# Genetic Parameters of Immune Response Estimated using Genetically Divergent Lines of Holstein-Friesian Dairy Heifers

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## Abstract

To test the hypothesis that immune responses are useful predictors of fertility in New Zealand (NZ) dairy cattle, we estimated genetic parameters for immune response using a small, experimental herd comprised of genetically divergent lines of Holstein-Friesian dairy heifers whose parents were selected for high or low fertility. Pedigree-based animal models fit using ASReml estimated the heritabilities of antibody-mediated immune response at days 14 and 21 (AMIR14 and AMIR21) and cell-mediated immune response (CMIR) as 0.44, 0.47 and 0.11. Genetic correlations between immune response traits varied: 0.67 for AMIR14 and AMIR21; -0.44 for AMIR14 and CMIR; and -0.07 for AMIR21 and CMIR, suggesting complex and time-dependent genetic relationships between the two types of immune responses. We also detected low to moderate genetic correlations between immune response traits and component traits of NZ's economic selection index, Breeding Worth (BW), which were close to zero for lowly heritable traits like fertility. These data indicate that immune response is unlikely to be a robust predictor trait for lowly heritable BW component traits, but may be a useful selection trait in its own right as consumer preferences and regulatory agencies accentuate animal health and welfare.

**Key words:** dairy cattle, immune response, fertility, genetic parameter

## Introduction

In dairy cattle, fertility is lowly heritable, but is included in selection indices due to the costs of infertility and historical overemphasis on production traits at the expense of fertility via antagonistic genetic correlations. The NZ fertility trait is an index of parity-specific binary traits PM21 (presented for mating within 21 days of planned start of mating) and CR42 (calving rate in first 42 days after planned start of calving), the average heritability of which is 0.03 (Harris *et al*., 2005). While the rate of genetic gain in fertility is limited by its low heritability, multi-trait models using highly heritable predictor traits with good genetic correlation with fertility can accelerate it. Immune response (IR) is an important part of a cow's post-partum recovery and reproductive function (Fair 2015), warranting investigation as a potential predictor trait for fertility, and as an important trait for selection in its own right.

Previous studies demonstrated that IR is moderately heritable with $h^2$ = 0.16 to 0.64 (Mallard *et al*., 1983; Wagter *et al*., 2000; Hernández *et al*., 2006; Thompson-Crispi *et al*., 2012). Genetic correlations between fertility-related traits and IR are low and with mixed sign in Canadian Holsteins, ranging from -0.19 to 0.20 (Thompson-Crispi *et al*., 2012).

Our objective was to estimate genetic parameters for three IR traits in NZ Holstein-Friesian dairy cattle, including genetic correlations with traits routinely evaluated in NZ, including fertility. We also sought to account for potential bias due to genetic divergence in fertility in our experimental herd, and to obtain valid genetic correlations using estimated breeding values (EBV) rather than raw phenotypes.

## Materials and Methods

### *Study animals*

The study population consisted of 535 Holstein-Friesian heifers born into 379 herds between June and September 2015 and produced by assortative mating of low or high fertility EBV dams and sires to generate divergent genotypes (Low line heifer EBVs: $n$=256, $\mu$=-5.10, $\sigma$=1.37; High line heifer EBVs: $n$=279, $\mu$=4.99, $\sigma$=0.76). We reared these heifers in four management mobs, each consisting of a mixture of high and low EBV heifers with a ratio of no

more than 40:60 either way (Meier *et al*., 2017). Some mobs were further partitioned into groups for immunity challenges during February and March 2016, such that each group was of similar age; there were seven immunity groups in total.

### *Immunization Protocol*

Heifers were immunized at an age of approximately 220 days old to induce immune response, following the protocol described by Thompson-Crispi *et al.* (2012). Briefly, we used serum antibody IgG1 production to a type-2 test antigen, hen egg white lysozyme (HEWL), as an indicator of AMIR, and inflammation due to delayed-type hypersensitivity to *Candida albicans*, a type-1 test antigen, as an indicator of CMIR. Both antigens were injected intramuscularly (0.5mg each with adjuvant in 1mL PBS) on the right side of the rump on days 0 and 14, with an additional 0.1mg intradermal injection of *C. albicans* in 0.1mL of PBS to the right tail fold, and 0.1mL of PBS control to the left tail fold, on day 21.

### *Data*

We sampled blood on days 0, 14 and 21 for evaluation of IgG1 concentration by modified ELISA. IgG1 concentration at days 14 and 21 (AMIR14 and AMIR21) post-challenge were used as response variates in our models, with IgG1 concentration at day 0 (AMIR0) as a pre-challenge control covariate. The log-transformed ratio of skinfold thickness at day 21 and day 23 (48 hours later), at either the treatment or control site, was used as a model response variate (CMIRt) or control covariate (CMIRc), respectively.

