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# Proliferation of peripheral blood mononuclear cells from healthy piglets after mitogen stimulation as indicators of disease resilience

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## Abstract

Disease resilience refers to the productivity of an animal under disease. Given the high biosecurity of pig nucleus herds, traits that can be measured on healthy pigs and that are genetically correlated with disease resilience, that is, genetic indicator traits, offer a strategy to select for disease resilience. Our objective was to evaluate mitogen stimulation assays (**MSA**s) on peripheral blood mononuclear cells (**PBMC**s) from young healthy pigs as genetic indicators for disease resilience. Data were from a natural disease challenge in which batches of 60 or 75 naïve Yorkshire × Landrace piglets were introduced every 3 wk into a continuous flow barn that was seeded with multiple diseases. In this environment, disease resilience traits, including growth, treatment, and mortality rates, were recorded on 3,136 pigs that were genotyped with a high-density marker panel. PBMCs from 882 of these pigs from 19 batches were isolated from whole blood collected prior to the disease challenge and stimulated with five mitogens: concanavalin A (**ConA**), phytohemagglutinin (**PHA**), pokeweed mitogen (**PWM**), lipopolysaccharide (**LPS**), and phorbol myristate acetate (**PMA**). The proliferation of cells was evaluated at 48, 72, and 96 h and compared with unstimulated samples (rest count). Heritabilities of cell proliferation were estimated using a model with batch as a fixed effect and covariates of entry age; rest count; complete blood count proportions of lymphocytes, monocytes, eosinophils, and basophils; and pen, litter, and animal genetics as random effects. Heritability estimates were highest for response to ConA (0.30 ± 0.09, 0.28 ± 0.10, 0.17 ± 0.10, and 0.25 ±0.10 at 48, 72, and 96 h after stimulation and for area under the curve across the three time points, respectively). Estimates were in a similar range for response to PHA and PMA but low for PWM and LPS. Responses to ConA, PHA, and PMA were moderately genetically correlated with several disease resilience traits and in the expected direction, but individual estimates were not significantly different from zero due to large SEs. In conclusion, although validation is needed, MSAss, in particular based on ConA, show promise as genetic indicator traits for disease resilience.

**Key words:** disease resilience, mitogen, natural disease challenge, swine

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**Abbreviations**

| | |
|---|---|
| ADG | average daily gain |
| AllMOR | mortality rate for pigs in challenge nursery and finisher |
| AllTRT | number of health treatments per pig in challenge nursery and finisher |
| AUC | area under the curve |
| BIS | Blastogenic Index score |
| CBC | complete blood count |
| CDPQ | center de développement du porc du Québec |
| cNurADG | average daily gain in challenge nursery |
| ConA | concanvalin A |
| DRAA | disease resistance assay for animals |
| FinADG | average daily gain in finisher |
| FinMOR | mortality rate for pigs in finisher |
| FinTRT | number of health treatments per pig in finisher |
| HBSS | Hank's balanced salt solution |
| Hscore | health score |
| LPS | lipopolysaccharide |
| MOR | mortality rate |
| MSA | mitogen stimulation assay |
| NurMOR | mortality rate for pigs in challenge nursery |
| NurTRT | number of health treatments per pig in challenge nursery |
| PCBMC | peripheral blood mononuclear cell |
| PCVAD | porcine circovirus associated disease |
| PHA | phytohemagglutinin |
| PMA | phorbol myristate acetate |
| PRRS | porcine reproductive and respiratory syndrome |
| PWM | pokeweed mitogen |
| qNurADG | average daily gain in quarantine nursery |
| SNP | single-nucleotide polymorphism |
| TRT | number of health treatments |

## Introduction

Infectious disease remains a substantial problem in the U.S. swine industry. One such disease is porcine reproductive and respiratory syndrome (**PRRS**), which is estimated to cost the U.S. swine industry US$664 million each year (Holtkamp et al., 2013). PRRS virus is found in swine barns globally; can cause severe reproductive disease, interstitial pneumonia, and immune suppression; and increases the risk of secondary infections. Porcine circovirus-associated disease (**PCVAD**) was estimated to cost the British swine industry £52.6 million per year prior to the availability of a vaccine in 2008. In addition to PCVAD and PRRS, many other viral, bacterial, and parasitic pathogens affect commercial pig production, including *Mycoplasma hyopneumoniae*, swine influenza virus, porcine rotavirus, porcine epidemic diarrhea virus, *Escherichia coli*, *Lawsonia intracellularis*, *Streptococcus suis*, *Brachyspira hyodysenteriae*, and *Ascaris suum*.

Disease resilience is defined as the ability of an animal to maintain performance when exposed to disease (Albers et al., 1987) and has been identified as a preferred target for genetic improvement of livestock, compared with selection for resistance and/or tolerance (Mulder and Rashidi, 2017). Genetic improvement of disease resilience in pigs is complicated by the structure of the industry, with selection occurring in nucleus herds that have to be kept free of major diseases. A possible solution is the use of genetic indicators. A genetic indicator is a trait that is genetically correlated with a trait of interest (Chase and Lunney, 2019). A genetic indicator for disease resilience would be a trait that can be measured in the nucleus without infecting the nucleus herd. The main requirements for a genetic indicator are that it is variable and heritable and that it has a strong genetic correlation with the target trait. Possible genetic indicators for disease resilience are measures of immune response that can be taken on healthy animals (Wilkie et al., 2000). The immune system of the pig is complex but contains many components that are heritable (e.g., Flori et al., 2011). One method of measuring immune response is using mitogen stimulation assays (**MSAs**), which evaluates proliferation of peripheral blood mononuclear cells (**PBMCs**) following stimulation with a mitogen. Mitogens have a similar effect as antigens by resembling antagonistic pathogen exposure, but mitogen-induced mitosis of lymphocytes is triggered by activating mitogen-activated protein kinases (Ko et al., 1979). The proliferation of PBMCs following mitogen stimulation can be assessed in vitro to quantify cell-mediated immune response, reflecting the potential of an animal's immune response when exposed to infectious pathogens. In the early 2000s, a set of MSAs was developed for pigs based on stimulation of PBMCs with five mitogens (Hurnik et al., 2006). The MSA revealed significant differences in immune capacity among individuals. In the present study, the same five mitogens [1) concanavalin A [**ConA**], 2) phytohemagglutinin [**PHA**], 3) pokeweed mitogen [**PWM**], 4) lipopolysaccharide [**LPS**], and 5) phorbol myristate acetate [**PMA**]) were used to stimulate PBMCs isolated from the blood of nursery pigs prior to their exposure to a polymicrobial challenge. Concanavalin A has previously been documented to activate T-lymphocyte division in swine (Lin et al., 2012), while PHA has been found to activate T-lymphocyte mitosis in rats and humans (Piguet and Vassalli, 1972; Lamers et al., 1992) by activating the same subsets of T-lymphocytes as ConA. PWM and PMA are mitogens that are both known to activate both B- and T-lymphocytes in swine (Paul et al., 1979; Stepanova et al., 2019). Lastly, LPS is an endotoxin that is present in all Gram-negative bacteria, acts as a stimulator of the innate immune system, and has been shown to activate B-lymphocytes in a wide range of mammals (Tough et al., 1997).

MSAs have been used to measure the genetic basis of immune cell proliferation in two previous studies. Edfors-Lilja et al. (1994) used blood from 124 Yorkshire pigs at 8 wk of age and reported the heritability of ConA-induced immune cell proliferation in vitro at 48 h post-stimulation to be 0.38 ± 0.21. A similar study was performed by Flori et al. (2011), who investigated responses to an expanded set of mitogens, including ConA, PMA, and LPS, of 443 Yorkshire pigs at 8 wk of age, 3 wk after vaccination against *M. hyopneumoniae*. They estimated the heritability of immune cell proliferation in response to ConA, PMA, and LPS at 48 h to be 0.36 ± 0.20, 0.27 ± 0.20, and 0.31 ± 0.19, respectively.

Hypothesizing that the MSAs described in Hurnik et al. (2006) can be used as genetic indicators for disease resilience in pigs, the present study aimed to determine whether these MSAs can be used as genetic indicators for resilience to polymicrobial disease by estimating 1) heritabilities of immune cell proliferation following mitogen stimulation and 2) phenotypic and genetic correlations among immune cell proliferation responses and with disease resilience traits. Samples and data from a large-scale natural polymicrobial disease challenge study of grow-finish pigs (Putz et al., 2018; Cheng et al., 2020) were used for this study.

# Materials and Methods

## Natural disease challenge model

This study was carried out in accordance with the Canadian Council on Animal Care (CCAC) guidelines (https://www.ccac.ca/en/certification/about-certification). The protocol was approved by the Protection Committee of the Centre de Recherche en Sciences Animales de Deschambault (CRSAD) and the Animal Care and Use Committee at the University of Alberta (AUP00002227). The project was conducted and overseen by the Centre de développement du porc du Québec (**CDPQ**) under the oversight of the herd veterinarian together with project veterinarians. Data collection for this study started in late 2015 and concluded in early 2019.

