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Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy cows

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1 **Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy**
2 **cows.**

3 *By Denholm et al.* Dairy cow health and fertility represent a major constraint on production and are
4 significant causes of poor welfare. Recently, there has been a growing interest in identifying
5 immune-associated and immune-response traits in livestock which are linked with disease
6 conditions. Our results provide evidence that cellular immune-associated traits are heritable and the
7 noticeable variation between animals would permit selection for altered trait values. Results also
8 show that genetic selection for cellular immune-associated traits could lead to a useful tool in
9 improving animal health, fitness and fertility. Our work expands on previous results and adds to the
10 growing area of identifying measurable immune related traits to act as markers for health and welfare
11 in livestock systems.

12

13 **GENETIC PARAMETERS OF IMMUNE-ASSOCIATED TRAITS**

14

15 **Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy**
16 **cows**

17

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29 ABSTRACT

30 Data collected from an experimental Holstein-Friesian research herd were used to determine genetic
31 and phenotypic parameters of innate and adaptive cellular immune-associated traits. Relationships
32 between immune-associated traits and production, health and fertility traits were also investigated.
33 Repeated blood leukocytes records were analyzed in 546 cows for 9 cellular immune-associated
34 traits, including %T cell subsets, B cells, NK cells and granulocytes. Variance components were
35 estimated by univariate analysis. Heritability estimates were obtained for all 9 traits, the highest of
36 which were observed in the T cell subsets % CD4⁺, % CD8⁺, CD4⁺:CD8⁺ ratio and % NKp46⁺ cells
37 (0.46, 0.41, 0.43 and 0.42, respectively) with between-individual variation accounting for 59% to
38 81% of total phenotypic variance. Associations between immune-associated traits and production,
39 health and fertility traits were investigated with bivariate analyses. Strong genetic correlations were
40 observed between % NKp46⁺ and stillbirth rate (0.61), and lameness episodes and % CD8⁺ (-0.51).
41 Regarding production traits, the strongest relationships were between CD4⁺:CD8⁺ ratio and weight
42 phenotypes (-0.52 for liveweight; -0.51 for empty bodyweight). Associations between feed
43 conversion traits and immune-associated traits were also observed. Our results provide evidence that
44 cellular immune-associated traits are heritable, and repeatable, and the noticeable variation between
45 animals would permit selection for altered trait values, particularly in the case of the T cell subsets.
46 The associations we observed between immune-associated, health, fertility and production traits
47 suggest that genetic selection for cellular immune-associated traits could provide a useful tool in
48 improving animal health, fitness and fertility.

49 **Keywords:** dairy cow, immune-associated trait, heritability, variance

50

51

INTRODUCTION

52

53 Dairy cow health represents a major constraint on production and is a significant cause of
54 poor welfare. This is particularly true in the case of the modern high-yielding dairy cow where
55 periods such as early lactation carry a heightened risk of disease and susceptibility to mastitis and
56 other mammary infections is increased (Collard et al., 2000; McDougall et al., 2007). Genetic
57 selection for increased milk yield has been highly successful, however, it has also resulted in
58 unforeseen negative impacts on health, longevity and production (Pryce et al., 2004; Oltenacu and
59 Broom, 2010; Koeck et al., 2013; Pritchard et al., 2013). The ability to predict the occurrence of
60 disease in dairy cows is crucial in maintaining a high level of production within a herd as well as
61 ensuring any financial loss is kept to a minimum (Huijps et al., 2008). Two examples of approaches
62 to improve dairy cow health are to identify phenotypic markers (*i.e.*, biomarkers) which can be used
63 to predict the occurrence of health events and allow early intervention, and/or to identify heritable
64 traits which are associated with improved health function for use in future genetic selection programs
65 aimed at reducing disease incidences and health conditions. Recently, there has been a growing
66 interest in identifying and measuring immune-associated (**IA**) phenotypes in livestock which could
67 then be associated with disease/health conditions. Such IA phenotypes could be used to estimate an
68 individual's susceptibility to disease and/or act as biomarkers of concurrent disease (Park et al.,
69 2004; Clapperton et al., 2005, 2008, 2009; Flori et al., 2011a; b; Thompson-Crispi et al., 2012a; b;
70 van Knegsel et al., 2012; Banos et al., 2013). Previous research has looked at either steady state
71 measurements such as circulating leukocyte populations, acute phase proteins and serum cytokine
72 levels (Park et al., 2004; Glass et al., 2005; Clapperton et al., 2005, 2008; Flori et al., 2011a; b;
73 Banos et al., 2013), or *in vitro* measurements of immune responsiveness focusing on innate and
74 adaptive arms of the immune response (Thompson-Crispi et al., 2012a; b; Heriazon et al., 2013;
75 Thompson-Crispi et al., 2014b; Mallard et al., 2015). Moreover, it has been suggested that including
76 measurable immune response (**IR**) phenotypes in selection indices may be a viable option in

77 decreasing disease and improving animal health (Abdel-Azim et al., 2005; Thompson-Crispi et al.,
78 2012a; Mallard et al., 2015)

79 Previously, using a cohort of 248 lactating Holstein-Friesian dairy cows sampled repeatedly
80 over a ten month period, we identified a number of cellular IA traits within the circulating leukocyte
81 population which were significantly associated with important health, fertility and lactation traits,
82 including mastitis, lameness and infertility. These included a negative association between the
83 $CD4^+ : CD8^+$ T lymphocyte ratio and subclinical mastitis, a negative association between % $CD8^+$ T
84 lymphocytes within the total circulating leukocyte population and fertility, and a positive association
85 between % $NKp46^+$ leukocytes and lameness. Furthermore, these cellular measures were highly
86 repeatable and displayed significant between-animal variation, suggesting they may be altered by
87 genetic selection (Banos et al., 2013).

