals. Later, the genome-wide association studies (GWAS) of CBC traits in this thesis (**Chapter 5**) revealed the genetic control of CBC traits and found that they were polygenic traits controlled by a large number of quantitative trait loci (QTL) with small effects. Therefore, genomic prediction on CBC traits under disease could be a promising approach to address the issues of making genetic improvement of disease resilience. Genomic prediction allows for the early selection of elite breeding animals from the high health nucleus herds (validation population) without CBC records under disease. This is because it predicts the genomic estimated breeding value (GEBV) of an animal by summing up all single nucleotide polymorphisms (SNPs) marker effects over the whole genome (Meuwissen

et al., 2001; Samorè and Fontanesi, 2016). The marker effects can be estimated as a regression of phenotypes on genotypes of animals (training population from the commercial environment) that have both CBC records under disease and genomic information (Samorè and Fontanesi, 2016).

To date, several traits have been targeted in genomic prediction studies of pigs, for example, production traits of average daily gain, average daily feed intake, loin muscle depth, backfat thickness, and carcass weight (Akanno et al., 2014; Badke et al., 2014; Jiao et al., 2014; Guo et al., 2016; Zhang et al., 2018); meat quality traits such as pH (Miar et al., 2014); and maternal traits of litter size and number born alive (Tusell et al., 2013; Akanno et al., 2014). However, genomic prediction studies of the host response to infectious challenges are limited. Although the prediction accuracies of the host response (viral load, weight gain, and antibody level) to porcine reproductive and respiratory syndrome (PRRS) has been assessed by Boddicker et al. (2014) and Serão et al. (2016), the genomic prediction accuracy of disease resilience to a polymicrobial challenge commonly found in commercial pig farms remains largely unknown. Therefore, estimating genomic prediction accuracy of disease resilience based on CBC traits collected from pigs in the natural disease challenge model (NDCM) with the polymicrobial infectious challenge may be of particular interest and can provide possible solutions for improving disease resilience in the pig industry. Therefore, this study aimed at assessing the genomic predictability of CBC traits collected from the NDCM to explore if they can be further used as a part of developing predictions for disease resilience.

7.2. Material and Methods

7.2.1 NDCM and CBC traits

The NDCM and the collection of CBC traits from the NDCM have been described in detail in **Chapter 3**. Briefly, animals with genotypes (n = 3205), approximately 400 to 500

animals from each genetics supplier, were used for this study. Animals were introduced in 50 batches at 3-weeks interval, and each batch consisted of approximately 65 or 75 pigs from one of the genetic suppliers. Every seven batches constituted one cycle for cycle 1 to cycle 6, and the last eight batches (43 to 50) formed cycle 7. Detailed information for batch, genetic supplier, and cycle can be found in **Chapter 3**, **Table 1**. CBC traits measured from three sets of blood samples drawn at 2-weeks before (Blood 1), and at 2- and 6-weeks (Blood 3 and Blood 4) after exposure to the challenge were the phenotypes in this study. In addition, the changes of CBC traits were also calculated for each animal and used as phenotypes, which were referred to as Δ13 for the change from Blood 1 to Blood 3, Δ34 for the change from Blood 3 to Blood 4, and Δ14 for the change from Blood 1 to Blood 4.

7.2.2 Genotype data and quality control

The genotyping of animals was performed at Delta Genomics (Edmonton, AB, Canada) using the 650K Affymetrix Axiom® Porcine Genotyping Array. In total, 658,692 SNPs were included on the chip. Raw Affymetrix SNP data for each cycle were processed separately at Delta Genomics with the Axiom Analysis Suite, using all defaults. Missing genotypes were imputed using FImpute with the reference genome Sscrofa 11.1 (Sargolzaei et al., 2014). Quality control was performed using the preGSf90 software in the BLUPF90 family of programs to remove SNPs with a minor allele frequency lower than 0.05 and call rates lower than 0.90 (Misztal et al., 2002). Overall, 435,172 SNPs passed quality control for 3205 animals and were used for analysis.

7.2.3 GRM

The preGSf90 software in the BLUPF90 family of programs was used to build the GRM (Misztal et al., 2002). The GRM was constructed using $ZZ'/2\sum p_i(1-p_i)$, where Z contains

centered genotypes codes, and p_i is the minor allele frequency for locus *i* (VanRaden, 2008). Firstly, a conventional intact GRM was constructed using all genotyped animals (n = 3205), the genomic relationships were estimated between all animals based on their genotypes. All genotyped animals were regarded as one population with the same p_i for locus *i* for the intact GRM. However, since the animals were provided by seven genetic suppliers, they can be regarded as seven populations corresponding to seven genetic suppliers. This is because different genetic suppliers usually have different breeding strategies on animal selection and mating, and p_i for locus *i* could change accordingly. Therefore, the GRM was built separately for each genetic supplier using the formula above and then combined into one large custom matrix in which genomic relationships between animals in different genetic suppliers were set to zero as non-related (Chen et al., 2020; Cheng et al., 2020). The custom GRM was expected to only focus on variances within each genetic supplier, and allow the marker effects to be estimated independently within each genetic supplier to predict the GEBV of animals from the same genetic supplier in the validation population. The pooled heritability and genomic prediction accuracy for the trait based on the estimates of each genetic supplier were obtained by using custom GRM.

7.2.4 Statistical model

The genomic best linear unbiased prediction (GBLUP) was used to estimate heritability (h^2) and GEBV for each CBC trait collected from the NDCM. The GBLUP model for CBC traits can be described by the following equation:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e}$$

where **y** is a vector of observations on a CBC trait for all individuals; **b** is a vector of fixed effects, including the effect of batch and the covariate of bleeding age; **X** is a design matrix

relating observations to the fixed effects; **a** is a vector of breeding values; **Z** is a design matrix that relates observations to breeding values; and **e** is a vector of residual effects. Vector **c** represents a stack of vectors ($\mathbf{c_{Litter}}$, $\mathbf{c_{Pen1}}$, $\mathbf{c_{Pen2}}$, and $\mathbf{c_{Pen3}}$) of independent and uncorrelated random environmental effects, including litter ($\mathbf{c_{Litter}}$) and pen effects in the quarantine unit ($\mathbf{c_{Pen1}}$), in the challenge nursery ($\mathbf{c_{Pen2}}$), and in the challenge grow-to-finish stage ($\mathbf{c_{Pen3}}$). These random environmental effects were tested using the intact GRM and fitted in each CBC trait model when significant (*p-value* < *0.05*). Matrix **W** ($\mathbf{W_{Litter}}$, $\mathbf{W_{Pen1}}$, $\mathbf{W_{Pen2}}$, and $\mathbf{W_{Pen3}}$) is a stack of incidence matrices that relate observations to the corresponding random environmental effects.

Assuming the random effects **c** and **e** are uncorrelated and identically distributed, the (co-) variances of random effects for univariate models are:

$$\text{var}\begin{bmatrix}\mathbf{a}\\ \mathbf{c}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G}\sigma_a^2 & 0 & 0\\ 0 & \mathbf{I}\boldsymbol{\sigma}_\mathbf{c}^\mathbf{2} & 0\\ 0 & 0 & \mathbf{I}\sigma_e^2\end{bmatrix}$$

where **G** is the GRM, the intact GRM was first used to estimate the h^2 and GEBV for each CBC trait, and then the intact GRM was changed to custom GRM for the second estimate of h^2 and GEBV for each trait; **I** is the identity matrix, σ_a^2 is the additive genetic variance; $\boldsymbol{\sigma}_\mathbf{c}^\mathbf{2}$ represents a stack of random effect variances (e.g. $\boldsymbol{\sigma}_\mathbf{c}^\mathbf{2} = \begin{bmatrix}\sigma^2_{c_{\text{Litter}}} & 0\\ 0 & \sigma^2_{c_{\text{Pen1}}}\end{bmatrix}$, when the random effects $\mathbf{c_{Litter}}$ and $\mathbf{c_{Pen1}}$ are significant and fitted in the model for a trait); and σ_e^2 is the residual variance. Heritability ($h^2 = \sigma_a^2/\sigma_a^2 + \sigma_c^2 + \sigma_e^2$) was estimated using ASReml 4.1 (Gilmour et al., 2015). As described in **Chapter 4**, the likelihood ratio test in ASReml 4.1 was used to determine the significance of heritability estimates (Gilmour et al., 2015). Estimation of GEBV was conducted using the BLUPF90 family of programs (Misztal et al., 2002).