This natural disease challenge study consisted of three phases, as previously described (Putz et al., 2018; Cheng et al., 2020): 1) a quarantine nursery, where the weaned piglets were first housed for around 19 d; (2) a challenge nursery, where the naïve piglets were first exposed to the disease challenge for around 28 d after quarantine; and 3) an adjoining grow-finishing unit, where the pigs recovered from the challenge and were raised until slaughter for on average 100 d. Every 3 wk, a new batch of 60 or 75 naïve Yorkshire × Landrace barrows was introduced into the quarantine nursery, alternating between the seven members of the PigGen Canada consortium, for a total of 3,139 pigs in 50 batches. The pigs that entered the natural disease challenge were considered naïve, originating from high health multiplication farms that were free of major infectious swine diseases, such as PRRS virus, porcine epidemic diarrhea virus, or *M. hyopneumoniae*. This was confirmed by routine testing by the multiplication farms, completion of a health survey prior to enrollment in the project, and a negative PRRS virus test prior to the entry of each batch in the quarantine nursery. All pigs were genotyped using a commercial 650K genotyping panel (Affymetrix), consisting of 658,692 single-nucleotide polymorphisms (**SNPs**). For more details on the genotyping and imputation of missing genotypes, see Putz et al. (2018).

The various pathogens of the natural disease challenge were initially established in the challenge barn by introducing infected pigs from nearby farms. The natural disease challenge was maintained through fence line contact of pigs from the new batch with pigs from the previous batch in the challenge nursery. By using natural transmission of common swine pathogens, the natural disease challenge replicated a severe diseased environment in a commercial swine barn. The following viruses were identified in the challenge barn (nursery and grow-finish combined): three strains of the PRRS virus, two serotypes of swine influenza virus, porcine circovirus type 2, and porcine rotavirus A. Bacterial pathogens identified included *M. hyopneumoniae, S. suis*, *Glasserella (Haemophilus) parasuis*, *Brachyspira hampsonii*, *Salmonella* spp., *Erysipelothrix rhusiopathiae*, and *Staphylococcus hyicus*. The presence of PRRS virus for each batch was confirmed by PCR testing at 21 or 28 d after entry into the disease challenge or by enzyme-linked immunosorbent assay (ELISA) 42 d after entry into the disease challenge. The presence of *Mycoplasma* and all influenza type A strains was confirmed by testing for antibodies by ELISA 18 wk after entry into the disease challenge. The presence of all other diseases was detected by passive surveillance, such as necropsy examination, or by fecal, oral fluid, or additional blood sampling. This was primarily done in response to specific health situations that warranted examination, such as high mortality, unusual deaths, or diarrhea outbreaks. The presence of the major pathogens in the disease challenge was generally consistent across batches. Influenza type A was below detectable levels before batch 11, but 25 of the 31 batches between batches 11 and 41 were found to be positive for influenza type A. The virus became undetectable again starting batch 42. Actinobacillus pleuropneumonia (APP) was identified in batch 9, brought in from one of the multiplier farms, but became undetectable after batch 20.

## Mitogen stimulation assays

Pigs used for the MSA included a randomly chosen 882 pigs from batches 13 to 38. MSA data were obtained only on a subset of pigs from these batches due to limitations in the number of samples that could be assayed. The assayed pigs included on average 2.3 (±1.1) piglets per litter from on average 1.03 (±1.02) litters per sow. On average, 3.4 (±1.02), 5.1 (±1.7), and 7.7 (±4.4) pigs were evaluated per pen in the quarantine nursery, the challenge nursery, and the finisher, respectively.

Blood samples used for the MSA were collected in the quarantine nursery, before exposure to the natural challenge. For batches 13 to 24, blood samples were collected at ~27 d of age, 6 d after arrival in the quarantine nursery. For logistical reasons, blood samples were collected at ~41 d of age for batches 25 to 38. Complete blood count (**CBC**) data were collected on all pigs from blood samples collected at ~27 d of age, as described by Bai et al. (2020).

Blood for the MSA was collected by CDPQ staff in 10 mL vacutainer sodium salt heparinized tubes placed immediately on crushed ice and transported on ice packs to the University of Laval on the same day. PBMCs were purified from the samples on the day of receipt by gradient centrifugation in 50 mL conical tubes at 400 × *g* for 10 min to separate the plasma, followed by mixing 1:1 in Hank's balanced salt solution (**HBSS**) and 12 mL of lymphocyte separation medium (Wisent Inc., St-Bruno, Québec, Canada, #cat: 305-010-CL), and then centrifugation at 600 × *g* for 30 min at 4 °C. The PBMC cell layer was then transferred into a new 50-mL conical tube with a Pasteur pipet and diluted by adding 40 mL of HBSS before centrifugation at 400 × *g* for 10 min. The cell pellets were then gently suspended in 2 mL $NH_4Cl$ solution for 5 min at 37 °C to lyse contaminating erythrocytes. Lysis was stopped by adding 20 mL of HBSS and centrifugation at 400 × *g* for 10 min. Each PBMC sample was then resuspended in 5 mL RPMI1640 (Wisent Inc., St-Bruno, Québec, Canada, #cat: 350045036). Cells were enumerated using the Trypan blue exclusion procedure (Rao and Otto, 1992). PBMCs were then diluted to a final concentration of $1.33 \times 10^5$ cells/mL in complete RPMI1640 containing 10% of decomplemented exosome-free fetal bovine serum (Sigma-Aldrich, St. Louis, MO), glutamine, nonessential amino acids, sodium pyruvate, and penicillin/streptomycin (Wisent Inc., St-Bruno, QC, Canada). The cultured cells were maintained in a $CO_2$ incubator (5%) at 37 °C in a humidified atmosphere.

For the MSA, five mitogens (ConA, PHA, PWM, LPS, and PMA) were strategically chosen to evaluate the proliferation capability of different types of immune cells (Supplementary Table S1). PBMCs were plated in 9-well plates (Costar round bottom) at 20,000 cells/well/150 µL, each row containing PBMCs from one pig. The first and last rows were empty cells used for background or positive DNA control measurements. Each mitogen was added to six wells in columns 7 to 12 at a 4× concentration in 50 µL of complete Roswell Park Memorial Institute (RPMI). PBMCs in columns 1 to 6 were used as technical replicates for resting or unstimulated cells, by adding 50 µL of complete RPMI. For each sample (columns 1 to 6), there were six wells for unstimulated cells and six wells for each mitogen at a specific time point,

resulting in six technical replicates per animal per mitogen per time point. The plates were incubated at 37 °C in 5% of $CO_2$ for 48, 72, or 96 h. After incubation, cell proliferation was quantified based on fluorometric estimation of total DNA content of cell populations using the Hoechst reagent, which is directly related to the number of cells in the assayed population (Strober, 2001). Briefly, the 96-well plates were centrifugated for 5 min at 400 × *g*, and the supernatant was gently aspirated. The cells were rinsed twice with 200 µL phosphate buffered saline containing $Ca^{++}$ 0.8 mM and $Mg^{++}$ 0.8 mM, before centrifugation. Cells were lysed by adding 100 µL lysis buffer (0.02% sodium dodecyl sulfate in 1X saline-sodium citrate) to each well, except for eight wells in rows 1 and 8, which were reserved for the DNA standard and background (four wells each). The plates were then incubated at 37 °C for 1 h with occasional swirling. Then, 100 µL of 40 µg/mL DNA was added to the four DNA standard wells and 100 µL of 1X SSC buffer to the four blank wells in rows 1 and 8. Then, 100 µL of 4 µg/mL Hoechst 33258 reagent in 1X SSC buffer was added to each well. The plates were then incubated with gentle agitation at room temperature for 5 min, wrapped in aluminum foil, and stored at −70 °C until fluorescence measurement. The latter was at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, altered as appropriate depending on the specific instrument used (either Cytofluor, Millipore, or Galaxy, BMG labs).

## Disease resilience traits

Disease resilience traits evaluated in the natural disease challenge were average daily gain in the challenge nursery (**cNurADG**) and in the finisher (**FinADG**); number of treatments in the challenge nursery (**NurTRT**), in the finisher (**FinTRT**), and across the challenge nursery and finisher (**ALLTRT**); and mortality in the challenge nursery (**NurMOR**), in the finisher (**FinMOR**), and across the nursery and finisher (**ALLMOR**). Although disease resilience is strictly defined based on the change in a trait (growth rate, number of treatments, and mortality) as a result of disease, for simplicity, we refer to these traits measured under disease as disease resilience traits. Treatments and mortalities were recorded by CDPQ staff. The number of treatments was a count of the total number of individual treatments that a pig received, which included any drug administered intramuscularly by injection but excluded group- and batch-level treatments administered via water or feed or by parenteral administration. If a pig received multiple drugs at the same time, each drug was considered a treatment. To account for the smaller number of days at risk for pigs that died, the number of treatments was scaled to a standard length of 180 d for the number of treatments to average age at slaughter, following Putz et al. (2018), to 27 d for number of treatments in the challenge nursery, and to 100 d for number of treatments in the finisher. Mortality was also measured as a phenotype for pigs that died in the challenge nursery, the finisher, or across both, with "0" for pigs that survived until the end of that phase and "1" for pigs that died prior to completing the phase. Subjective health scores were assigned to each pig by trained personnel at four time points, as described by Cheng et al. (2020): at 5 d after entry into the quarantine nursery (qHScore1, at ~26 d of age); 19 d after entry into the quarantine nursery (QHScore 2, at ~41 d of age); 2 wk after entry into the challenge nursery (cHScore); and 6 weeks after entry into the finisher (FinHScore). Scores were assigned on a scale of 1 (severe clinical symptoms of disease) to 5 (healthy condition).