88 The aim of the present study was to add to the previous findings by using a larger dataset, and
89 corresponding pedigree information, to estimate genetic and phenotypic variance components for
90 various subsets of blood leukocytes. Further, we investigate the genetic and phenotypic associations
91 between these cellular IA traits and health, fertility, production and functional traits (e.g., somatic
92 cell count, feed intake, liveweight, body condition score) in dairy cows.

93

94 **MATERIALS AND METHODS**

95 *Animals*

96 All animals in the study population were Holstein-Friesians from the Langhill lines of dairy
97 cattle housed at the SRUC Dairy Cattle Research Centre at Crichton Royal Farm, Dumfries in
98 Scotland. Cows were born between January 2003 and September 2012 and were between their 1st
99 and 5th lactation (inclusive). Cows in the Langhill herd are routinely and extensively monitored for
100 productivity, health, welfare and reproduction, generating a wealth of phenotypic data for use in
101 statistical analyses. Full pedigree spanning seven generations was available.

102 Langhill cows are involved in an on-going selection experiment in a 2 by 2 approach (genetic
103 line x feeding systems) that has been running for over 30 years (Veerkamp et al., 1994). Cows are
104 divided equally between two genetic groups, a control and a select. Those in the control group were
105 daughters of sires selected with the UK average genetic merit for milk fat and protein. In contrast,
106 cows in the select group were from sires selected with the highest genetic merit for milk fat and
107 protein (Pryce et al., 1999; Bell et al., 2011). Within each genetic group cows were also divided
108 among two distinct feed groups that aimed to be divergent in terms of energy content. From 2002 to
109 2009 animals were split between an indoor non-grazing/low forage system with a target ME of 12.3
110 MJ/kg DM with the other half of the herd receiving a high forage diet with summer grazing with a
111 target ME of 11.5 MJ/kg DM. From September 2009 cows moved to different diets, either a home-
112 grown forage diet (home-grown) or on a bought-in by-product feed (by-product). Over summer, the
113 animals on the home-grown forage diet were at grass during the day and overnight they were being
114 offered a feed of appropriate home-grown ingredients to balance the high protein and relatively low
115 neutral detergent fibre of the grass. The by-product diet was based on ingredients available following
116 a primary production process and not normally used for human food (March et al., 2016).

117

118 ***Data***

119 Detailed animal performance data were collected on the cows routinely while they were on
120 the genetic line x feeding systems. The present study included 546 cows with IA trait information. Of
121 these, 256 were previously included in Banos et al., (2013). An additional 246 cohorts without IA
122 trait information were also available and included in the bivariate analyses, resulting in a total of 792
123 cows with yield, reproductive and health measures. The data are summarized in Table 1 and
124 described in further detail below.

125 ***Immune-associated Traits.*** Blood samples were collected on 12 separate occasions from 358
126 animals (2,266 total samples). Samples were collected at bi-monthly intervals between April 2013

127 and March 2015 and included summer and winter samplings. Blood leukocyte sub-populations in
128 each sample were analyzed by flow cytometry to derive 9 cellular IA traits: % Peripheral blood
129 mononuclear cells (**PBMC**), % Eosinophils, % Lymphocytes, % Monocytes, % Neutrophils, %
130 CD4⁺, % CD8⁺, CD4⁺:CD8⁺ ratio and % NKp46⁺.

131 The additional data from Banos et al., (2013) were collected at bi-monthly intervals on 5
132 separate occasions between July 2010 and March 2011. Cellular IA trait information from the Banos
133 et al. (2013) data were available for animals within both genetic groups but only those on the high
134 concentrate diet (Banos et al., 2013). This additional data accounted for approximately 25% of the
135 total IA trait dataset.