7.2.5 Cross-validation

The genomic prediction accuracy of CBC traits was estimated using the sevenfold cycle-based cross-validation, which involved model training on six cycles and validating on the left-out cycle. This was repeated until all seven cycles were used as the validation dataset. The cycle-based cross-validation was used rather than random cross-validation (randomly partitions the sample into subsamples) because: (1) each cycle consisted of 7 or 8 batches to provide reasonable amounts of animals in training and validation populations for genomic prediction; and (2) the introduction and management of the entire batch of animals together in each cycle were close to the all-in-all-out system operated in swine production. Here, the genomic prediction accuracy was defined as:

$$Accuracy = \frac{r\ (GEBV, y^*)}{\sqrt{\overline{h^2}}}$$

where *r (GEBV, y*)* is the correlation of GEBV with adjusted phenotypes (y^*) in the validation population, the GEBV estimated with the intact GRM and the custom GRM was used to estimate the intact GRM prediction accuracy and the custom GRM prediction accuracy, respectively; $\overline{h^2}$ is the average of two heritability estimates for each trait, one estimated with the intact GRM and the other one estimated with the custom GRM. Phenotypes were adjusted for estimates of the fixed effect of batch and the covariate of bleeding age.

For the sevenfold cycle-based cross-validation, the pooled *r (GEBV, y*)* was calculated as a weighted average correlation across seven validation cycles (Serão et al., 2016):

$$r\ (GEBV, y^*) = \frac{\sum_{i=1}^{7} n_i r_i\ (GEBV, y^*)}{\sum_{i=1}^{7} n_i}$$

where $r_i\ (GEBV, y^*)$ is the correlation of GEBV (intact GRM estimates or custom GRM as described above) with y^* in the *i*th validation cycle (i = 1 to 7); n_i is the number of animals in

the *i*th validation cycle. The $r_i\ (GEBV, y^*)$ was estimated using the R package 'stats (version 3.6.2) (R Core Team, 2020).

A paired t-test was conducted between the genomic prediction accuracies, $\frac{r_i\ (GEBV, y^*)}{\sqrt{\overline{h^2}}}$, estimated by the intact GRM and the custom GRM for all seven validation cycles using R to explore if there are any significant differences in between (Kim, 2015, R core team, 2020). The raw *p-values* for all CBC traits were corrected using the Benjamini and Hochberg correction (*FDR*) to control false positives from multiple comparisons (Benjamini et al., 1995; R core team, 2020)

The standard error of genomic prediction accuracy was estimated as (Altman and Bland, 2005):

$$se = \frac{s}{\sqrt{n}}$$

where s is the sample standard deviation of genomic prediction accuracies, $\frac{r_i\ (GEBV, y^*)}{\sqrt{\overline{h^2}}}$, across the seven validation cycles, and n is the sample size of seven.

7.3. Results

7.3.1 Genomic relationships in the intact GRM and the custom GRM

Both intact GRM and custom GRM were n × n matrices, where n was the number of genotyped animals (n = 3205) in this study. Each diagonal value in both intact GRM (**Figure 48A**) and custom GRM (**Figure 48B**) indicated the genomic relationship for each animal with itself. The diagonal genomic relationship estimates in the intact GRM ranged from 0.54 to 1.34, with an average of 1 (estimated as no inbreeding in the population across seven genetic suppliers). The diagonal genomic relationship estimates in the custom GRM ranged from 0.42 to 1.26, with an average of 0.89 (genomic inbreeding was estimated to be negative indicating the

animals are “more heterozygote than what is expected”) (Legarra et al., 2018). The off-diagonal values for both intact GRM (**Figure 48C**) and custom GRM (**Figure 48D**) were the pairwise genomic relationships for animals with the others. The off-diagonal relationship estimates in the intact GRM ranged from -0.19 to 1.01, with the average close to 0 (-9.19e-07, the property of the centered GRM), approximately 64% of genomic relationships were negative. Most negative genomic relationships in the intact GRM were found between animals from different genetic suppliers (**Figure 48E**), and the majority of animals from the same genetic supplier had positive genomic relationship estimates in the intact GRM (**Figure 48F**). For the off-diagonal of the custom GRM, 9% of the genomic relationship estimates were negative among the off-diagonal values in the custom GRM, the genomic relationships were set to zero for animals from different genetic suppliers (86% of the off-diagonal values) to allow the marker effects to be estimated independently within each genetic supplier. The off-diagonal genomic relationships in the custom GRM for animals from the same company ranged from -0.23 to 0.95, with the average close to 0 (-0.0003, the property of the centered GRM).

7.3.2 Estimates of heritability by intact GRM and custom GRM

Heritability estimates for CBC traits using intact GRM and custom GRM models are shown in **Figure 49** for Blood 1, Blood 3, and Blood 4 and in **Figure 50** for Δ13, Δ34, and Δ14. Similar to what has been identified with six cycles of animals in **Chapter 4**, most CBC traits in Blood 1, Blood 3, and Blood 4 were heritable for both models with seven cycles of animals here, shown as significant heritability estimates (*p-value < 0.05*). Several red blood cell traits in Blood 3 and Blood 4 were moderately to highly heritable (0.25 ± 0.04 to 0.60 ± 0.04). However, CBC traits in Δ13, Δ34, and Δ14 were less heritable, shown as relatively low heritability estimates with both models, and many were not significantly different from zero (*p-value > 0.05*). For the

comparison of heritability estimates between intact GRM and custom GRM models, heritability estimates with the custom GRM were slightly higher than the intact GRM for most CBC traits. Only eosinophil concentration (EOS), mean corpuscular volume (MCV), and red blood cell distribution width (RDW) in Blood 4 had higher estimates of heritability with intact GRM models than custom GRM models. A few CBC traits had the same heritability estimates in intact GRM and custom GRM models, including white blood cell concentration (WBC) in Blood 1, monocyte concentration (MONO) and platelet concentration (PLT) in Blood 3, mean corpuscular volume (MCH) and PLT in Blood 4, the Δ13 of lymphocyte concentration (LYM) and MONO, and the Δ14 of hemoglobin concentration (HGB). However, higher standard errors were found with custom GRM (an average of 0.04) than intact GRM (an average of 0.03) models for most traits that had the same heritability with the two models.

7.3.3 Estimates of genomic prediction accuracy with intact GRM and custom GRM

The genomic prediction was only conducted for CBC traits that had significant (*p-value* < 0.05) estimates of heritability. The genomic prediction accuracies calculated based on the weighted average correlation across validation sets are shown in **Figure 51** for CBC traits in Blood 1, Blood 3, and Blood 4, and in **Figure 52** for Δ13, Δ34, and Δ14. In general, genomic prediction accuracies for white blood cell traits ranged from 0.15 to 0.3 with an average standard error of 0.05 for both models in all blood samples. Higher genomic prediction accuracies, ranging from 0.2 to 0.4 with an average standard error of 0.05, were found for most red blood cell traits in Blood 1, Blood 3, and Blood 4 with both models, especially those that showed moderate to high heritability (0.25 ± 0.04 to 0.60 ± 0.04). The genomic prediction accuracies were found to be lower for most red blood cell traits in Δ13, Δ34, and Δ14 (ranging from 0.1 to 0.2 with an average standard error of 0.05) compared to Blood 1, Blood 3, and Blood 4 with both

models, which corresponded to their lower heritability estimates (ranging from 0.07 ± 0.03 to 0.26 ± 0.05). According to the t-test results for the comparison of genomic prediction accuracies between intact GRM and custom GRM models, basophil concentration (BASO) in Blood 1, and MONO and red blood cell concentration (RBC) in Blood 3 showed significantly (*FDR < 0.05*) higher genomic prediction accuracy with the intact GRM model compared to the custom GRM model.