For the finishing traits, FinADG, FinTRT, and ALLTRT, two sets of phenotypes were analyzed. The first set consisted of data for traits other than mortality for pigs that survived to slaughter, henceforth referred to as the survival data. The second set (expanded data) also included phenotypes on some pigs that died prior to slaughter in the finisher by imputation and expansion of their incomplete phenotypes, as described by Cheng et al. (2020). Only incomplete records that were imputed with sufficient accuracy, as defined by Cheng et al. (2020), were included in the expanded data set.

## Statistical analyses

For the MSA, two methods were used to adjust cell counts from the stimulated wells for each pig, mitogen, and time point based on the average cell counts in the corresponding unstimulated wells with resting cells at that time point, which will be referred to as the Rest Count: 1) the Blastogenic Index score (**BIS**), computed by dividing the average cell count of the stimulated wells by the Rest Count and 2) by including Rest Count as a covariate in the model for analysis of the average cell count of the stimulated wells, which will be referred to as the Stimulated Means data. A BIS score greater than one indicates greater cell proliferation, whereas a BIS score less than one indicates greater cell death (Han and Dadey, 1978). Data obtained using both approaches were edited by removing outliers that were more than three times the standard deviation away from the mean of the dataset. In total, 292 and 171 data points out of 13,230 total data points were removed for BIS and Stimulated Means, respectively. After outlier removal, the residuals from the model were checked for normality. Distributions were found to be skewed to the right and, therefore, a $\log_2$ transformation was applied to both the BIS and the Stimulated Means data prior to further analyses.

Data across the three time points were also combined into an area under the curve (**AUC**) for pigs and mitogens for which all three time points were available. The AUC for each pig and each mitogen was calculated by fitting a quadratic function to the log-transformed BIS or Stimulated Mean data by regression analysis and integrating the resulting quadratic function between the limits of 48 and 96 h to calculate the area under the quadratic function. For AUC based on the Stimulated Means data, in order to account for the effect of Rest Count at each time point, the Stimulated Mean at each time point was first adjusted for Rest Count based on the estimate of the covariate from the statistical model described in the following. Changes in log-transformed BIS or adjusted Stimulated Means between time points (delta traits) were also computed and analyzed for each mitogen (delta 72 – 48, 96 – 72, and 96 – 48 h).

The following univariate model was used to estimate the heritability of BIS for each mitogen at each time point, using ASReml 4.0 (Gilmour et al., 2009):

$$\begin{aligned} Y_{ijklm} &= \mu + \text{Batch}_j + b_1 * \text{Age}_i + b_2 * \text{Lymphocytes}_i \\ &+ b_3 * \text{Monocytes}_i + b_4 * \text{Eosinophils}_i + b_5 * \text{Basophils}_i \\ &+ \text{Animal}_i + \text{Litter}_k + \text{Pen}_{l(j)} + \text{Plate}_{m(j)} + e_{ijklm} \end{aligned}$$

where $Y_{ijklm}$ = the $\log_2$(BIS) for animal i for a given mitogen at a given time point; Batch = fixed effect of batch; Age = the age at entry into the quarantine nursery; lymphocytes, monocytes, eosinophils, and basophils = the proportion of each immune cell type from the CBC data; Animal = random animal genetic effect of the ith individual, assumed distributed ~N(0, $\mathbf{G}\sigma_g^2$), with **G** equal to the genomic relationship matrix constructed using SNP genotypes, with pigs from different companies assumed unrelated (see Cheng et al., 2020), and $\sigma_g^2$ equal to the genetic variance; Litter = litter effect; Pen = random effect of

pen nested within Batch (50 levels); Plate = random effect of plate nested within batch (146 levels with six pigs per plate); and e = residual. For analysis of the Stimulated Mean data, Rest Count was added to the model as a covariate. Proportions of cell types from the CBC data were used as covariates to relate the MSAs back to the individual animal response by compensating for differences in the whole blood subset population for each animal. By controlling for cell counts in the assay, an animal's individual response is related to proliferative response and the proportion of lymphocytes in the whole blood. The proportions of neutrophils and of "large unidentified cells," as obtained from the CBC data, were not included in the model because the proportion of neutrophils was highly correlated with the proportion of lymphocytes ($r$ = 0.91), while the "large unidentified cells" were determined to be mostly agglutinated cells. Fitting separate cell count covariates for MSA conducted on blood collected at ~27 vs. ~41 d of age, with CBC always taken at the ~27 d time point, did not result in significant differences in covariate effects and, therefore, only one covariate per cell type was fitted in the final model. Variance component estimates from the univariate analyses were used to estimate the narrow-sense heritability ($h^2$) for each MSA trait, as well as variance due to litter effects as a proportion of phenotypic variance ($c^2$). Phenotypic variance was estimated as the sum of estimates of variances due to animal genetics, litter, and residuals.

Bivariate analyses were conducted to obtain estimates for four sets of genetic and phenotypic correlations. The first set consisted of correlations between the BIS and the corresponding Stimulated Mean phenotypes to compare the two approaches to account for Rest Count. The second set consisted of correlations between time points and AUC for the same mitogen for the BIS and the Stimulated Mean data. The third set consisted of correlations between mitogens at the same time point and for AUC for the BIS and Stimulated Mean data. The fourth set consisted of correlations of the MSA traits with each of the disease resilience traits evaluated. Correlations were estimated using bivariate models in ASReml 4.0, using the model described above for the MSA traits and the models described in Cheng et al. (2020) for the disease resilience traits. For the latter, resilience data from all 3,139 pigs across 50 batches were used, using their genomic relationships with pigs with MSA data.

The first set of correlations could not be estimated using ASReml 4.0 (no convergence) and were, instead, estimated by analyzing the sum of the BIS and the Stimulated Mean data as a phenotype, using the univariate model described above to estimate its phenotypic and genetic variances. Prior to summing, the Stimulated Mean data were pre-adjusted for Rest Count using the estimate of the regression coefficient that was obtained from the univariate analysis of each MSA trait. The covariance (genetic or phenotypic) between the BIS and Stimulated Mean phenotypes was then estimated by rearranging the formula for the variance of a sum as:

$$\mathrm{Var}(\mathrm{BIS}+\mathrm{StimMean}) = \mathrm{Var}(\mathrm{BIS}) + \mathrm{Var}(\mathrm{StimMean}) + 2\mathrm{Cov}(\mathrm{BIS}, \mathrm{StimMean})$$

$$\mathrm{Cov}(\mathrm{BIS}, \mathrm{StimMean}) = \frac{\mathrm{Var}(\mathrm{BIS}+\mathrm{StimMean}) - \mathrm{Var}(\mathrm{BIS}) - \mathrm{Var}(\mathrm{StimMean})}{2}$$

Estimates of genetic and phenotypic variances for the individual phenotypes used in these derivations were as obtained from the univariate analyses.

# Results

## Basic statistics of the MSA phenotypes

Descriptive statistics of the MSA assay measurements are presented in Table 1. For average Rest Count, ConA showed a general increase from 48 to 96 h. This proliferation of unstimulated cells is unexpected, since growth factors are in limited supply in the blood sample. For the other four mitogens, the average Rest Count either went down or was relatively stable between 48 and 96 h, as expected, since cells expend energy to maintain homeostasis in a resting state. For the stimulated wells, there were large numerical changes in cell counts and observable patterns between 48 and 96 h. For PWM, the largest mean cell count was at 48 h, while for ConA, the largest count was at 72 h. For PHA, LPS, and PMA, the largest count was at 96 h. The within-sample coefficient of variation (CV) was between 13% and 17% for the resting cell counts but generally higher, up to 29%, for the stimulated cell counts. These sizeable CVs were

**Table 1.** Mean and within-sample CV across six replicates for the number of live cells in unstimulated (Rest Count) and stimulated samples (Stim Mean)[1]

| Mitogen | Time | Number of samples | Rest Count | | Stim Mean | |
|---|---|---|---|---|---|---|
| | | | Mean | CV, % | Mean | CV, % |
| ConA | 48 | 860 | 4,971.9 | 15.0 | 6,700.0 | 21.5 |
| | 72 | 873 | 5,590.9 | 12.9 | 8,313.7 | 21.3 |
| | 96 | 871 | 5,719.7 | 13.1 | 6,679.8 | 16.3 |
| PHA | 48 | 860 | 5,814.7 | 13.1 | 6,548.6 | 14.0 |
| | 72 | 872 | 5,029.5 | 13.3 | 7,289.5 | 22.6 |
| | 96 | 855 | 4,224.8 | 14.0 | 7,640.8 | 24.6 |
| PWM | 48 | 866 | 4,003.3 | 15.4 | 8,482.5 | 28.7 |
| | 72 | 858 | 3,970.6 | 15.0 | 5,091.5 | 19.6 |
| | 96 | 855 | 4,052.4 | 15.7 | 4,578.6 | 15.6 |
| LPS | 48 | 859 | 3,999.7 | 15.2 | 7,116.5 | 25.0 |
| | 72 | 862 | 3,811.4 | 15.9 | 6,768.2 | 24.8 |
| | 96 | 856 | 4,043.4 | 15.3 | 7,608.4 | 26.0 |
| PMA | 48 | 854 | 4,063.1 | 16.1 | 4,737.9 | 18.6 |
| | 72 | 868 | 4,039.1 | 16.0 | 4,412.2 | 15.5 |
| | 96 | 869 | 4,201.6 | 16.8 | 6,856.8 | 24.9 |

[1]Each sample refers to the number of pigs that were available for that mitogen and time point.

offset by the six replicates that were run per sample, mitogen, and time point.