136

137 ***Flow Cytometry.*** For flow cytometric analysis of circulating leukocyte populations, blood
138 samples were collected into EDTA Vacutainers (BD, Franklyn, NJ). Red blood cell lysis and
139 antibody labeling was performed in 96 well round bottomed plates as follows: 25µl of EDTA whole
140 blood was added per well and subsequently incubated with 125µl ammonium chloride lysis buffer
141 (0.15M NH₄Cl, 10mM NaHCO₃, 1mM Disodium EDTA, pH 7.4). After red blood cell lysis was
142 complete, leukocytes were pelleted by centrifugation at 850 x g and washed twice with FACS buffer
143 (5% FBS and 0.02% sodium azide in PBS) before incubating at 4°C for 30 minutes in FACS buffer
144 containing 10% heat-inactivated normal mouse serum (Invitrogen Caltag). Cell measurements were
145 focused on cell types which had been shown to correlate with health and productivity traits in our
146 previous study (Banos et al, 2013). Cells were then incubated at 4°C for 30 minutes with the
147 following monoclonal antibodies: anti-bovine CD4 conjugated to Alexa Fluor® 647 (clone CC8,
148 mouse IgG2a, AbD Serotec), anti-bovine CD8 conjugated to R-phycoerythrin (clone CC63, mouse
149 IgG2a, AbD Serotec) and anti-bovine CD335 conjugated to Alexa Fluor® 488 (clone AKS1, mouse
150 IgG1, AbD Serotec). Unstained control cells and isotype stained cells (mouse IgG1 conjugated to
151 Alexa Fluor® 488, mouse IgG2a conjugated to R-phycoerythrin, mouse IgG2a conjugated to Alexa

152 Fluor® 647, all eBioscience, San Diego, CA) were included on each plate. After final washes in
153 FACS buffer then PBS, cells were fixed in 1% paraformaldehyde in PBS for 10 min at RT and then
154 re-suspended in PBS prior to analysis on a MACSQuant® flow cytometer (Miltenyi Biotech). Data
155 analyses were performed using FlowJo version 7.6.1 analysis software (TreeStar, San Carlos, CA).
156 The results were expressed as a percentage of cells within the peripheral blood mononuclear cell
157 (PBMC) population which were positive for each surface marker. In addition, differential cell counts
158 were performed by analysis of unstained cells and identifying leukocyte populations by their size
159 (forward scatter), granularity (side scatter), and auto-fluorescence as previously described (Lun et al.,
160 2007).

161

162 ***Lactation, Feed Intake, Production and Functional Traits.*** A phenotypic dataset was
163 created and matched to the immune profile of each individual animal if IA trait information was
164 available. This data contained lactation traits recorded at the daily level and included milk yield
165 (kg), fat and protein percentage (%), feed intake (kg), dry matter intake (kg), feed to milk ratio, dry
166 matter to milk ratio, empty body weight (kg), live weight (kg), body condition score (0 to 5) and
167 somatic cell count ($\times 10^3/\text{ml}$). Daily records were averaged over the week to give data for each week
168 in milk. Information relating to record date (year, month), calving date (year, month), age at calving
169 (months), Holstein percentage, lactation number, number of weeks in milk (**WIM**), diet group and
170 genetic group were also included.

171

172 ***Health Traits.*** Detailed health records were available for each cow in the study population. A
173 phenotypic dataset containing health event information (expressed as binary traits) was created and
174 matched to the immune profile of each individual animal. Health events were grouped into 4 groups:
175 mastitis, reproductive problems, lameness and other. Due to the low incidence of metabolic and other
176 disorders/diseases within the Crichton herd these conditions (including ketosis, displaced abomasum,

177 hypocalcaemia, hypomagnesaemia, pyelonephritis etc.) were grouped into the “other” health
178 category. Health events were then matched such that animals were scored as 0 or 1 for absence or
179 presence of a condition or treatment within \pm one week of the immune sample date. Additionally, the
180 number of distinct mastitis, reproductive and lameness episodes per lactation was calculated for each
181 animal. Distinct episodes were calculated according to consecutive treatments more than 7, 21 and
182 28 days apart for mastitis, reproductive problems and lameness respectively (Banos et al., 2013).

183

184 ***Fertility Traits.*** A fertility timeline was created for each animal and included information for
185 each lactation such as calving date, calving interval, days to first heat, days from first to last heat,
186 number of heats, days to first service, days from first to second service, days from first to last
187 service, number of services, dystocia and stillbirth rate. This information was matched to each cow’s
188 immune profile in the lactation the cow was sampled for immunological analysis. Calving interval
189 referred to the interval between the date of calving of the previous lactation and the current calving.
190 Number of services referred to the total number of artificial inseminations before positive
191 conception. Dystocia and stillbirth referred to the calving previous to the current lactation and were
192 expressed as binary (0/1) traits. Dystocia was scored as of 0 for a normal calving else 1 and stillbirth
193 was scored as 0 if calves were born alive and 1 if born dead (or died within 24 hours).

194

195 ***Statistical Analysis***

196 Statistical analysis of cellular IA traits was carried out using a repeated measures mixed
197 linear animal model with a pedigree relationship matrix fitted to account for the genetic relationships
198 between animals:

199

$$200 \quad y_{ijklmnopq} = \mu + F_j + G_k + W_m + T_n + Y_q + L_l + C_i + a_o + p_o + e_{ijklmnopq}$$

201

(1)

202

203 Where $y_{ijklmnopq}$ is the trait record; μ is the overall mean; F_j is the fixed effect of the j^{th} diet group; G_k
204 is the fixed effect of the k^{th} genetic group; W_m is the fixed effect of the m^{th} lactation week; T_n is the
205 fixed effect of the n^{th} assay technique, fitted to account for the variation between the methods used to
206 generate the IA trait data; Y_q is the fixed effect of the q^{th} year by month of record interaction; L_l is
207 the fixed effect of the l^{th} lactation number by age at calving; C_i is the fixed effect of the i^{th} year by
208 month of calving interaction; a_o is the random additive genetic effect of the o^{th} individual cow
209 including pedigree data (2793 animal in pedigree, see Table 1 for further details); p_o is the random
210 permanent environmental effect of the o^{th} individual cow, fitted to account for the use of repeated
211 measures of the same animal; and $e_{ijklmnopq}$ is the random residual effect.