7.4 Discussion

This study estimated the heritability and assessed the genomic prediction accuracy for CBC traits collected in the NDCM before and after exposure to the polymicrobial infectious challenge. Analyses were conducted on all genotyped animals (n = 3205) in seven cycles using GBLUP with intact GRM and custom GRM. Although genomic prediction can be conducted by multiple methods, for example, Bayesian methods in addition to GBLUP, GBLUP was only used here for genomic prediction of CBC traits. The reason is that GBLUP assumes an infinitesimal model that all markers have the same and small contribution to the trait, but Bayesian methods assume that only a small proportion of SNPs have a large effect on the trait (Henderson 1984; Meuwissen et al., 2013; Mrode 2014). Based on the GWAS results in **Chapter 5**, CBC traits were mostly controlled by QTL with small effects throughout the genome. Therefore, GBLUP was used because its model assumption more closely represented the underlying genetic architecture of CBC traits.

7.4.1 Heritability estimates for CBC traits

Heritability estimates of CBC traits using intact GRM and custom GRM models for all genotyped animals in seven cycles were not precisely the same but close to the results in **Chapter 4** using genotyped animals in the first six cycles (n = 2593). The heritability estimate of

a trait was not precisely the same as **Chapter 4** because heritability is a population parameter (Wray and Visscher, 2008). The heritability estimate depends on population-specific factors, such as allele frequencies, the effects of gene variants, and variation due to environmental factors, which differ between the population of animals in all seven cycle of animals and the population of animals in the first six cycles (Visscher et al., 2008). However, both heritability estimates using the six cycles and all seven cycles indicated that many CBC traits collected before and after exposure to the polymicrobial challenge were heritable.

The heritability estimates for each CBC trait with the intact GRM and with the custom GRM were also close but not precisely the same, although the same genotyped animals in all seven cycles was used for both estimates. The change was caused by the difference between the intact GRM and the custom GRM. The heritability with the intact GRM was estimated based on the variances across all animals from seven genetic suppliers. However, the heritability with the custom GRM was estimated based on the pooled within-genetic supplier variances. It was still puzzling because numerically higher heritability estimates were generally found with the custom GRM model than the intact GRM model in this study. Intuitively, it may be caused by a larger number of negative genomic relationships in the intact GRM (approx. 64%) compared to the custom GRM (approx. 9%) (**Figures 48C and D**). The negative genomic relationships estimated based on genotypes of SNPs are also puzzling but have been validated and accepted as reasonable estimates with no error in the GRM (Toro et al., 2011; Thompson, 2013; Legarra et al., 2018). Thompson (2013) suggested that the GRM constructed based on SNPs reinforced the interpretation of relatedness (potentially negative) rather than a probability (necessarily positive) as a genomic relationship. Legarra et al. (2018) further demonstrated that the negative relatedness could be due to the animals carrying different SNP genotypes. Indeed, most negative

genomic relationships in the intact GRM were found between animals from different genetic suppliers (**Figures 48E and F**). However, whether and how this factor affected the heritability estimates needs to be further explored with additional studies on two GRMs, and the algorithm for constructing the GRMs and solving the models.

7.4.2 Genomic prediction accuracy estimates for CBC traits

Most CBC traits did not show any significant differences ($FDR > 0.05$) between the intact GRM and the custom for the estimates of genomic prediction accuracy. The intact GRM regarded all animals from seven genetic suppliers as one population and estimated the SNP marker effects to be the same for all seven genetic suppliers. The custom GRM considered the fact that marker effects can differ among different genetic suppliers. The genomic prediction with the custom GRM allowed the marker effects to be estimated within each genetic supplier independently to predict the GEBV of the validation population from the same genetic supplier. However, the genomic prediction accuracy for each CBC trait did not show a significant improvement with the custom GRM. Instead, the genomic prediction accuracy was found to be significantly higher with the intact GRM for BASO in Blood 1, and for MONO and RBC in Blood 3 (**Figure 51**).

Since the marker effects were estimated within each genetic supplier with the custom GRM, the small training population (approximately 300 animals) within each genetic supplier for each fold of cross-validation may impair the accuracy of estimating the marker effects. Although the intact GRM overlooked the differences of marker effects among genetic suppliers, the training population (approximately 1995 animals) was enlarged by combining animals across all genetic suppliers for each fold of cross-validation. Multiple previous studies suggested that maximizing training population size by combining data across populations (e.g. breeds, genetic

suppliers) was especially valuable for improving genomic prediction accuracy when the number of phenotypes per population is small (Roos et al., 2008, 2009; Brøndum et al., 2011; Zhou et al., 2014; Iheshiulor et al., 2016; Meuwissen et al., 2021). However, such improvement of genomic prediction accuracy can only be achieved when the animals in the training population and the validation population are closely related or the high-density marker data is used, as both strategies could ensure a relatively high linkage disequilibrium (LD) between SNPs marker and QTL (Meuwissen et al., 2021). In this study, although the animals from different genetic suppliers were less likely to be closely related, a relatively high-density marker date (n = 435,172 SNPs) was used. Therefore, genomic prediction with the intact GRM provided an opportunity to improve the genomic prediction accuracy because the animals from different genetic suppliers were combined to enlarge the validation population.

Of note, the most significant improvement of genomic prediction accuracy with the intact GRM model compared to the custom GRM model was observed in BASO in Blood 1 (**Figure 51**). Due to the mechanical problems of the Hematology System used to measure BASO from Blood 1 samples, the BASO measures from batches 31 (genetic supplier E) and 34 (genetic supplier D) in cycle 5 and the batch 42 (genetic supplier B) in cycle 6 (**Table 1** in **Chapter 3** for the relation of the batch, cycle, and genetic supplier) were extraordinarily high and regarded as outliers. Approximately 50 measures of BASO in Blood 1 were removed from each of the three genetic suppliers. The significantly lower genomic prediction accuracy with the custom GRM model for BASO in Blood 1 may be caused by further reducing the training population size within these three genetics suppliers, which affected the accuracy of estimating the marker effects (Goddard and Hayes, 2009; VanRaden et al., 2009). However, the intact GRM model provided an opportunity to use phenotypes across all genetics suppliers in the training population

to estimate the marker effects, which may be less impacted by the reduced training population within these three particular genetic suppliers.

In addition, animals can be genetically correlated although they were sourced from different genetic suppliers. It may be biased to set the genomic relationship between animals in different genetic suppliers to zero as non-related in the custom GRM, which resulted in the reduction of genomic prediction accuracy compared to the intact GRM.

7.4.3 Improve disease resilience with CBC traits

Some CBC traits under disease were expected to have the potential to be developed as phenotypes for genomic prediction of disease resilience in **Chapter 4** based on their significant genetic correlations with resilience traits of grow-to-finish growth rate (GFGR) and treatment rate (TR) (details of GFGR and TR are described in **Chapter 3**). This study further supports this idea as three CBC traits under disease that had significant genetic correlations with TR, including LYM in Blood 3, neutrophil concentration (NEU) in Blood 4, and RDW in Blood 4, showed moderate genomic prediction accuracies (0.12 ± 0.04 to 0.28 ± 0.03, **Figure 51**). The moderate negative genetic correlation between the LYM in Blood 3 with TR (-0.38 ± 0.18, **Table 11** in **Chapter 4**) indicating selection on a higher level of LYM in Blood 3 may lead to a desirable decrease in TR. High positive genetic correlations for NEU in Blood 4 and RDW in Blood 4 with TR (0.50 ± 0.23 and 0.89 ± 0.26, respectively, **Table 11** in **Chapter 4**) suggested that selection on lower levels of NEU and RDW traits in Blood 4 may lead to lower TR.

Regarding the importance and need to reduce antimicrobial use and antimicrobial resistance in commercial farms of the swine industry for "One Health" (**Chapter 2, section 2.2.3**), genomic selection for resilient animals with low TR in response to disease challenge is of particular interest. However, the low heritability for TR (0.04 ± 0.01) makes it difficult to be

directly used for genomic selected for resilient animals with lower TR because the low heritability indicating the TR will be primarily affected by environmental effects rather than genetics. Therefore, developing those three CBC traits (LYM in Blood 3, NEU and RDW in Blood 4) with higher heritability estimates (0.06 ± 0.03 to 0.26 ± 0.04) and moderate to high genetic correlations with TR in the selection index for disease resilience may be more promising to a desirable decrease in TR than a selection directly and solely on TR. In addition, according to the negative genetic correlation between TR and GFGR (-0.50 ± 0.16) found in **Chapter 4**, the genomic selection for resilient animals with lower TR may potentially improve GFGR in response to the challenge simultaneously.