Descriptive statistics of the log-transformed BIS and Stimulated Mean phenotypes are presented in Table 2. The averages of the Stimulated Mean phenotypes were comparable across the five mitogens, ranging from 12.0 to 12.7 for individual time points, from 1,671 to 1,759 for AUC, and from −0.4 to 0.2 for the delta phenotypes. This consistency was not evident for the BIS phenotypes. Notably, the means for individual time points and AUC were substantially larger for ConA, PHA, and PMA than for PWM and LPS. In addition, for each mitogen, the largest average BIS was at 72 h.

The CBC cell composition covariates were significant for a number of the MSA phenotypes (Supplementary Table S3) but generally explained a limited amount of variance for most of the mitogens and time points, including for AUC and the delta traits. Although eosinophils and basophils are not present in the isolated PBMCs that the assays were performed on, their proportions were included in the model for completeness as they only took up one degree of freedom each and they were significant for a number of traits.

Plate explained less than 0.2% of the total variance across all mitogens and time points, including AUC and the delta traits (see Supplementary Table S4). Pen on average explained 24.4% (range from 11% to 37%) of the total variance across all mitogens and time points, including AUC and delta traits.

## Heritability of MSA phenotypes

Heritability for each MSA phenotype was estimated for each mitogen for both the BIS and Stimulated Mean data and is presented in Table 2. In general, estimates of heritability were numerically very similar for the BIS and the Stimulated Means data, and, therefore, only estimates for BIS will be discussed in the remainder of this section. Estimates of litter effects were very small for most of the MSA phenotypes (Table 2), with most of the estimates being zero, suggesting that the environment provided by the sow had a limited impact on the MSA phenotypes.

Estimates of heritability at individual time points were moderate ($0.19 \pm 0.09$ to $0.28 \pm 0.10$) for ConA and PHA at all three time points and for PMA at 72 h ($0.29 \pm 0.09$) but low for PWM and LPS across all time points (<0.10) and for PMA at 48 ($0.12 \pm 0.08$) and 96 h ($0.04 \pm 0.06$). For all mitogens, except LPS, the largest estimate of heritability was at 72 h. This pattern was consistent with the BIS values being highest at 72 h for most of the mitogens.

Heritability estimates for AUC were similar to the highest estimate for individual time points for each mitogen, except for PHA, for which the estimate of heritability of AUC was higher. The delta phenotypes showed no clear patterns in heritability estimates. For ConA, PWM, and PMA, delta from 48 to 72 h had heritability estimates that were higher or as high as the most heritable individual time point. Estimates of heritabilities for most of the other delta phenotypes were of the same magnitude or smaller than those for individual time points.

## Correlations between BIS and stimulated mean phenotypes

Estimates of genetic and phenotypic correlations between BIS and their corresponding simulated mean phenotypes are presented in Table 2, comparing the two methods of accounting for Rest Count. Because of lack of convergence of estimates with ASReml, these correlations were calculated based on an alternate method. This method does not restrict estimates to the parameter space, resulting in some estimates to be greater than 1. In general, estimates of phenotypic correlations between BIS and the corresponding Stimulated Mean phenotypes were high. Estimates of genetic correlations were also high but very variable, reflecting their large SEs. The average of estimates of genetic correlations between the BIS and their corresponding Stimulated Mean phenotypes within a mitogen across all time points were 0.86, 1.11, 0.67, 1.27, and 1.20 for ConA, PHA, PWM, LPS, and PMA, respectively. These high genetic correlations suggest that selecting on BIS and on Stimulated Means would have similar effects.

## Correlations among time points within the same mitogen

Estimates of genetic correlations between time points and with AUC for the same mitogen are presented in Table 3, while the corresponding phenotypic correlations are presented in Supplementary Table S5. For both the BIS and the Stimulated Mean phenotypes, all genetic correlation estimates were positive. Some estimates were large but all had substantial SEs. Regardless of these large SEs, it is notable that estimates based on BIS were similar to corresponding estimates based on Stimulated Means for each pair of time points for ConA and PHA. This was as expected, as the BIS and the corresponding Stimulated Mean phenotypes had high genetic correlations for these mitogens (Table 2).

## Correlations among mitogens at the same time point

Estimates of genetic correlations among mitogens at the same time point are presented in Table 4, and corresponding phenotypic correlations are presented in Supplementary Table S6. Similar to the genetic correlations between time points for the same mitogen, several genetic correlations could not be estimated, especially those that involved PWM and LPS phenotypes. All genetic correlation estimates were positive and some were highly positive (>0.90). Considering the large SEs, genetic correlation estimates between mitogens based on BIS were similar to those based on Stimulated Mean phenotypes. Genetic correlation estimates between ConA and PHA for corresponding MSA phenotypes were consistently strong, which was as expected, since these two mitogens activate similar cell types.

## Correlations of MSA with disease resilience traits

Descriptive statistics of the evaluated performance and resilience traits are described in Cheng et al. (2020) for the full data set and presented in Supplementary Table S2 for the pigs with MSA phenotypes analyzed here. Reasons for treatments and mortalities and their frequencies are summarized in Supplementary Table S7. Infection and disease were responsible for nearly all of the prescribed treatments and mortalities. Respiratory distress accounted for 27.9% of treatments and 19.2% of mortalities. Noninfectious causes only accounted for a small portion of reasons.

Estimates of genetic correlations of MSA phenotypes with disease resilience traits are shown in Figures 1–4 and corresponding phenotypic correlations are presented in Supplementary Tables S8 for BIS and in Supplementary Table S9 for Stimulated Means data. Estimates of phenotypic correlations were low, suggesting limited opportunities to predict the resilience of an individual pig based on its MSA phenotypes.

**Table 2.** Descriptive statistics (mean and SD) and estimates of heritability ($h^2$) and litter effects ($c^2$) (SE in parentheses) for $log_{(2)}$-transformed BIS and stimulated mean phenotypes for each of five mitogens at three time points (48, 72, and 96 h after stimulation), and for AUC and for changes in phenotypes between time points (72 – 48, 96 – 48, and 96 – 72), as well as estimates of phenotypic ($r_p$) and genetic ($r_g$) correlations between corresponding BIS and Stimulated Mean phenotypes