212

213 Total phenotypic variances (σ_p^2), as well as corresponding additive genetic (σ_a^2), permanent
214 environmental (σ_{pe}^2), residual (σ_e^2) variance and covariance components were estimated by the
215 Restricted Maximum Likelihood (**REML**) approach using ASReml version 3 (Gilmour et al., 2009).
216 Univariate models were run initially for each trait to establish the correct model (the significance
217 levels of the fixed effects are presented in Supplementary Information Table S2) followed by a series
218 of bivariate models to estimate the genetic/phenotypic correlations between cellular IA traits and the
219 health, fertility and production traits. For all model outputs P-values <0.05 were considered
220 significant. The variance components were used in the calculation of the following genetic and
221 phenotypic parameters: the ratio of total phenotypic variance attributed to additive genetic variation
222 (heritability, h^2); the ratio of total phenotypic variance due to the individual animal (sum of additive
223 genetic and permanent environmental effects), *i.e.*, between-individual variation (repeatability, R);
224 and the ratio of total phenotypic variance due to permanent environmental variance (c^2).

225

226

RESULTS

227 The data used in this study are summarized in Tables 2 (IA traits); 3 (production and
228 functional traits); and 4 (health and fertility traits). Coefficients of variation of the traits were
229 substantial and ranged from 18% (% PBMC) to 95% (% eosinophils) for IA traits; 12% (protein %)
230 to 318% (somatic cell count) for production and functional traits; and 18% (calving interval) to
231 113% (days first to last service) for fertility traits. The coefficient of variation was used as an
232 indicator of trait variability, and as seen above, marked differences in variability amongst recorded
233 traits was observed. Consistent levels of variability in these traits was observed previously (Banos et
234 al., 2013).

235

236 Total phenotypic variances (σ_p^2), as well as corresponding additive genetic (σ_a^2), permanent
237 environmental (σ_{pe}^2) and residual (σ_e^2) variance components, and their standard errors, were
238 estimated and are presented in Table 5. Estimates of heritability (h^2), between-individual variation
239 (repeatability, R), and the ratio of total phenotypic variance due to permanent environmental variance
240 (c^2) are also presented in Table 5. Statistically significant ($P < 0.05$) heritability estimates were
241 obtained for all 9 IA traits. Heritability estimates ranged from 0.15 (% monocytes) to 0.46 (% CD4⁺)
242 with the majority above 0.2. The highest heritabilities were observed in the T cell and NK cell
243 subsets (% CD4⁺, % CD8⁺, CD4⁺:CD8⁺ ratio and % NKp46⁺; 0.46, 0.41, 0.43 and 0.42 respectively;
244 Table 5). Significant heritability estimates suggest these traits could be improved with selective
245 breeding.

246

247 All IA traits were shown to be repeatable and between-animal variation accounted for 18% to
248 81% of total phenotypic variance (Table 5). The most significant estimates of repeatability were
249 observed in % CD4⁺, % CD8⁺ CD4⁺:CD8⁺ ratio and % NKp46⁺ (0.70, 0.76, 0.81 and 0.59
250 respectively, see Table 5). These estimates were higher than those previously obtained from a
251 considerably smaller dataset (Banos et al., 2013).

252

253 The permanent environmental effect was fitted to estimate any between-animal variation over
254 and above that due to additive genetic effects. This could be due to long-term environmental effects
255 (*e.g.*, previous diseases) and/or non-additive genetic effects (*e.g.*, epigenetic) which pertain to
256 individual animals throughout their lives but are not passed on to the next generation. A significant
257 ratio of total phenotypic variance due to permanent environmental variance (c^2) was found for %
258 eosinophils, % CD4⁺, % CD8⁺, CD4⁺:CD8⁺ ratio, and % NKp46⁺ (0.22, 0.23, 0.34, 0.38 and 0.17
259 respectively). For all traits, with the exception of % eosinophils, estimates for c^2 were lower than the
260 heritability. Moreover, all traits appeared to show higher genetic variances in comparison with
261 permanent environmental variances (% eosinophils once again being the only exception). In the case
262 of % PBMC, % eosinophils, % lymphocytes, % monocytes and % neutrophils the largest proportion
263 of phenotypic variance was observed in the residual variance (Table 5). Significant repeatability
264 estimates may help to derive predictions of future animal performance. No significant estimates of c^2
265 were obtained for the remaining traits which may be a function of dataset size.