Many CBC traits were analyzed here, for example, CBC traits in Blood 1, although they were not significantly associated with the economically important resilience traits of GFGR or TR in response to the polymicrobial infectious challenge in the pig industry. The results of those CBC traits are anticipated to provide reference values for future studies because the genetic and genomic analyses of CBC traits collected from a polymicrobial challenge were rare or absent, and their genetic parameters were largely unknown. In addition, there are also other economically important resilience traits, such as carcass traits, mortality, health scores, that can be tested for the genetic correlation with CBC traits and whether CBC traits can be further developed as the indicator traits for the genomic selection of disease resilience.

7.5 Conclusion

In conclusion, several CBC traits collected before and after the polymicrobial infectious challenge were found to be predictable with GBLUP models. The effects of intact GRM and custom GRM on heritability and genomic prediction accuracy estimates are still puzzling, which need to be further explored with additional studies on two GRMs, and the algorithm for

constructing the GRMs and solving the models. For genomic prediction, using an intact GRM that includes the genomic relationships of all animals from multiple genetics suppliers in the GBLUP model was hypothesized to have the potential to improve the prediction accuracy of CBC traits due to the increased number of phenotypes that can be used for marker effects estimates in the training set. However, the hypothesis needs to be further tested also with some other traits. Genomic selection for a higher level of LYM in Blood 3 but lower levels of NEU and RDW in Blood 4 after exposure to the challenge may provide an opportunity to make genetic improvement of disease resilience by reducing TR so as to help reduce antimicrobial use and antimicrobial resistance in commercial farms of the pig industry. However, further research is needed to validate the results. Many other CBC traits were also found to be heritable and predictable, especially CBC traits in Blood 1 that can be directly measured from nucleus herds and used as the most cost-effective indicators of disease resilience. Therefore, these CBC traits in Blood 1 are still attractive and may be worthwhile to explore if they are associated with other economically important resilience traits (e.g. carcass traits, mortality, health scores) in addition to GFGR and TR.

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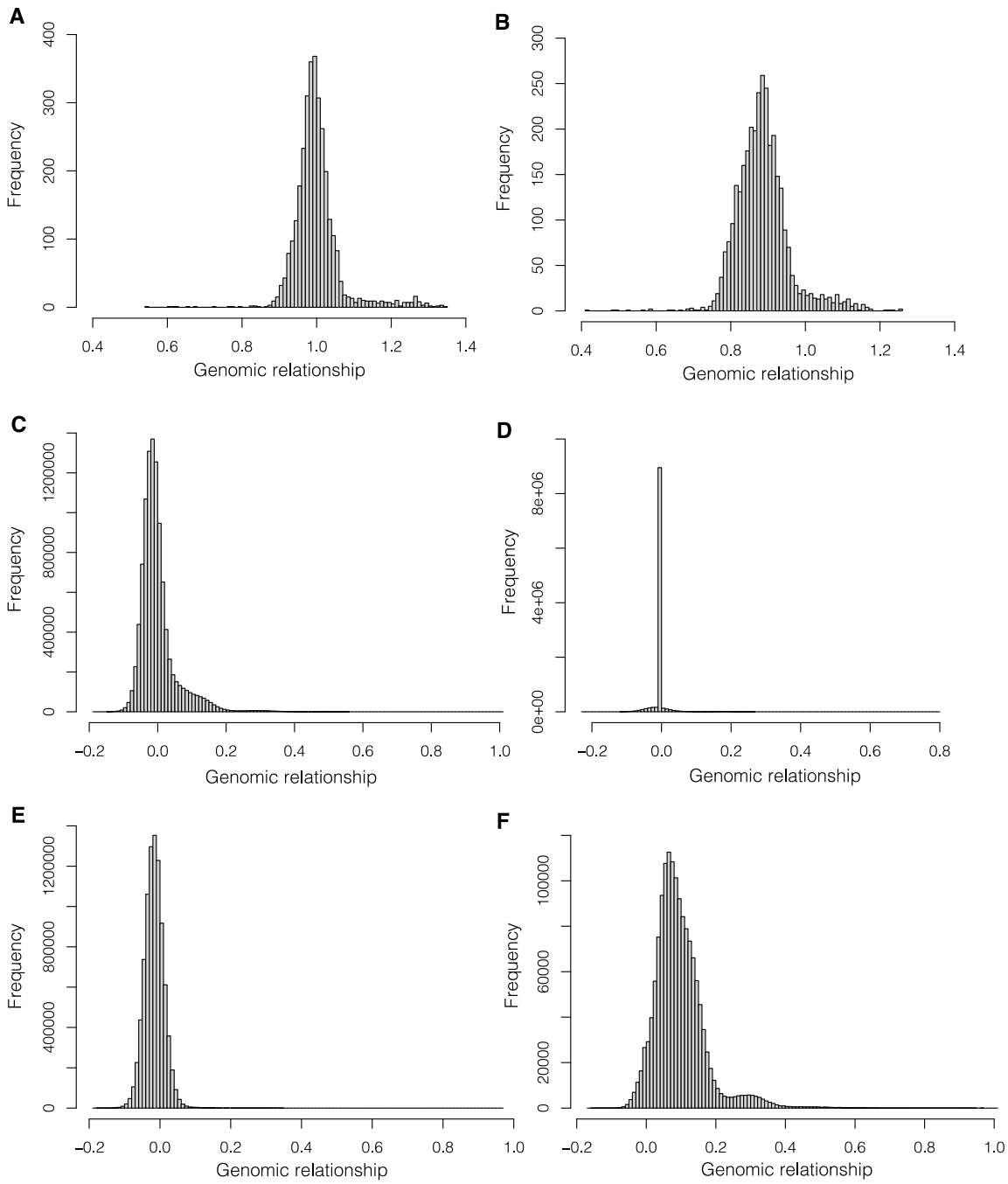

Figure 48. Genomic relationship estimates in the intact GRM and the custom GRM.

Diagonal genomic relationship estimates in the intact GRM (**A**) and the custom GRM (**B**) for each animals with itself.

Off-diagonal genomic relationship estimates in the intact GRM (**C**) and the custom GRM (**D**) for animals with the others.

Off-diagonal genomic relationship estimates in the intact GRM for animals with the others from different genetic suppliers (**E**) and for animals with the others in the same genetic supplier (**F**).