| | | BIS | | | | | Stimulated Mean | | | | | Correlation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mitogen | Time[1] | Number of pigs | Mean | SD | $h^2$ (SE) | $c^2$ (SE) | Number of pigs | Mean | SD | $h^2$ (SE) | $c^2$ (SE) | $r_p$ | $r_g$ |
| ConA | 48 | 860 | 0.3 | 0.37 | 0.24 (0.08) | 0.00 (0.00) | 863 | 12.4 | 0.9 | 0.30 (0.09) | 0.00 (0.00) | 0.89 | 0.62 |
| | 72 | 873 | 0.7 | 0.63 | 0.27 (0.09) | 0.00 (0.00) | 868 | 12.6 | 0.89 | 0.28 (0.10) | 0.00 (0.00) | 0.97 | 1.04 |
| | 96 | 871 | 0.66 | 0.61 | 0.23 (0.10) | 0.00 (0.00) | 866 | 12.5 | 0.83 | 0.17 (0.10) | 0.04 (0.05) | 0.89 | 0.93 |
| | AUC | 850 | 43.43 | 36.45 | 0.24 (0.09) | 0.00 (0.00) | 859 | 1,743 | 52.53 | 0.25 (0.10) | 0.01 (0.05) | 1.04 | 1.1 |
| | 72 – 48 | 860 | 0.39 | 0.56 | 0.37 (0.09) | 0.00 (0.00) | 861 | –0.4 | 0.78 | 0.36 (0.10) | 0.00 (0.00) | 1.21 | 0.71 |
| | 96 –72 | 871 | –0.03 | 0.53 | 0.14 (0.09) | 0.00 (0.00) | 865 | 0 | 0.75 | 0.12 (0.09) | 0.00 (0.00) | 1.04 | 0.82 |
| | 96 – 48 | 859 | 0.35 | 0.56 | 0.25 (0.10) | 0.00 (0.05) | 860 | 0.4 | 0.93 | 0.24 (0.09) | 0.00 (0.00) | 0.89 | 0.81 |
| PHA | 48 | 860 | 0.48 | 0.43 | 0.19 (0.09) | 0.02 (0.05) | 862 | 12.7 | 1.02 | 0.07 (0.09) | 0.02 (0.06) | 0.84 | 1.86 |
| | 72 | 872 | 0.85 | 0.73 | 0.28 (0.09) | 0.00 (0.00) | 866 | 12.7 | 0.97 | 0.33 (0.10) | 0.00 (0.00) | 0.89 | 1.07 |
| | 96 | 855 | 0.71 | 0.76 | 0.22 (0.10) | 0.04 (0.05) | 849 | 12.5 | 0.95 | 0.22 (0.10) | 0.04 (0.05) | 0.9 | 0.95 |
| | AUC | 836 | 54.85 | 44.89 | 0.34 (0.09) | 0.00 (0.00) | 857 | 1,759 | 60.78 | 0.28 (0.10) | 0.00 (0.00) | 0.9 | 0.75 |
| | 72 – 48 | 859 | 0.37 | 0.58 | 0.11 (0.08) | 0.00 (0.00) | 857 | –0.4 | 0.9 | 0.09 (0.08) | 0.00 (0.00) | 0.81 | 1.24 |
| | 96 – 72 | 854 | –0.15 | 0.55 | 0.12 (0.08) | 0.00 (0.00) | 846 | 0.1 | 0.63 | 0.10 (0.07) | 0.00 (0.00) | 1.01 | 0.89 |
| | 96 – 48 | 842 | 0.23 | 0.62 | 0.16 (0.09) | 0.03 (0.06) | 845 | 0.2 | 1.08 | 0.11 (0.10) | 0.01 (0.06) | 0.74 | 1.01 |
| PWM | 48 | 866 | 0.14 | 0.29 | 0.00 (0.00) | 0.01 (0.05) | 863 | 12.3 | 0.91 | 0.00 (0.09) | 0.01 (0.06) | 0.96 | 1.44 |
| | 72 | 858 | 0.32 | 0.32 | 0.10 (0.08) | 0.00 (0.00) | 864 | 12.2 | 0.58 | 0.15 (0.09) | 0.00 (0.00) | 0.83 | 0.62 |
| | 96 | 855 | 0.2 | 0.37 | 0.09 (0.08) | 0.00 (0.00) | 865 | 12.1 | 0.55 | 0.07 (0.08) | 0.00 (0.00) | 0.82 | 0.7 |
| | AUC | 848 | 20.11 | 19.14 | 0.03 (0.06) | 0.00 (0.00) | 848 | 1,684 | 37.33 | 0.09 (0.08) | 0.00 (0.00) | 0.74 | 2.1 |
| | 72 – 48 | 856 | 0.18 | 0.37 | 0.14 (0.09) | 0.00 (0.00) | 856 | –0.2 | 0.82 | 0.14 (0.08) | 0.00 (0.00) | 0.96 | 0.83 |
| | 96 – 72 | 856 | -0.12 | 0.42 | NE[2] | NE | 856 | 0.1 | 0.59 | NE | NE | NE | NE |
| | 96 – 48 | 848 | 0.06 | 0.38 | 0.15 (0.09) | 0.00 (0.00) | 855 | 0.1 | 1.02 | 0.03 (0.08) | 0.00 (0.00) | 0.65 | 0.21 |
| LPS | 48 | 859 | 0.13 | 0.23 | 0.00 (0.00) | 0.00 (0.00) | 863 | 12.3 | 0.88 | 0.00 (0.09) | 0.00 (0.00) | NE | NE |
| | 72 | 862 | 0.17 | 0.27 | 0.05 (0.07) | 0.00 (0.00) | 868 | 12.1 | 0.52 | 0.10 (0.09) | 0.00 (0.00) | 1.18 | 0.18 |
| | 96 | 856 | 0.13 | 0.29 | 0.07 (0.09) | 0.03 (0.06) | 864 | 12 | 0.52 | 0.00 (0.09) | 0.04 (0.06) | 1.34 | 2.02 |
| | AUC | 834 | 11.11 | 16.52 | 0.03 (0.06) | 0.00 (0.00) | 854 | 1,671 | 33.93 | 0.00 (0.09) | 0.08 (0.05) | 0.6 | 0.41 |
| | 72 – 48 | 849 | 0.04 | 0.27 | NE | NE | 849 | 0 | 0.72 | 0.10 (0.09) | 0.00 (0.00) | 1.71 | -0.43 |
| | 96 – 72 | 846 | –0.03 | 0.29 | NE | NE | 846 | 0 | 0.55 | 0.00 (0.00) | 0.00 (0.00) | NE | NE |
| | 96 – 48 | 844 | 0.01 | 0.29 | NE | NE | 844 | 0 | 0.99 | 0.00 (0.00) | 0.04 (0.05) | 1.28 | 4.17 |
| PMA | 48 | 854 | 0.39 | 0.42 | 0.12 (0.08) | 0.00 (0.00) | 864 | 12.5 | 0.89 | 0.10 (0.09) | 0.00 (0.01) | 1.04 | 0.86 |
| | 72 | 868 | 0.66 | 0.62 | 0.29 (0.09) | 0.01 (0.05) | 867 | 12.5 | 0.96 | 0.22 (0.09) | 0.01 (0.05) | 1.19 | 1.29 |
| | 96 | 869 | 0.55 | 0.7 | 0.04 (0.06) | 0.00 (0.00) | 867 | 12.4 | 0.89 | 0.03 (0.06) | 0.00 (0.00) | 0.97 | 1.48 |
| | AUC | 851 | 43.51 | 37.35 | 0.27 (0.10) | 0.04 (0.06) | 861 | 1,716 | 61.37 | 0.30 (0.10) | 0.00 (0.00) | 0.78 | 1.21 |
| | 72 – 48 | 852 | 0.27 | 0.53 | 0.26 (0.09) | 0.00 (0.00) | 861 | –0.3 | 0.76 | 0.14 (0.08) | 0.00 (0.00) | 1.13 | 1.51 |
| | 96 – 72 | 865 | –0.11 | 0.56 | 0.05 (0.06) | 0.00 (0.04) | 867 | 0.1 | 0.83 | 0.00 (0.00) | 0.00 (0.00) | 1.04 | 1.22 |
| | 96 – 48 | 852 | 0.15 | 0.64 | 0.08 (0.08) | 0.01 (0.05) | 861 | 0.2 | 0.9 | 0.13 (0.10) | 0.01 (0.05) | 1.03 | 0.86 |

[1]48, 48 h; 72, 72 h; 96, 96 h; 72 – 48, difference between 72 and 48 h; 96 – 72, difference between 96 and 72 h; 96 – 48, difference between 96 and 48 h.
[2]NE, not estimable.

**Table 3.** Estimates of genetic correlations for BIS (below diagonal) and Stimulated Means (above diagonal) (SE in parentheses) between time points (48, 72, or 72 h after stimulation) and for AUC for each of five mitogens

| ConA | 48 | 72 | 96 | AUC |
|---|---|---|---|---|
| 48[1] | | 0.42 (0.21) | 0.53 (0.23) | 0.87 (0.12) |
| 72[1] | 0.24 (0.25) | | 0.11 (0.66) | NE |
| 96[1] | 0.51 (0.22) | 0.78 (0.15) | | 0.82 (0.14) |
| AUC | 0.41 (0.22) | NE | 0.90 (0.10) | |
| PHA | 48 | 72 | 96 | AUC |
| 48 | | NE | 0.73 (0.24) | NE |
| 72 | 0.86 (0.14) | | 0.91 (0.09) | NE |
| 96 | 0.68 (0.17) | 0.83 (0.11) | | 0.92 (0.12) |
| AUC | 0.36 (0.22) | NE | 0.83 (0.10) | |
| PWM | 48 | 72 | 96 | AUC |
| 48 | | 0.53 (0.31) | 0.45 (0.55) | 0.56 (0.69) |
| 72 | NE[2] | | 0.41 (0.80) | 0.13 (0.91) |
| 96 | NE | 0.67 (0.43) | | NE |
| AUC | NE | NE | NE | |
| LPS | 48 | 72 | 96 | AUC |
| 48 | | 0.27 (0.41) | 0.29 (0.44) | 0.93 (0.25) |
| 72 | NE | | 0.44 (0.41) | 0.09 (0.84) |
| 96 | NE | NE | | 0.89 (0.56) |
| AUC | NE | NE | NE | |
| PMA | 48 | 72 | 96 | AUC |
| 48 | | 0.78 (0.24) | 0.24 (0.63) | NE |
| 72 | 0.65 (0.21) | | NE | 0.36 (0.46) |
| 96 | 0.46 (0.66) | NE | | 0.21 (0.45) |
| AUC | 0.76 (0.14) | NE | NE | |

[1]48, 48 h; 72, 72 h; 96, 96 h.
[2]NE, not estimable.