266

267 ***Correlations between Immune-Associated Traits and Production, Functional, Fertility and Health***
268 ***Traits***

269 Additive genetic correlations between traits of interest to this study are presented in Tables 6
270 and 7 along with their corresponding standard errors. Informative phenotypic correlations are
271 presented in Table 8. Less informative phenotypic correlations as well as permanent environmental
272 and residual correlations are summarised in Supplementary Tables S3 to S4, S5 to S6 and S7 to S8
273 respectively.

274

275 Milk fat percentage was found to have a moderate positive genetic correlation with % PBMC
276 (0.33) and % lymphocytes (0.36), and a negative association with neutrophils (-0.35). Moderate

277 negative genetic correlations were found between the CD4⁺:CD8⁺ ratio within the PBMC population
278 and liveweight (-0.52) and similarly, empty body weight (-0.52). Further significant genetic
279 correlations were between IA and feed conversion traits; these were low to moderate and are
280 presented in Table 6. Regarding fertility traits (Table 7) significant correlations were observed
281 between % NKp46⁺ and stillbirth rate (0.61). Analyses yielded no significant correlations with health
282 traits (Table 7), however, the following relationships were found to be approaching significance (i.e.,
283 $0.5 < P < 0.1$), highlighting the requirement for further investigation: lameness episodes and % CD8⁺ (-
284 0.51, $P=0.06$); lameness episodes and CD4⁺:CD8⁺ ratio (0.47, $P=0.08$); and mastitis and %
285 eosinophils (0.63, $P=0.09$).

286

287 The largest significant phenotypic correlations (Table 8) estimated between IA and
288 production traits were all negative and were between the CD4⁺:CD8⁺ ratio and liveweight, empty
289 body weight and body condition score, BCS, (-0.16, -0.15 and -0.11 respectively). For the fertility
290 traits a negative association between % CD4⁺ and time between first and second service was
291 identified (-0.14) as well as a positive relationship between % monocytes and calving interval (0.10).
292 The remaining phenotypic correlations were close to zero and are summarized in Supplementary
293 Tables S3 (production and functional traits) and S4 (fertility and health).

294

295 Regarding production and functional traits, the only statistically significant permanent
296 environmental correlations were moderate, negative and between % eosinophils and milk yield (-
297 0.47), feed intake (-0.50), dry matter intake (-0.52) and metabolizable energy intake (-0.53).
298 Additionally, % CD4⁺ was found to be negatively correlated with BCS (-0.43). In the health traits a
299 negative association between % eosinophils and reproductive episodes (-0.25) as well as a positive
300 association between % CD4⁺ and mastitis (0.30) were noted. Finally in the case of the fertility traits
301 permanent environmental correlations were found to be moderate and negative between CD4⁺:CD8⁺

302 ratio and number of heats/services (-0.25, -0.27 respectively). Moreover, positive relationships were
303 found to exist between % CD8⁺ and number of services, number of heats and the time between first
304 and last service (0.26, 0.23 and 0.23 respectively). Permanent environmental correlations may be
305 used to develop optimal management practices regarding future animal performance (see
306 Supplementary Tables S5 to S6 for production, fertility and health traits respectively).

307

308 Residual correlations, *i.e.*, correlations which relate to covariation unexplained by the model
309 of analysis, were generally low or close to zero in all traits (see Supplementary Tables S7 to S8).

310

311 **DISCUSSION AND CONCLUSIONS**

312 Previously, Thompson-Crispi et al. (2012b) showed antibody- and cell-mediated immune-
313 response traits in Holstein-Friesian dairy cows to be heritable, with estimates of 0.29 and 0.19
314 respectively, this was further confirmed by Heriazon et al. (2013), however, these studies focused on
315 immune-response traits rather than the steady state measured IA traits presented here. In the present
316 study, significant genetic and phenotypic associations were observed between T cell subsets and
317 fertility as well as lameness events. T cell subsets such as CD4 T helper cells produce cytokines and
318 chemokines and play an important role in immune protection, interacting with many other immune
319 cells such as B cells, eosinophils, basophils macrophages, and neutrophils (Zhu and Paul, 2009).
320 Earlier work by Saama et al. (2004) also highlighted the potential importance lymphocyte subsets as
321 indicators of immune competence in dairy cattle. As highlighted above, the T cell subsets showed the
322 most promising heritabilities, which were consistent with previous studies (Saama et al., 2004;
323 Clapperton et al., 2009). Specifically, heritability of %CD4⁺ has been previously reported as 0.69 in
324 pigs (Clapperton et al., 2009). Moreover, Ahmadi et al. (2001) reported a heritability of 0.54 in
325 humans. The Ahmadi et al. (2001) study measured actual CD4⁺ cell counts in contrast to CD4⁺
326 measured as a proportion of PBMC (*i.e.*, % CD4⁺) as in Clapperton et al. (2009) and the present

327 study. Comparison of the genetic variance estimates from all three studies suggests they are similar
328 regardless of whether total numbers or proportions are used, presumably as the numbers of
329 PBMC/ml blood are not changing considerably between individuals.