We obtained the full pedigree of the heifers from the New Zealand Dairy Industry Good Animal Database (DIGAD), which consisted of 10,992 records, up to 18 generations deep. We also extracted from DIGAD the most recent EBVs (January 2017) for the eight component traits of the national selection index (BW or Breeding Worth), to be used alongside IR traits in bivariate analyses. These EBVs included milk protein yield, milk fat yield, milk volume, liveweight, fertility, somatic cell score (SCS), residual survival (RSv), and body condition score (BCS).

### *Models*

We fit univariate animal BLUP models for each of the three IR traits with fixed effects for immunity group, age-in-days and a control covariate, to estimate genetic variance and heritability. If the divergence in fertility genetics present between the two heifer lines was also present within the founding population, and if a particular trait was genetically correlated with fertility, then an appropriate model for that trait would need to assume two distinct distributions in genetic variance. This could be achieved by introducing a fertility line term into the model (either via genetic groups in the pedigree or a binary fixed term in the model), which would pool genetic variance into a single distribution, similar to accounting for breed divergence in multi-breed models. An examination of the heifer portion of the A-matrix revealed that the distribution of the off-diagonal values of either the within- or between-line relationship coefficients were largely similar (0.07 on average), apart from some >0.25 values for half- and full-sib relationships within lines, suggesting that the pedigree was of sufficient depth to assume a single distribution of genetic merit for fertility, and therefore for any other trait also. Nonetheless, we trialled models with a binary fertility line fixed effect, but because the resulting heritabilities only differed from models without a fertility line effect by a few percent, models with the effect were not considered any further in this analysis.

We estimated genetic correlations ($r_g$) from bivariate animal BLUP models that included fixed effects for immunity group, age-in-days and a control covariate, where age-in-days and the control covariate were applied only to IR response variate(s). Bivariate models with an EBV response variate would inevitably fail to converge properly due to the EBV "trait" having $h^2 \approx 1$. To address this, we initially deregressed the EBVs (dEBV) by dividing by their reliabilities (Garrick *et al*., 2009), but as reliabilities were nearly equal (0.3-0.4), the resulting dEBVs were essentially scaled by a constant, and so most models would still fail to

converge. To rectify this, we added additional noise variance to the dEBVs (nEBV) by randomly sampling a normal distribution with the known residual variance prior to each run, running the model 100 times per pair of response variates, and taking the average estimated $r_g$ and its SE across the 100 runs. Although it would be preferable to multiply the noise vector by the Cholesky decomposition of the prediction error variance/covariance matrix to simulate heterogeneous residual variance, given the limitations of our dataset (small and divergent), we opted to simply assume homogeneity of noise variance. Runs that failed to converge after 20 iterations, yet estimated all effect solutions and estimated IR $h^2$ consistent with the univariate models, were permitted.

To verify these estimates, we also calculated $r_g$ between IR and EBVs using a Pearson correlation in which IR genetic variance came from the univariate model, and EBV trait genetic (co)variances were the residual (co)variances from a bivariate model with the IR trait, but without random effects; these residual (co)variances could be considered as genetic (co)variances as the EBV is already a genetic estimate. However, standard errors would not be easily estimated for $r_g$ calculated in this way.

ASReml (Gilmour *et al.* 2015) was used to perform regression model analyses; R (R Core Team, 2017) was used for data pre- and post-processing, including iterative handling of ASReml runs.

## Results and Discussion

The estimated heritabilities of the three IR traits, and the estimated phenotypic and genetic correlations between them are presented in Table 1. The two AMIR traits have moderate heritabilities and a moderately high genetic correlation between them. CMIR has a low heritability and negative genetic correlations with the AMIR traits, and its $r_g$ with AMIR14 is much stronger than with AMIR21. However, the standard error (SE) of these $r_g$ are large. These results are consistent with those of previous findings (Thompson-Crispi *et al*., 2012), apart from AMIR14 $h^2$ which was previously lower at 0.14 ± 0.09, and its $r_g$ with AMIR21 was previously higher at 0.91 ± 0.21.

The negative genetic correlations between AMIR and CMIR are consistent across studies and species (Thompson-Crispi *et al*., 2012), as the cytokines which promote CMIR tend to dampen AMIR and vice versa (Brown *et al*., 1998). Any breeding strategy aiming to increase immunity should, therefore, incorporate both types of immune response to avoid adverse correlated responses to selection. Despite the antagonistic genetic correlations between them, the fact that the $r_g$ are low to moderate means that genetic improvement in both is simultaneously possible, particularly for AMIR21 which has a low $r_g$ with CMIR.

**Table 1.** Estimated heritabilities (diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations of immune response traits, with standard error.

| | AMIR14 | AMIR21 | CMIRt |
|---|---|---|---|
| AMIR14 | 0.44 ± 0.14 | 0.67 ± 0.17 | -0.44 ± 0.43 |
| AMIR21 | 0.44 ± 0.04 | 0.47 ± 0.15 | -0.07 ± 0.40 |
| CMIRt | -0.03 ± 0.05 | 0.01 ± 0.05 | 0.11 ± 0.10 |

Estimated genetic correlations between the three IR traits and the eight component traits of BW are presented in Table 2, along with the known heritabilities of the BW traits. The correlations estimated using a noise resampling schema generally aligned well with those determined via a Pearson correlation, except for CMIR with production traits (milk protein, fat and volume), where the resampling schema provided much more negative correlations. The standard errors were large for all correlations, particularly for residual survival. Although our dataset is limited in size, the results still offer novel insight into the correlations that exist between immunity and routinely evaluated traits in NZ. Positive genetic correlations are considered beneficial in NZ for all BW traits except somatic cell score.