**Table 4.** Estimates of genetic correlations for BIS (below diagonal) and Stimulated Mean (above diagonal) data (SE in parentheses) between mitogens at a given time point (48, 72, or 72 h after stimulation) and for AUC

| Trait | ConA 48 | PHA 48 | PWM 48 | LPS 48 | PMA 48 |
|---|---|---|---|---|---|
| ConA 48 | | 0.96 (0.72) | NE | 0.00 (0.00) | NE |
| PHA 48 | 0.65 (0.19) | | NE | 0.00 (0.00) | 0.25 (0.79) |
| PWM 48 | NE[1] | NE | | 0.00 (0.00) | NE |
| LPS 48 | NE | NE | NE | | NE |
| PMA 48 | NE | 0.73 (0.32) | NE | NE | |
| Trait | ConA 72 | PHA 72 | PWM 72 | LPS 72 | PMA 72 |
| ConA 72 | | 0.74 (0.13) | 0.00 (0.00) | 0.40 (0.29) | 0.92 (0.19) |
| PHA 72 | 0.55 (0.17) | | 0.00 (0.00) | 0.77 (0.27) | 0.89 (0.16) |
| PWM 72 | 0.00 (0.00) | 0.00 (0.00) | | NE | NE |
| LPS 72 | 0.65 (0.70) | NE | NE | | 0.53 (0.34) |
| PMA 72 | 0.71 (0.16) | 0.91 (0.12) | 0.47 (0.31) | 0.67 (1.17) | |
| Trait | ConA 96 | PHA 96 | PWM 96 | LPS 96 | PMA 96 |
| ConA 96 | | 0.64 (0.19) | 0.87 (0.33) | 0.65 (1.19) | NE |
| PHA 96 | 0.62 (0.16) | | 0.44 (0.83) | NE | NE |
| PWM 96 | 0.28 (0.50) | 0.88 (0.37) | | 0.45 (0.71) | 0.57 (0.74) |
| LPS 96 | 0.60 (0.47) | 0.13 (0.43) | NE | | NE |
| PMA 96 | NE | 0.34 (0.72) | NE | NE | |
| Trait | ConA AUC | PHA AUC | PWM AUC | LPS AUC | PWM AUC |
| ConA AUC | | 0.74 (0.12) | 0.96 (0.21) | 0.15 (0.44) | 0.87 (0.17) |
| PHA AUC | 0.60 (0.15) | | 0.98 (0.11) | 0.74 (0.36) | 0.78 (0.11) |
| PWM AUC | 0.99 (0.89) | NE | | 0.92 (0.50) | NE |
| LPS AUC | 0.66 (0.94) | 0.04 (0.74) | NE | | 0.92 (0.50) |
| PMA AUC | 0.76 (0.16) | 0.86 (0.11) | NE | NE | |

[1]NE, not estimable.

For genetic correlations, estimates are only presented for ConA, PHA, and PMA because the low heritabilities for PWM and LPS resulted in genetic correlation estimates with very large SEs or that could not be estimated. Even the estimates for the ConA, PHA, and PMA had large SEs and no individual estimate was statistically significantly different from 0 ($P > 0.05$) based on a likelihood ratio test (King, 2010). Nevertheless, several MSA phenotypes showed consistent trends in estimates across time

points and across resilience traits and in the expected direction, and these are described in more detail later. For the finisher traits, estimates of genetic correlations were similar between the survival and expanded data (Supplementary Figure S1). Therefore, only estimates for the survival data will be described in the following.

### *Growth rate*

Estimates of genetic correlations of BIS phenotypes with growth rate in the quarantine nursery (**qNurADG**), the challenge nursery (cNurADG), and in the finisher (FinADG) are shown in Figure 1. The overall trend of estimates of genetic correlations of BIS phenotypes with growth rate in the quarantine nursery

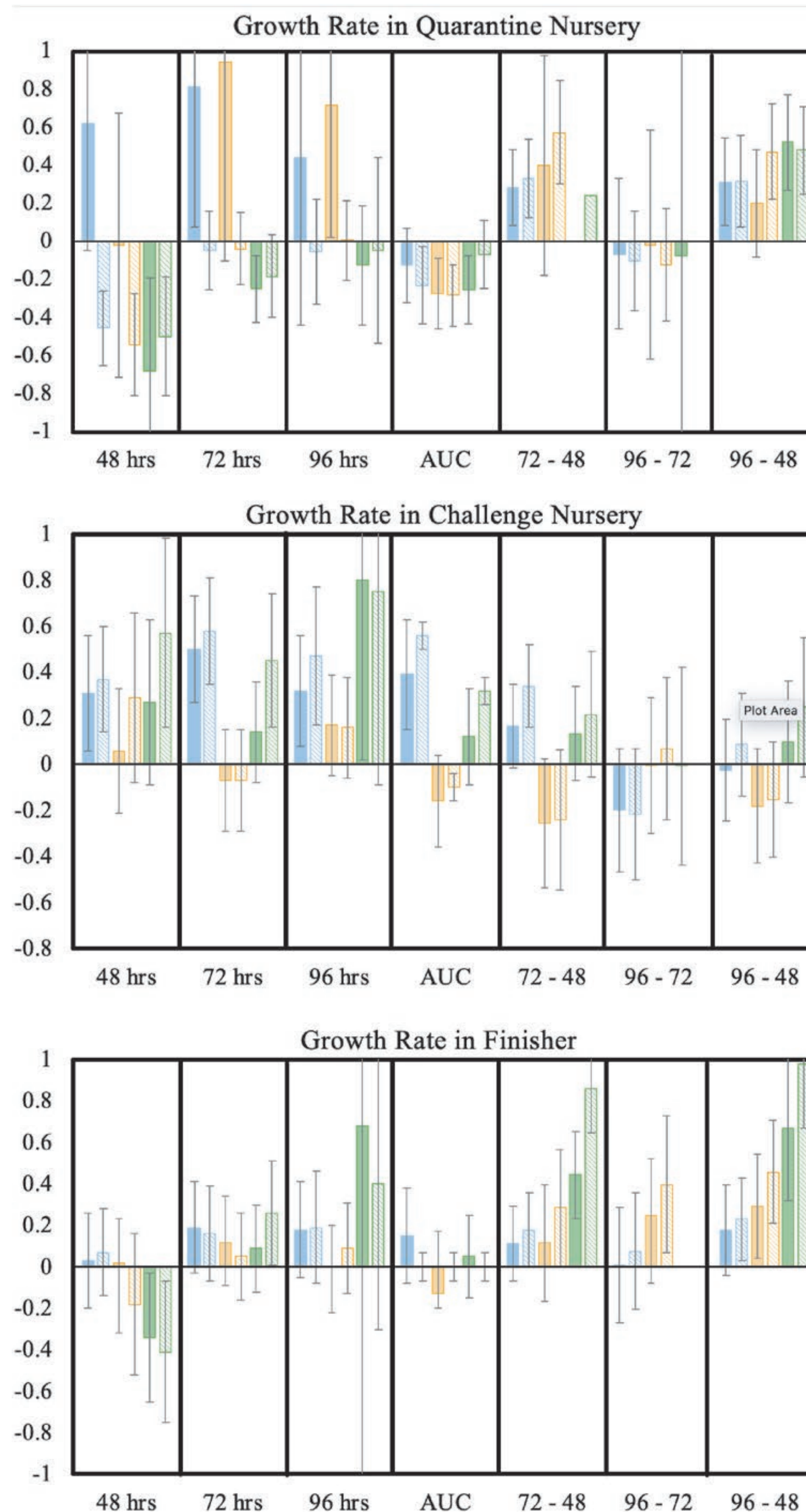


**Figure 1.** Estimates of genetic correlations (and SE bars) of MSA phenotypes with growth rate traits for ConA (blue), PHA (yellow), and PMA (green), using BIS (solid bars) and Stimulated Mean (striped bars) MSA phenotypes.

was inconclusive. Associations of MSA phenotypes with growth rate in the quarantine nursery were expected to be weak since exposure to disease-causing pathogens was expected to be minimal at this stage. Some genetic correlation estimates with qNurADG differed substantially and had opposite signs when based on BIS vs. Stimulated Mean data, for example, for ConA and PHA at 48, 72, and 96 h. However, SEs were large. Estimates of genetic correlations of qNurADG were negative but weak with AUC for all three mitogens but generally positive with delta 72 – 48 and 96 – 48 and negative for 96 – 72.

Estimates of genetic correlations with cNurADG were positive for ConA and PMA at individual time points and for AUC (Figure 1), as expected. Corresponding estimates for PHA were weak and variable in sign. The delta phenotypes also had weak and variable genetic correlation estimates with cNurADG. Differences between estimates based on BIS vs. Stimulated Means were generally small, except for PHA at 48 h, for ConA delta 96 – 48, and for PHA delta 96 – 72.

Estimates of genetic correlations of MSA phenotypes at individual time points and for AUC with growth rate in the finisher were generally weak (Figure 1). Genetic correlations with AUC based on stimulated means did not converge. Estimates of genetic correlations of FinADG with the delta phenotypes were all positive. Among the three mitogens, PMA phenotypes had the strongest estimates of genetic correlations with FinADG but with large SEs. Differences between estimates based on BIS and Stimulated Means were minimal for FinADG, except for PMA at 72 and 96 h.

### *Treatment rates*

Estimates of genetic correlations of MSA phenotypes with numbers of treatments in the challenge nursery are shown in Figure 2. The majority of genetic correlations with number of treatments in the finisher could not be estimated because the heritability of this resilience trait was very small and is, therefore, not shown. Correlations that could be estimated had extremely high SEs and showed no clear trend.