330 Previously, $CD4^+ : CD8^+$ ratio was shown to have a negative phenotypic correlation with milk
331 somatic cell count in cows (-0.56, Banos et al., 2013). The present study estimated a genetic
332 correlation between $CD4^+ : CD8^+$ and SCC of -0.31 ($P=0.17$). The present study utilized a much
333 larger dataset (four fold increase in IA records) collected over a longer period and incorporated the
334 original data collected previously (Banos et al., 2013). Although not significant, the result suggests at
335 a genetic level animals with lower values of $CD4^+ : CD8^+$ ratios will have higher somatic cell counts.
336 Moreover, a high somatic cell count in milk is often considered as an indicator of mastitis and other
337 intra-mammary infections in cattle (Mrode and Swanson, 1996, 2003); many countries currently use
338 SCC (or somatic cell score) to indirectly breed for mastitis resistance (Miglior et al., 2005). In the
339 present study, the genetic correlation between SCC and mastitis was 0.67 with corresponding
340 phenotypic correlation of 0.12. A lower value of $CD4^+ : CD8^+$ can be indicative of a chronic infection
341 and a higher value indicative of fighting a major/viral infection. A low $CD4^+ : CD8^+$ ratio may
342 potentially indicate the presence of mastitis infection, either by sequestering circulating $CD4^+$ T cells
343 into the mammary gland (e.g., Taylor et al., 1997; Tassi et al., 2013), or preferentially expanding
344 both circulatory and mammary populations of $CD8^+$ T cells, as these have been shown to play a key
345 role in protection against intra-mammary infection (Denis et al., 2011). Evidence of such an
346 association has also been reported (Park et al., 2004).

347 Additionally, the $CD4^+ : CD8^+$ ratio, a cell-mediated adaptive IA trait that decreases with age
348 (Wikby et al., 1998; Hadrup et al., 2006; Strindhall et al., 2007), has been found to exhibit a high
349 level of heritability across species, for example 0.65 in humans (Hall et al., 2000) and; 0.64 in pigs
350 (Flori et al., 2011a). The $CD4^+$ cells are associated with fighting against infections whereas the $CD8^+$
351 cells are killer cells of the immune system. The $CD4^+ : CD8^+$ ratio gives an indication of the strength

352 of the immune system such that declining ratios are associated with immune dysfunction and
353 increased risks of severe infections and malignancies (Wikby et al., 1998; Strindhall et al., 2007; Lu
354 et al., 2015). In humans, the CD4⁺:CD8⁺ ratio can be used as a marker of HIV to AIDS progression
355 (Fahey et al., 1990; Serrano-Villar et al., 2015). Other human health conditions that have been
356 previously associated with the CD4⁺:CD8⁺ ratio include chronic lymphocytic leukaemia (Bartik et
357 al., 1998; Gonzalez-Rodriguez et al., 2010), infectious mononucleosis and other viral infections
358 (Karcheva et al., 2008; Salih, 2009), Hodgkin disease (Gupta, 1980; Poppema, 1996; Gorczyca et al.,
359 2002; Hernandez et al., 2005), aplastic anaemia (Zhang et al., 2007), as well as neurological
360 disorders like multiple sclerosis (Pender et al., 2014) and myasthenia gravis (Berrih et al., 1981;
361 Matsui and Kameyama, 1986). Further, there is substantial evidence that this trait is under genetic
362 control in mice, chickens and humans (Kraal et al., 1983; Clementi et al., 1999; Amadori et al., 1995;
363 Myrick et al., 2002; Ewald et al., 1996).

364 The present study also identified a moderately strong genetic correlation between CD4⁺:CD8⁺
365 ratio and lameness (0.51, $P=0.06$) which was only identified at the phenotypic level in our previous
366 study (Banos et al., 2013). This suggests that animals with higher steady state values of CD4⁺:CD8⁺
367 ratios are genetically predisposed to higher incidences of lameness. Additional moderate genetic
368 correlations were found between SCC and CD8⁺ (0.36, $P=0.12$) and monocytes (0.48, $P=0.08$) but
369 were not statistically significant. As CD4⁺:CD8⁺ is useful in particular types of infections it would be
370 interesting to explore if the relationship is consistent with different mastitis and/or lameness causing
371 pathogens and duration of said health events.

372 A strong genetic correlation was found between % NKp46⁺, a natural killer (NK) cell marker
373 (Sivori et al., 1997; Storset et al., 2004) and stillbirth (0.61, $P=0.04$), which is a novel finding in
374 cattle. This is in agreement with literature concerning human studies which have consistently
375 identified a relationship between NK cells and reproductive outcomes, with higher percentages of
376 NK cells within the circulating lymphocyte pool being associated with poor reproduction (Kwak-

377 Kim and Gilman-Sachs, 2008; Kwak-Kim et al., 2010; Seshadri and Sunkara, 2014; Michou et al.,
378 2003). NK cells are a type of innate immune cell with potent cytotoxic activity that are important in
379 controlling intracellular pathogens (Storset et al, 2004). Circulating NK cells can traffic into the
380 uterus and their association with reproductive failure is thought to be due to unregulated NK-
381 mediated cytotoxicity within the uterine environment (Kwak-Kim and Gilman-Sachs, 2008).