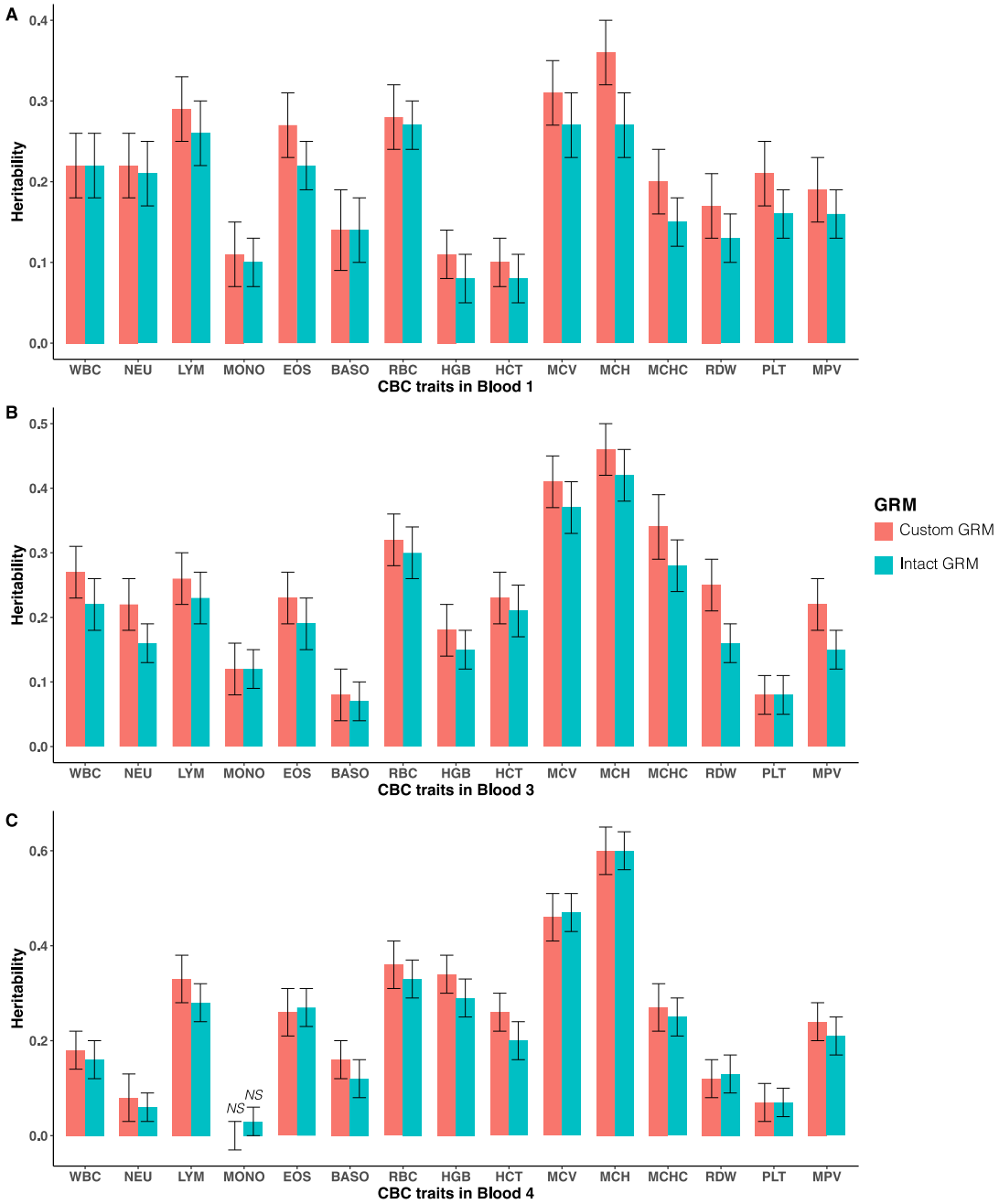

Figure 49. Heritability estimates of all complete blood count (CBC) traits in Blood 1 (A), Blood 3 (B) and Blood 4 (C) using intact genomic relationship matrix (GRM) and custom GRM models.

The *NS* indicates a non-significant (*p-value > 0.05*) estimate of heritability. The error bar represents one standard error of the estimate.

WBC (total white blood cell concentration), NEU (neutrophil concentration), LYM (lymphocyte concentration), MONO (monocyte concentration), EOS (eosinophil concentration), BASO (basophil concentration), RBC (red blood cell concentration), HGB (hemoglobin concentration), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin),

MCHC (mean corpuscular hemoglobin concentration), RDW (red blood cell distribution width), PLT (platelet concentration), MPV (mean platelet volume).

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after exposure to the challenge, respectively

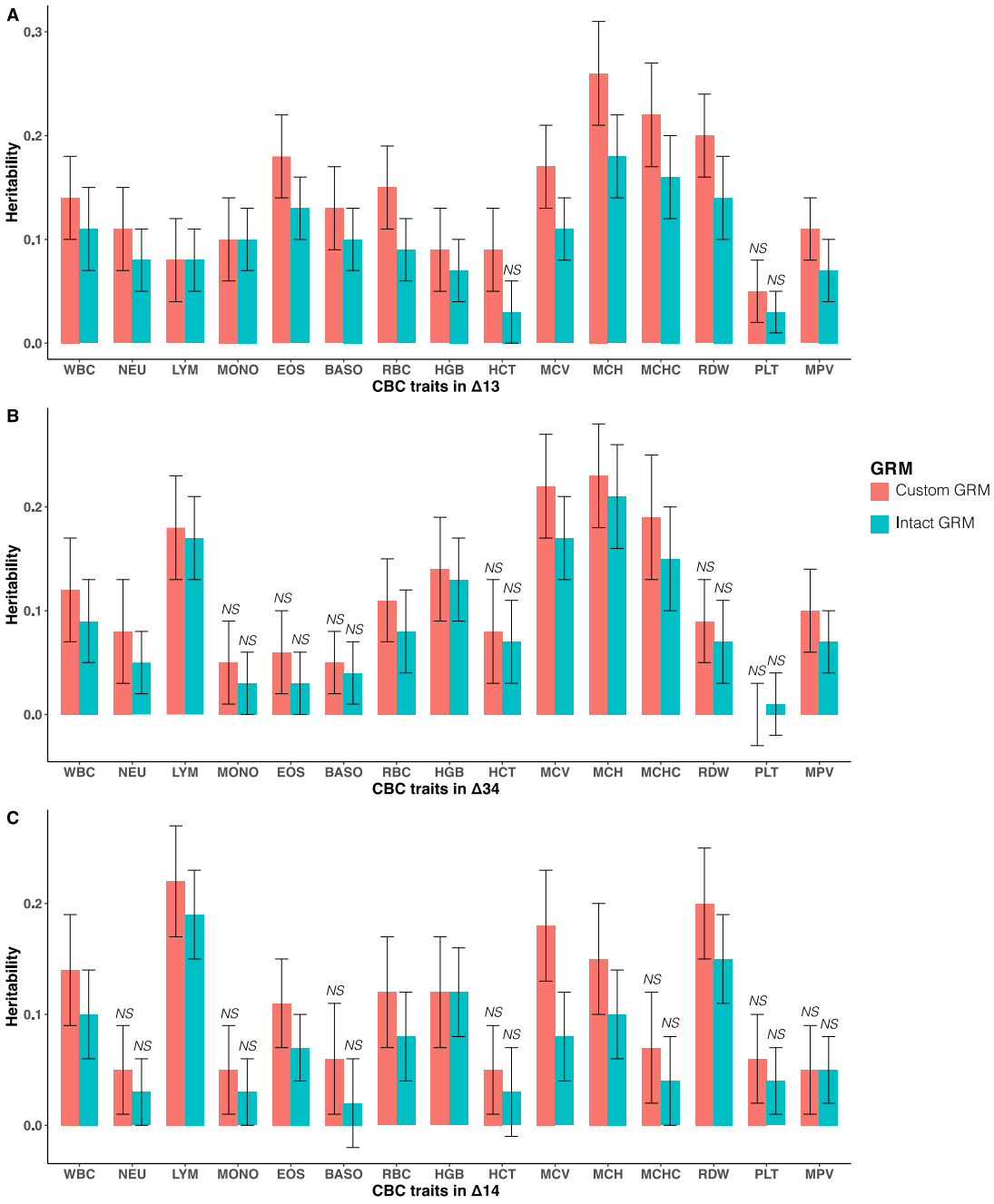

Figure 50. Heritability estimates of all complete blood count (CBC) traits in Δ13 (A), Δ34 (B), Δ14 (C) using custom genomic relationship matrix (GRM) and intact GRM models.

The NS indicates a non-significant (*p-value > 0.05*) estimate of heritability. The error bar represents one standard error of the estimate.

WBC (total white blood cell concentration), NEU (neutrophil concentration), LYM (lymphocyte concentration), MONO (monocyte concentration), EOS (eosinophil concentration), BASO (basophil concentration), RBC (red blood cell concentration), HGB (hemoglobin concentration), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin),

MCHC (mean corpuscular hemoglobin concentration), RDW (red blood cell distribution width), PLT (platelet concentration), MPV (mean platelet volume).

Δ13 for the change from Blood 1 to Blood 3; Δ34 for the change from Blood 3 to Blood 4; Δ14 for the change from Blood 1 to Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after the challenge, respectively.