Estimates of genetic correlations with number of treatments in the challenge nursery were generally negative for MSA phenotypes at individual time points and for AUC (Figure 2). For the delta MSA phenotypes, genetic correlation estimates with number of treatments in the challenge nursery were generally negative for ConA and PMA but positive for PHA. Differences between estimates based on BIS and Stimulated Means were small. However, for PMA at 48 and 96 h, the sign of the estimates differed between these two approaches. Estimates of genetic correlations of the number of treatments across the challenge nursery and finisher with MSA phenotypes generally followed similar trends as observed for treatments in the challenge nursery (Figure 2), except for the delta phenotypes.

### *Mortality*

Estimates of genetic correlations of MSA phenotypes with mortality are shown in Figure 3. For mortality in the challenge nursery, estimates of genetic correlations with MSA phenotypes generally followed expected trends, with negative estimates for individual time points and AUC. The estimates were negative for delta 72 – 48 and 96 – 48 for all three mitogens but positive for delta 96 – 72, but with large errors.

For mortality in the finisher, estimates of genetic correlations with MSA phenotypes at individual time points were similar to those observed for mortality in the nursery (Figure 3), but the estimates with AUC were much weaker with mortality in the finisher than in the challenge nursery. Estimates of mortality in the finisher with delta phenotypes were positive for ConA and PHA but negative for PMA. Differences in estimates based on BIS and Stimulated Means were small for most of the MSA phenotypes, except for PHA and PMA at 48 h.

Patterns in estimates of genetic correlations of mortality across the nursery and finisher with MSA phenotypes at individual time points were very similar to those for mortality in the challenge nursery and in the finisher but with more positive estimates (Figure 3). Estimates of the delta phenotypes with mortality across the nursery and finisher tended to be similar to those for mortality in the nursery rather than to estimates for mortality in the finisher.

### *Health scores*

Estimates of genetic correlations of MSA phenotypes with health scores are shown in Figure 4, with corresponding phenotypic correlations in Supplementary Table S10. For the two health scores collected in the quarantine nursery, all pigs scored either as 4 or 5 (Cheng et al., 2020), and there were no expected trends in the correlation estimates because of the high health status at this stage. Estimates of genetic correlations for health scores in the challenge nursery and finisher were nearly all positive for all MSA phenotypes and across all three mitogens (Figure 4). Some estimates were strong but all had large SEs. Generally, differences between estimates based on BIS and Stimulated Means were small, but with some exceptions.

## Discussion

Commercial pigs typically face a wide variety of infectious diseases that are generally not observed in the nucleus herds that breeding programs are executed in. This structure of the North American swine production system and the lack of substantial numbers of identified quantitative trait loci for disease for marker-assisted selection complicate selection for disease resilience. Identification of genetic indicator traits for disease resilience that can be measured on young healthy pigs can be a pragmatic strategy to increase disease resilience of commercial pigs without requiring swine breeders to infect nucleus animals with disease or collect disease data in commercial herds.

In this study, we measured the proliferation of PMCs isolated from the blood of young healthy pigs resulting from mitogen stimulation as possible genetic indicators for disease resilience. Of the five mitogens investigated, three were found to result in in vitro PBMC stimulation phenotypes that were moderately heritable: ConA, PHA, and PMA. These three mitogens are commonly used in in vitro assays to stimulate specific pathways and to assess the immune response of an organism (Paul et al., 1979; Lamers et al., 1992; Tough et al., 1997; Lin et al., 2012; Stepanova et al., 2019).

Heritability is an important genetic parameter that determines to what extent the measured phenotype of an animal is affected by genetics and, therefore, selection on traits with higher heritabilities is expected to lead to a greater response to selection (Lush, 1937). Our estimates of heritability for ConA at 48 h (0.24 ± 0.08) were consistent with the findings of Edfors-Lilja et al. (1994; 0.38 ± 0.21) and Flori et al. (2011; 0.36 ± 0.20). However, our heritability estimates for PMA and LPS at time 48 (0.12 ± 0.08 and 0.00 ± 0.00) were substantially lower than those estimated by Flori et al. (2011; 0.27 ±0.20 and 0.31 ± 0.19). These differences may be attributed to multiple factors. First, Flori et al. (2011) evaluated the proliferation of PBMCs isolated from pigs that were vaccinated for *M. hyopneumoniae* 3 wk earlier. Second,

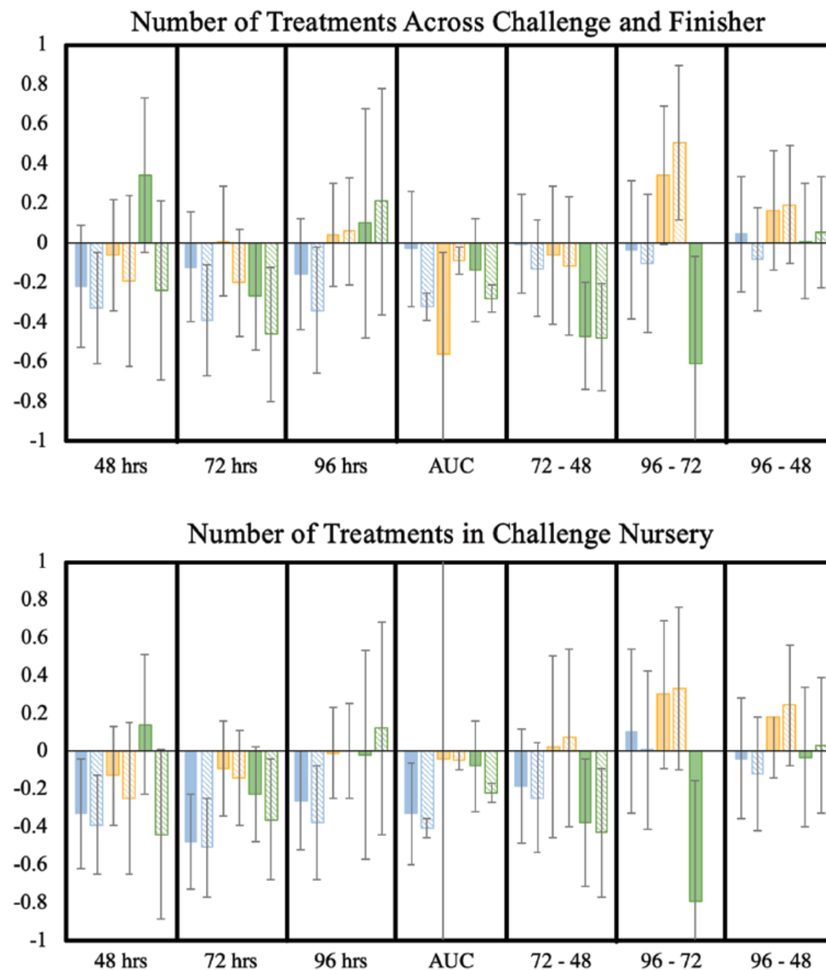


**Figure 2.** Estimates of genetic correlations (and SE bars) of MSA phenotypes with number of treatments for ConA (blue), PHA (yellow), and PMA (green), using BIS (solid bars) and Stimulated Mean (striped bars) MSA phenotypes.

the sample size in the study of Flori et al. (2011) was roughly half that of the current study, which resulted in SEs for estimates of heritability to be roughly twice as large as those obtained here. Third, different breeds were used, Large White by Flori et al. (2011) vs. Landrace × Yorkshire crossbreds in our study.

For responses to the three mitogens that resulted in moderate heritabilities in our study (ConA, PHA, and PMA), estimates of genetic correlations of the MSA phenotypes with disease resilience followed expected trends in most of the cases. MSA phenotypes were expected to have positive genetic correlations with growth rate and health scores under disease because a faster and greater mitogen-induced immune cell proliferation is expected to reflect a more effective immune response. Biologically, higher rates of PBMC proliferation in blood upon stimulation may coordinate a more efficient and effective immune response, expediting the expression of PBMCs and thus mitigating the antagonistic physiological consequences of pathogenic infection. Similarly, genetic correlations of MSA phenotypes with treatment and mortality rates were expected to be negative. Estimates of genetic correlations among the disease resilience traits evaluated here have been described in Cheng et al. (2020).

Although none of the individual genetic correlation estimates of MSA phenotypes with disease resilience traits were significantly different from zero, the trends in estimates across mitogens and disease resilience traits agreed with the expected directions of the genetic correlations. These trends suggest that selection for higher MSA response to these three mitogens (ConA, PHA, and PMA) may result in greater growth rate, higher health scores, fewer health treatments, and lower rates of mortality under a polymicrobial challenge and, therefore, in commercial herds with subclinical or clinical disease.

In order to determine which of the five mitogens would be the best to use for an MSA as a genetic indicator trait for disease resilience, we first considered estimates of heritability of the MSA phenotypes. On this basis, LPS and PWM were removed from consideration because their MSA phenotypes had low estimates of heritability. There were no clear differences in heritability estimates for MSA phenotypes of the other three mitogens, although estimates for ConA were moderate for all three time points, while PHA, and especially PMA, had lower estimates at 48 and 96 h. The AUC and delta phenotypes did not provide higher estimates of heritabilities for these three mitogens than individual time points.