382 Previous research has demonstrated that the occurrence of metabolic and infectious disease in
383 dairy cows classed as high immune responding (**HIR**) is lower than non-HIR cows (Thompson-
384 Crispi et al., 2012a, 2013). Results from the present study support opinions in the literature that
385 genetic selection of measurable immune-associated phenotypes may be possible (Thompson-Crispi
386 et al., 2012b; Heriazon et al., 2013), could provide a useful tool in monitoring and improving disease
387 resistance and animal health (Thompson-Crispi et al., 2014a; Mallard et al., 2015, 2011) and may not
388 negatively impact production (Stoop et al., 2016). Results also highlight the importance of blood
389 leukocyte subsets with respect to reproduction and fertility in dairy cows, the strong association
390 found between stillbirth and % NKp46⁺ is promising and gives a foundation for further investigation.

391 One limitation of the current study is that no functional assessment has been performed on
392 the various leukocyte subsets measured. Many of these subsets exhibit a wide range of functional
393 capabilities, which will be related to their previous antigenic experience (particularly for lymphocyte
394 subsets) or other environmental and host factors (e.g. nutritional, reproductive or disease status). For
395 example, while CD8⁺ T cells largely target intracellular pathogens through killing of infected cells
396 and production of antiviral cytokines (Bevan, 2004), CD4⁺ T cells differentiate into a number of
397 distinct helper-T cell subsets including T-helper (T_H)-1, T_H-2, T_H-9, T_H-17 and regulatory T cells, all
398 of which exhibit different functionalities in relation to the types of pathogens they target or their role
399 in regulating the immune response (Nakayamada et al., 2012). Thus, associations between the
400 cellular traits used in this study and health traits may be weaker and/or absent in other study
401 populations, and consequently the health benefits of selection for these cellular traits in dairy cattle

402 may be unpredictable. In future studies, IA traits involving additional cellular markers and/or
403 immune assays which better reflect immune function (e.g. naïve vs. memory T cell markers, cytokine
404 release profiles) should be explored. Such an approach would be similar to, but less labour intensive
405 than that taken by other studies (Thompson-Crispi et al, 2013, 2014a), in which proposed selection
406 is based on antibody-mediated immune response traits (broadly representing T_H -2 immunity) and
407 cell-mediated immune response traits (broadly representing T_H -1 immunity) obtained following
408 immunization of cattle with specific antigens.

409 In addition to the immunological measures of blood leukocyte subsets considered in the
410 present study, serological immune phenotypes measurable in both bovine milk and blood may also
411 be of value in improving the health and welfare of dairy cows. Associations between IA traits found
412 in blood and milk have been highlighted, for example, in natural antibodies (de Klerk et al., 2015)
413 and haptoglobin (Hiss et al., 2009). Furthermore, supporting the results of the present study
414 serological IA traits are considered beneficial, an association between haptoglobin and mastitis has
415 previously been highlighted (Banos et al., 2013). Moreover, obtaining data collected out with the
416 research herd used in the present study would be advantageous and provide a means of validating our
417 results.

418 In the present study we have provided evidence that cellular IA traits derived from
419 measurable blood leukocyte populations are heritable and would permit selection for altered trait
420 values, particularly in the case of the T cell subsets. Moreover, the associations observed between
421 IA, health, fertility and production traits suggest that genetic selection for cellular IA traits could lead
422 to a useful tool in improving animal health, fitness and fertility.

423

424

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432

433 **APPENDIX**

434 Supplementary Information accompanies this paper and is available on the Journal of Dairy Science
435 website (www.journalofdairyscience.org)

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667 **Table 1.** Description of phenotype dataset used in all model analyses

Description	Total
Weekly production and functional phenotypic records	92,153
Weekly cellular immune-associated, health and fertility records	3,581
Animals in data set	792
Animals with immune data	546
Animals with phenotypic data only	246
Lactations	3 ¹
Years (2005-2015)	10
Animals in pedigree	2,793
Sires	539
Dams	1,813
Generations	7

668 ¹ 1,785 total lactations. Note: lactations ≥ 3 are grouped into the lactation 3 class.

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671 **Table 2.** Descriptive statistics of the 9 cellular immune-associated traits obtained via flow cytometric analysis

Trait	No. Records	Min	Max	Mean	Std. Dev	CV ¹ (%)
% PBMC ^{2,3}	2,266	18.00	89.50	58.39	10.24	17.54
% Eosinophils ³	2,266	0.07	35.20	3.61	3.43	95.06
% Lymphocytes ³	2,265	7.70	79.70	44.25	12.35	27.90
% Monocytes ³	2,265	3.03	55.40	13.99	8.25	58.98
% Neutrophils ³	2,266	8.09	81.10	37.76	10.10	26.74
% CD4 ⁺ ⁴	2,232	3.39	46.00	25.52	6.28	24.61
% CD8 ⁺ ⁴	2,260	2.51	28.00	11.29	3.42	30.28
CD4 ⁺ :CD8 ⁺ ratio	2,232	0.48	6.12	2.38	0.73	30.67
% NKp46 ⁺ ⁴	2,262	0.01	16.50	2.32	1.58	67.95