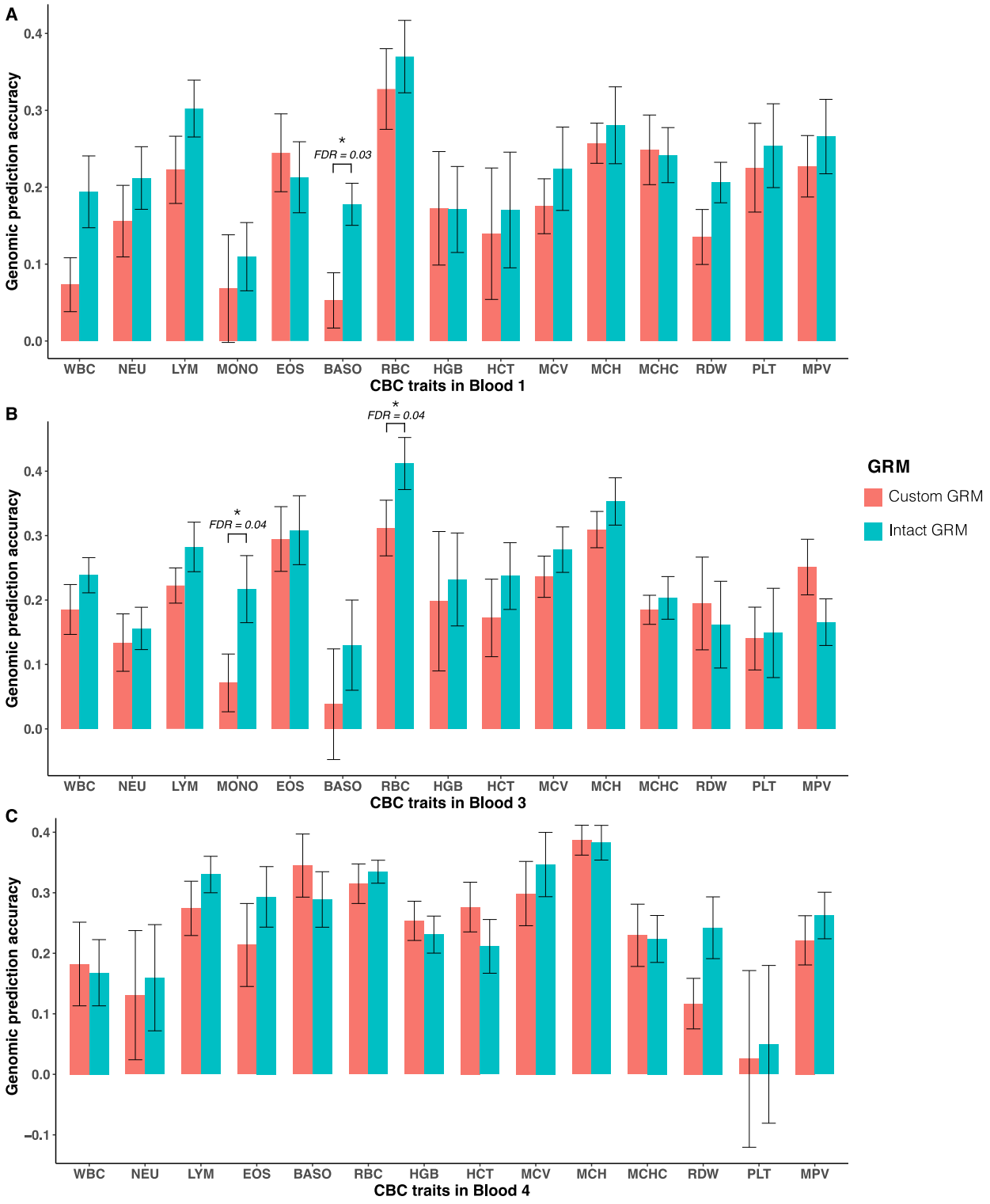

Figure 51. Genomic prediction accuracy for complete blood count trait that has significant heritability estimates (*p-value < 0.05*) in Blood 1 (A), Blood 3 (B), and Blood 4 (C) using intact genomic relationship matrix (GRM) and custom GRM models.

*CBC traits showed significantly different (*FDR < 0.05*) genomic prediction accuracy estimates between the intact GRM and the custom GRM. *FDR* is the Benjamini-Hochberg correction of false discovery rate on the *p-value*.

The error bar represents one standard error of the estimate.

WBC (total white blood cell concentration), NEU (neutrophil concentration), LYM (lymphocyte concentration), MONO (monocyte concentration), EOS (eosinophil concentration), BASO (basophil concentration), RBC (red blood cell concentration), HGB (hemoglobin concentration), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red blood cell distribution width), PLT (platelet concentration), MPV (mean platelet volume).

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after the challenge, respectively.

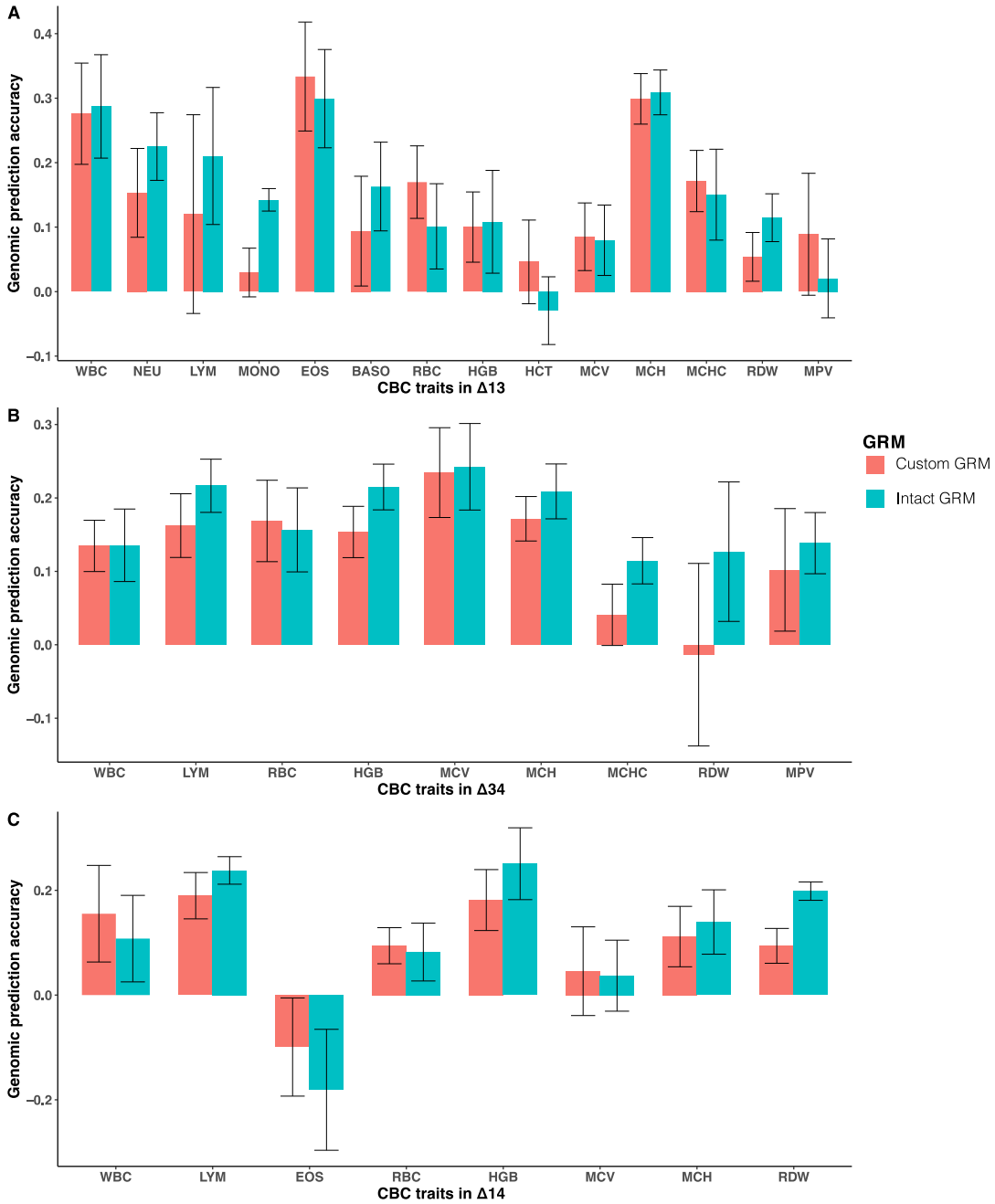

Figure 52. Genomic prediction accuracy for CBC trait for complete blood count trait that has significant in heritability estimates (*p-value < 0.05*) in Δ13 (A), Δ34 (B), Δ14 (C) using intact genomic relationship matrix (GRM) and custom GRM models.

The error bar represents one standard error of the estimate.