Trends in estimates of genetic correlations of the MSA with disease resilience traits shown in Figures 1–4 provide additional insight into the suitability of the different MSA phenotypes and their associated mitogens as indicator traits for disease resilience. MSA phenotypes based on ConA generally showed more consistent estimates of genetic correlations and in the

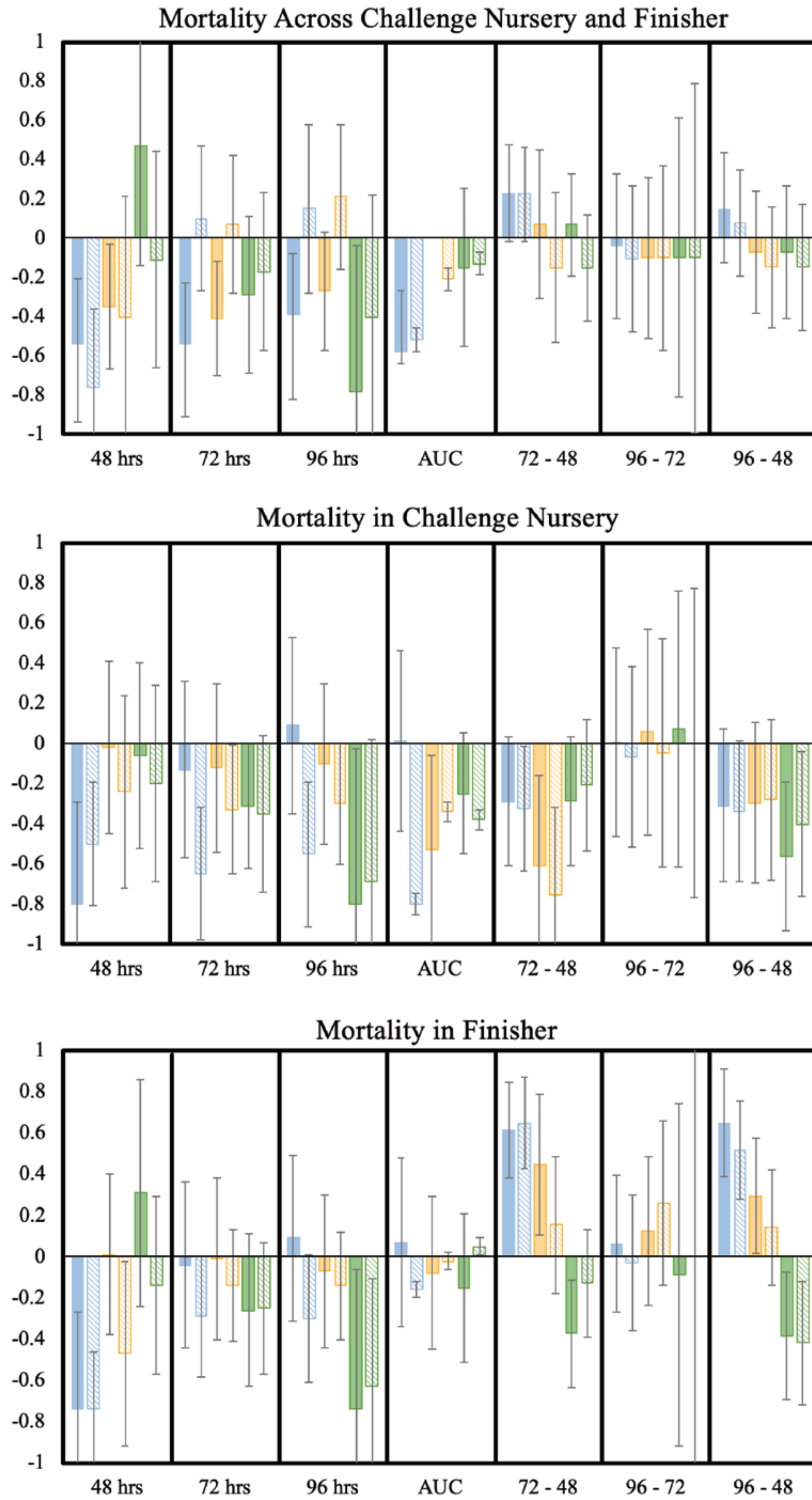


**Figure 3.** Estimates of genetic correlations (and SE bars) of MSA phenotypes with mortality for ConA (blue), PHA (yellow), and PMA (green), using BIS (solid bars) and Stimulated Mean (striped bars) MSA phenotypes.

expected direction across time points. Although PMA resulted in the numerically highest genetic correlation estimates, estimates for PMA and PHA were not as consistent relative to expectations as ConA and showed large SEs. Estimates for PMA also differed from those of ConA and PHA for several resilience traits, which likely reflects that PMA activates different pathways than ConA and PHA, which target the same biological pathways. Thus, although ConA, PHA, and PMA all showed promise in generating MSA that could serve as genetic indicators for disease resilience, ConA showed the most promise because of the consistent

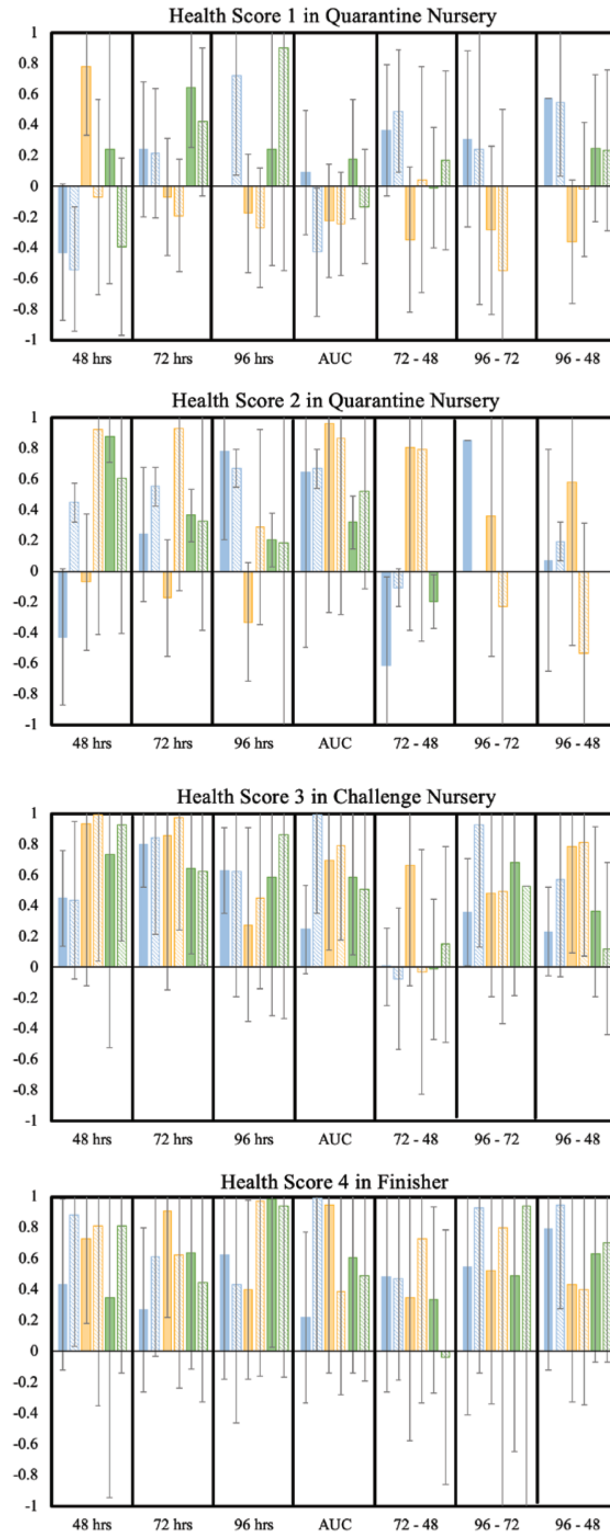


**Figure 4.** Estimates of genetic correlations (and SE bars) of MSA phenotypes with health score for ConA (blue), PHA (yellow), and PMA (green), using BIS (solid bars) and Stimulated Mean (striped bars) MSA phenotypes.

desirable genetic correlation estimates of its MSA response phenotypes with most disease resilience traits.

When comparing BIS and Stimulated Means as the MSA phenotypes for ConA, there were few substantial differences and genetic correlations estimates with disease resilience traits were generally similar for both measures. Therefore, selection on BIS or Stimulated Mean is expected to yield similar results.

## Conclusions

MSAs of PBMCs from blood collected on young healthy piglets were found to have moderate heritability for three of the five mitogens tested. MSA phenotypes also tended to be genetically correlated with performance under a polymicrobial disease challenge, with pigs with higher MSA phenotypes genetically having greater growth rate and lower treatment and mortality rates. This suggests that swine breeders can use MSA phenotypes of PBMCs isolated from the blood of young healthy nucleus pigs as genetic indicators of disease resilience. MSAs with the mitogen ConA showed the most promise as genetic indicators because of their consistent desirable estimates of genetic correlations with most disease resilience traits. However, further studies are required not only to validate these results, given the large SEs of the genetic correlation estimates, but also to determine which time point following stimulation is the most appropriate to assess the mitogen stimulation response of the isolated PBMCs. A limitation of the MSA phenotypes is that the assays that must be conducted on fresh blood are time consuming.

## Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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## Conflicts of interest statement

The authors declare no real or perceived conflicts of interest.

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