We identified negative associations between all IR and production traits, whereas other studies have reported both negative and positive associations: Thompson-Crispi *et al*. (2012) report $r_g$ = 0.16 between CMIR and milk yield; Heriazon (2007) report $r_g$ = -0.15 for CMIR and

protein% and $r_g$ = 0.18 for AMIR and fat%; and Wagter *et al*. (2003) report negative phenotypic associations between AMIR and protein or fat yields but positive phenotypic associations between AMIR and milk yield. This variety of findings, along with the fact that the $r_g$ are all low with high SE, indicate that selecting on immunity is unlikely to negatively affect production.

**Table 2.** Genetic correlations between immune response traits and BW component traits, estimated by either a noise resampling schema (with standard error) or a Pearson correlation.

| | | AMIR14 | | AMIR21 | | CMIRt | |
|---|---|---|---|---|---|---|---|
| BW trait | $h^2$ | Resampling | Pearson | Resampling | Pearson | Resampling | Pearson |
| Protein | 0.31 | -0.10 ± 0.22 | -0.05 | -0.13 ± 0.21 | -0.06 | -0.39 ± 0.31 | -0.05 |
| Fat | 0.33 | -0.22 ± 0.21 | -0.15 | -0.10 ± 0.21 | -0.03 | -0.24 ± 0.29 | 0.05 |
| Volume | 0.36 | -0.12 ± 0.20 | 0.00 | -0.08 ± 0.20 | 0.02 | -0.40 ± 0.32 | -0.08 |
| Liveweight | 0.35 | -0.15 ± 0.17 | -0.16 | -0.22 ± 0.17 | -0.18 | [a] | 0.33 |
| Fertility | 0.03 | 0.09 ± 0.22 | 0.10 | -0.17 ± 0.21 | -0.05 | -0.04 ± 0.32 | -0.07 |
| SCS | 0.12 | 0.05 ± 0.25 | -0.01 | 0.03 ± 0.25 | -0.03 | 0.10 ± 0.39 | 0.06 |
| RSv | 0.04 | 0.03 ± 0.62 | -0.01 | -0.08 ± 0.41 | -0.01 | 0.17 ± 0.58 | 0.19 |
| BCS | 0.19 | 0.02 ± 0.19 | 0.05 | -0.15 ± 0.18 | -0.09 | 0.19 ± 0.27 | 0.08 |

[a]For all 100 CMIRt with Liveweight runs, poor CMIRt $h^2$ estimates, and lack of effect solutions for most runs

We report here a weak positive association between fertility and AMIR14, but negative associations between fertility and AMIR21 or CMIR. Thompson-Crispi *et al*. (2012) generally describe positive associations between IR and the Canadian fertility traits which indicated that breeding for IR could aid fertility, however, we cannot conclude the same for NZ dairy cattle.

We also report low to moderate associations between liveweight and IR traits; negative for AMIR but positive for CMIR. Although the cytokines of CMIR are associated with growth inhibition during infection (Johnson, 1997), their complex role in cellular signalling, particularly for metabolic pathways, may mean they are involved more generally in increased animal growth.

Overall, only weak genetic correlations were evident between IR traits and component traits of BW in NZ. Selection on IR is unlikely to negatively affect existing BW traits, although caution would be required given that most correlations were unfavourable. Conversely, selection on existing BW traits will likely have little impact on IR genetics. IR is unlikely to be a useful predictor trait for fertility or any other trait in NZ's BW index.

## Conclusions

Despite limited data, our results indicate that IR is a moderately heritable trait, consistent with current literature. AMIR is more heritable than CMIR, but they have a negative genetic correlation with each other, and so CMIR ought to also be included in any IR index. Weak genetic correlations with routinely evaluated traits in NZ means that selection for IR may not be detrimental to existing breeding objectives and vice versa. However, caution is required as most of these correlations, although insignificant, were unfavourable. With only moderate heritabilities and weak genetic correlations with fertility, IR traits are unlikely to be of use as predictor traits for fertility in dairy cattle.

Measurement of IR is not practical on a national scale and therefore not feasible for pedigree-based selection, however, it may be feasible within a reference population of a genomic selection scheme. Further research is required to obtain more robust genetic parameter estimates, including economic analysis to determine whether potential reduction in disease-related costs outweighs any potential loss due to reduced genetic gain in productivity. Selection for IR may also make

both quantitative and qualitative gains in meeting the expectations of both consumers and regulatory agencies with regards to animal health and welfare.

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