WBC (total white blood cell concentration), NEU (neutrophil concentration), LYM (lymphocyte concentration), MONO (monocyte concentration), EOS (eosinophil concentration), BASO (basophil concentration), RBC (red blood cell concentration), HGB (hemoglobin concentration), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red blood cell distribution width), PLT (platelet concentration), MPV (mean platelet volume).

Δ13 for the change from Blood 1 to Blood 3; Δ34 for the change from Blood 3 to Blood 4; Δ14 for the change from Blood 1 to Blood 4.

Blood 1, Blood 3, and Blood 4 were collected at 2-weeks before, and at 2- and 6-weeks after the challenge, respectively.

Chapter 8. General Discussion

The productivity of pigs has been primarily organized in the pig production system or pyramid by selecting superior parents from nucleus herds to multiply and breed commercial pigs. However, health status degrades from nucleus farms to commercial farms, caused by more frequent movements of pigs, feed, and pork products at local, national, and international scales in commercial farms. The productivity of pigs in commercial farms can decline when challenged by endemic and or emerging pathogens. Therefore, managing infectious diseases and maintaining herd productivity in commercial farms are critical to the profitability of the swine industry. Conventional methods, including strict biosecurity, vaccines, and antimicrobials, are being used in commercial farms to manage diseases and maintain animal performance and health. However, they are not always effective as discussed in **Section 2.2**. Briefly, biosecurity needs to be continuously improved regarding gaps in knowledge of epidemiology for emerging diseases and diverse ways for pathogen transmission. The co-infections by multiple pathogens, and the high recombination rate for some of the viruses (e.g. PRRSV) and their interaction with the host immune system impair vaccine efficacy. And the use of antimicrobials to maintain or return pig health need to be reduced due to the concern of antimicrobial resistance. Regarding the limitations of conventional methods, breeding disease resilient pigs that can maintain relatively undepressed performance and productivity in commercial farms is anticipated to be a practical method to address these issues. To date, many research studies have been dedicated to breeding disease resilient pigs, but it is not yet a common objective in practical pig breeding programs due to the lack of valuable and cost-effective traits for breeding (Knap, 2005; Hermesch et al., 2015; Harlizius et al., 2020; Knap and Doeschl-Wilson, 2020).

Thus, in my thesis studies, I aimed to explore the potential of complete blood count (CBC) to generate valuable and cost-effective CBC traits for breeding disease resilient pigs. To do this I analyzed longitudinal CBC data collected from the natural disease challenge model (NDCM) at 2-weeks before and 2- and 6-weeks after the polymicrobial challenge. Overall, the results of this thesis suggested good potential to use CBC under disease or commercial conditions as phenotypes for breeding disease resilient pigs and also helped to further understand the genetic basis and immunological mechanisms of disease resilience in pigs. Furthermore, this thesis provides many reference values for future studies, including heritability, genetic correlation and genomic prediction accuracy estimates for CBC traits. These reference values are valuable because the genetic and genomic analyses of CBC traits collected from a polymicrobial challenge model were rare or absent prior to the work, and their genetic parameters were mostly unknown.

8.1. Feasibility of collecting CBC traits from commercial farms

Collecting blood samples from commercial pig farms for CBC can be "costly" in terms of the requirements for proper training and skilled technicians to limit the injury and stress of pigs caused by blood sampling. Also, fresh blood samples are crucial to successful CBC, and most laboratories require delivery of blood samples for CBC within 24 hours after sampling.

Intuitively, mortality in response to disease challenge seems to be a phenotype that is easier to be collected than CBC traits. However, mortality is a notoriously tricky trait with low heritability, which makes it difficult to be used for breeding. The disease pressure in the NDCM was relatively high, with an average mortality of 26%, and the heritability estimate for mortality was only 0.09 ± 0.03 (Cheng et al., 2020). Typically, the average mortality in a wean-to-finish pig barn is expected to be 6% to 8%, and the finishing barn may only experience a 4% to 6%

mortality on average (Stalder, 2018). This means the heritability estimate for mortality at the commercial level will be even lower than the estimate from NDCM due to the lack of phenotypic variance. Accordingly, the genomic prediction for mortality with low heritability is less likely to be accurate. Recording mortality traits can also be laborious and costly as it is not a simple "yes or no" trait. Instead, the precision tracking of mortality for more accurate breeding requires the date and reason of death to be recorded along with the pig ID or the tag number. In commercial farms, the reason for death can be complex or even mysterious. For example, pigs can die with clinical signs caused by co-infection with multiple pathogens. Moreover, sudden death with no signs of illness (also observed in NDCM) can occur at any age in commercial farms. In these cases, necropsy by veterinarians is needed to explore and identify the reason for death, which is costly.

In addition, feed intake, carcass traits, and growth performance collected from the NDCM, have also been investigated as phenotypes for disease resilience. Moderate heritability estimates (0.08 ± 0.03 to 0.23 ± 0.05) were found for resilience traits derived from longitudinal feed intake data of each animal in the NDCM finishing barn (Putz et al., 2019; Cheng et al., 2020). However, the high cost of installing and maintaining electronic feeding equipment is a significant issue associated with the collection of feed intake data in commercial farms (Putz et al., 2019). Moderate to high heritability estimates were found for carcass traits (0.12 ± 0.04 to 0.54 ± 0.06) and growth performance (0.15 ± 0.04 to 0.30 ± 0.05) under disease (Bai et al., 2020; Cheng et al., 2020). However, collecting either carcass traits or growth performance for each pig is not expected to be necessarily less laborious than CBC traits.

Typically, nothing is ever easy and cheap to collect at commercial farms. CBC traits from commercial farms have the advantage of being further explored as phenotypes of disease

resilience because many CBC traits have moderate to high heritability (0.1 to 0.5) and genomic prediction accuracy (0.1 to 0.4). The cost-effectiveness of CBC traits can be further improved by exploring time points that are more practical for blood sample collection in commercial farms. In this thesis, only two sets of blood samples were collected at 2-weeks and 6-weeks after exposure to the challenge regarding cost and animal welfare. Still, they are not necessarily the optimal time points for all commercial farms when the pathogen types and management strategies are different. In addition, the polymicrobial infectious challenge always existed in the challenge barn of the NDCM and animals were exposed to the challenge as soon as they entered the barn. However, the outbreak of infectious diseases in commercial farms can be more sporadic and complex. The time point for animals being exposed to the challenge may be hard to determine in commercial farms, making it difficult to follow the suggested time points of 2- and 6-weeks after exposure to the challenge for blood sampling and collecting of CBC traits. Instead, it may be more practical to collect blood samples for CBC traits based on "pig signals" captured in commercial farms. For example, a set of blood samples can be collected as soon as the drop of health score or clinical signs has been observed in commercial farms. With increasing advocacy for precision pig farming and burgeoning research of new remote monitoring technology (e.g. cameras, microphones, and accelerometers), more "pig signals" can be captured timely and efficiently through sensors, images, sounds and movements (Benjamin and Yik, 2019). The continuous development of precision farming in the swine industry will enhance the swine specialist's eyes, ears, and nose to capture "pig signals" in everyday farming to make use of CBC more practical and help to improve the cost-effectiveness of CBC traits.

8.2. Disease resilience and its effect on the infection itself

Disease resilience has been primarily defined and quantified by animal productivity and performance regardless of pathogen burden. Therefore, its effect on the infection itself, such as pathogen transmission and disease prevalence, remains unknown. This property of disease resilience makes it to be regarded as a practical strategy with the contribution of both disease resistance and tolerance, because recording pathogen burden can be more difficult and costly than collecting some other animal performance traits. This is because pathogens can be distributed, non-uniformly, throughout multiple different cells, tissue, or organ compartments of the body, many of which are difficult to sample (Cunnington, 2015). Therefore, measuring pathogen burden is constrained to use samples that are readily accessible such as blood, urine, and feces by assuming that these are representative of total pathogen load (Cunnington, 2015). In the face of multiple pathogens in commercial pig farms, multiple different samples and methods (e.g. reverse-transcription or real-time polymerase chain reaction and enzyme-linked immunosorbent assay for some viruses and bacteria, plating and culture bacteria, fecal egg/worm count for parasites, etc.) may need to be used regarding the epidemiology of different pathogens. Although having animals (e.g. disease resistant animals) that exert control on pathogen burden can limit disease transmission in the population, it is not always necessary or cost-effective for the swine industry regarding the difficulty of monitoring pathogen burden. For example, there is no concern on disease transmission when the basic reproduction ratio (R_0, expected number of cases directly generated by one case in a completely susceptible population) of infection is lower than 1 (Bishop and MacKenzie, 2003; Heffernan et al., 2005). In that case, less than one naïve pig gets infected during the infectious period of a pig on average, and the disease will die out on its own (Heffernan et al., 2005). The epidemic can arise in the population when $R_0 > 1$ as

more than one naïve pig gets infected during the infectious period of a pig, and disease will propagate to susceptible animals (Bishop and MacKenzie, 2003; Heffernan et al., 2005). However, the epidemic may not necessarily be a concern for the profitability of the swine industry as long as the animals have been selected for disease resilience and can maintain high productivity regardless of pathogen burden.

Pathogen burden and disease transmission should not be overlooked regarding zoonotic pathogens. Pigs and pork products with high zoonotic pathogen burden can threaten human health, especially for workers in pig farms and consumers. In this case, another advantage of developing CBC traits for breeding disease resilient pigs arises. Although the pathogen burden is not directly measured for disease resilience, CBC traits, especially white blood cell traits, are measures of immune responses that play essential roles in controlling the pathogen lifecycle. Based on the results of this thesis, disease resilient animals are also likely to be primed to initiate more efficient immune responses during the infection, which will help to defend against pathogens and limit transmission of infection. However, since the pathogen burden was not recorded for this thesis, the effect of disease resilience on pathogen burden and infection transmission will need to be further explored and validated in the next step.

Furthermore, pathogen virulence evolution in response to changing host genotypes is often raised as a risk with genetic disease control strategies. Pathogen virulence is defined as the damage of pathogens done to the host, which is often measured by the magnitude of the morbidity and the increase of mortality resulting from the colonization and proliferation of the pathogen in a host (Mackinnon and Read, 2004; Margolis and Levin, 2008). The immune mechanisms employed by the host to defend against pathogens were thought to cause harmful effects on pathogen fitness and impose selection pressure on pathogens (Margolis and Levin,

2008). Therefore, it may fuel the arms race and co-evolution between host and pathogens, stimulate pathogen evolution toward high virulence or ways of evading and subverting the host immune defence (Gandon and Michalakis, 2000; Woolhouse et al., 2002). Although the hosts evolve simultaneously, pathogens could adapt to the environment much more rapidly than the hosts due to their much shorter generation intervals and the horizontal gene transfer in bacteria (Read et al., 2008; Carrillo-Bustamante et al., 2015). Breeding for disease resistance that the host exert control over pathogen lifecycle is likely to fuel the arms race between host and pathogen (Casadevall and Pirofski, 2001; Glass, 2012). In contrast, disease tolerance may form stable host-pathogen associations (mutualism) that give neither hosts nor pathogens an evolutionary incentive because there are no competitive mechanisms (Roy and Kirchner, 2000; Little et al., 2010). Disease resilience with contribution of both disease resistance and tolerance may balance the benefits and concerns to some degree, showing a tendency to fuel the arms race between host and pathogen, but less likely to be as significant as disease resistance. Therefore, continuous monitoring and collecting CBC data and pig productivity from commercial farms will be necessary to re-estimate genetic parameters and prediction accuracies of CBC traits on disease resilience frequently and repeatedly in future studies. In addition, random sampling of pathogen burden from a proportion of animals throughout infection, continuous monitoring of pig signals and clinical signs, and recording of morbidity and mortality may also need to be considered. All these together will help ensure the animals are being selected and bred in the right direction regarding pathogen virulence evolution and its associated changes of disease challenge.

8.3. CBC traits as phenotypes for breeding general resilience (robustness)

Apart from the infectious challenge, non-infectious environmental challenges, including animals' social status and stress, deterioration in environmental conditions and management,

such as extreme climatic conditions, poor air quality, low feed quality, may further intensify adverse effects on animal performance and productivity in response to the disease challenge on commercial farms (Knap, 2005; Nakov et al., 2019). For example, it has been found that the average daily gain of grower pigs can be reduced by 12% to 30% when aerial ammonia levels in the barn increased from 50 ppm to 150 ppm (Drummond et al., 1980). High ammonia exposure and *Ascaris suum* (one of the most common nematode parasites of pigs) infection together was found to result in a significantly higher percentage reduction (61%) in the average daily gain of pigs compared to either ammonia-exposed (32%) or infection (28%) alone (Drummond et al., 1981). Stress hormones (glucocorticoids) could disrupt the T-cell homeostasis and the balance between T_H1 and T_H2 by inducing suppression or enhancement of innate immune response and cytokine production (e.g. interleukins-4, -5, -6, -12, and interferon-γ), therefore, disturb immune function and increase animals' susceptibility to disease (Sapolsky et al., 2000; Salak-Johnson and McGlone, 2007). Heat stress is also a major economic concern in addition to the disease challenge, which decreases pork production, increases mortality, and more importantly, reduces feed intake of pigs (Gabler and Pearce, 2015; Cross et al., 2018, 2020). The estimated annual loss due to heat stress is nearly US$900 million in the U.S. pig industry alone (Pollmann, 2010; Mayorga et al., 2018).

In line with the above, a more general resilience (robustness) refers to the high production potential with high resilience to perturbations in intensive pig commercial farms (e.g. pathogens and stress caused by weaning, housing conditions, social environment, and heat) is becoming one of the most desirable attributes of pigs (Knap and Bishop, 2000; Knap, 2005). This thesis primarily focused on resilience to disease challenges as the high polymicrobial challenge level in the NDCM was expected to have the most significant effect on animal

performance. The stress effect on productivity has not been dissected in this study, although the stressors, for example, heat stress may exist in both quarantine barn and challenge barn during the summer season in the NDCM (**Chapter 3, section 3.3.4**), which may also impact animal performance in addition to infection.

Alterations in immune indices, for example, the elevated CBC trait of neutrophil concentration, have been found to be associated with pigs that had aggressive and submissive responses to heat and social stress (Morrow-Tesch et al., 1994). Additionally, the neutrophil concentration to lymphocyte concentration ratio (NLR) calculated based on CBC measures has also been gaining increasing attention as higher NLR can be a phenotype of physiological stress (Zahorec, 2001). Increased endogenous cortisol induced by stress has been known to increase the neutrophil concentration while simultaneously decreasing the lymphocyte concentration (Onsrud and Thorsby, 1981). Therefore, CBC traits may also have the potential to be further explored as valuable indicators and or phenotypes of robustness, including resilience to physiologic stress in addition to pathogens.

8.4. Overall summary of the discussion

Disease resilience is a complex trait composed of multiple biological functions, such as production, health, nutrient status, and other dynamic elements, including the efficiency of immune response and the rate of recovery from infection, which makes it hard to be appropriately characterized. Therefore, further exploration and integration of multiple phenotypes will assist with a better characterization of disease resilience. This thesis identified the potential of using CBC traits under disease as phenotypes of disease resilience. It also helps to explore the immunological mechanisms and genetic basis for disease resilience because the blood cells play multiple roles in the immune system to help defend against pathogens. This

thesis highlighted the potential of CBC traits that can be further investigated as valuable phenotypes for breeding disease resilience as CBC is a robust test that is routinely available in veterinary laboratories. More research is also needed to investigate the opportunity to use CBC traits for breeding more general resilience, including resilience to stressors, such as weaning, social interaction, housing, and heat stress, in addition to disease challenges. Collecting indicator traits and phenotypes of resilience can be laborious and costly. However, due to the high economic losses caused by pathogens and stressors in the pig industry, it would be valuable to further explore and integrate resilience into the breeding scheme. The promise of new automated, remote, monitoring technologies may make this even more useful. All of these together will contribute to the improvement of resilience (roburtness) to perturbations induced by pathogens and stressors, therefore, they are anticipated to improve the profitability and animal health of the pig industry. Last but not least, the improvement of disease resilience may also help reduce antimicrobial use and antimicrobial resistance, which will ultimately contribute to improving the "One Health" of the world and sustainability of the pork industry.

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