672 ¹ Coefficient of variation

673 ² % Peripheral Blood Mononuclear Cells

674 ³ % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

675 ⁴ % of PBMC that were CD4, CD8 and NKp46 positive

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678 **Table 3.** Descriptive statistics of the 12 production and functional traits

Trait	No. Records	Min	Max	Mean	Std. Dev	CV ¹ (%)
Milk (kg)	90,750	3.00	65.54	28.71	9.10	31.71
Fat (%)	72,433	0.23	9.94	3.78	0.71	18.83
Protein (%)	72,433	0.20	6.65	3.21	0.39	12.00
Feed intake (kg)	64,970	2.21	182.31	42.39	11.71	27.63
Dry matter intake (kg)	64,970	1.03	57.27	16.62	5.22	31.39
Metabolizable energy intake (MJ)	64,970	10.47	641.47	186.56	59.15	31.70
Empty body weight (kg)	88,345	237.00	782.00	483.00	67.50	13.98
Live weight (kg)	88,345	310.00	953.00	605.00	79.51	13.14
Body condition score (0-5)	69,703	0.50	4.25	2.11	0.43	20.33
Somatic cell count (x10 ³ /ml)	74,288	2.67	7,865.00	110.64	351.76	317.93
Feed intake:Milk (ratio)	64,919	0.07	271.50	1.61	1.98	122.92
Dry matter intake:Milk (ratio)	64,919	0.03	136.56	0.62	0.84	135.82

679 ¹ Coefficient of variation

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683 **Table 4.** Descriptive statistics of the 3 health traits and 9 fertility traits

Health Trait	Incidence ¹	Mean ²	Std. Dev ²	Max ²
Mastitis	0.01	0.17	0.38	10
Reproductive problems	0.12	0.83	0.59	11
Lameness	0.12	0.61	0.59	12

Fertility Trait	No. Records	Min	Max	Mean	Std. Dev	CV ³ (%)
Calving interval (days)	663	189	737	404.41	74.15	18.34
Days to first heat (days)	855	2	205	58.64	30.68	52.32
Days first last heat (days)	855	0	731	86.33	93.48	108.28
Number of heats	861	0	15	3.76	2.79	74.04
Days to first service (days)	850	4	205	66.66	25.95	38.92
Days first second service (days)	634	1	206	33.71	24.13	71.58
Days first last service (days)	850	0	662	75.72	85.68	113.15
Number of services	861	0	14	3.41	2.65	77.70
Dystocia score (0/1)	861	-	-	0.22	0.41	-
Stillbirth score (0/1)	861	-	-	0.09	0.29	-

684 ¹ Proportion of cows experiencing a health event on the week of immune sampling. Measured as a binary trait

685 ² Based on number of distinct episodes per lactation

686 ³ Coefficient of variation

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689 **Table 5.** Results from univariate analysis. Additive genetic (σ_a^2), permanent environmental (σ_{pe}^2), residual (σ_e^2), and
690 phenotypic variances (σ_p^2), with standard errors, are presented for the 9 cellular immune-associated traits. Heritability
691 estimates (h^2), ratio of permanent environmental variance (c^2) and repeatability (R), with standard errors are also
692 provided. Statistically significant values ($P < 0.05$) are given in bold.

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Trait	σ_a^2	σ_{pe}^2	σ_e^2	σ_p^2	h^2	c^2	R
% PBMC ^{1,2}	22.45 (5.828)	3.21 (3.997)	50.42 (1.725)	76.08 (3.510)	0.30 (0.065)	0.04 (0.053)	0.34 (0.030)
% Eosinophils ²	1.18 (0.490)	1.56 (0.423)	4.41 (0.151)	7.16 (0.320)	0.17 (0.065)	0.22 (0.059)	0.38 (0.028)
% Lymphocytes ²	23.66 (5.655)	0.22 (3.679)	44.60 (1.563)	68.48 (3.331)	0.35 (0.071)	0.00 (0.054)	0.35 (0.032)
% Monocytes ²	1.19 (0.416)	0.29 (0.327)	6.52 (0.227)	8.00 (0.298)	0.15 (0.049)	0.04 (0.041)	0.18 (0.026)
% Neutrophils ²	20.62 (5.403)	3.42 (3.766)	51.80 (1.772)	75.85 (3.369)	0.27 (0.064)	0.05 (0.050)	0.32 (0.030)
% CD4 ⁺ ³	8.49 (2.258)	4.26 (1.531)	5.56 (0.193)	18.31 (1.261)	0.46 (0.101)	0.23 (0.090)	0.70 (0.023)
% CD8 ⁺ ³	4.14 (1.126)	3.44 (0.828)	2.45 (0.085)	10.04 (0.677)	0.41 (0.095)	0.34 (0.088)	0.76 (0.018)
CD4 ⁺ :CD8 ⁺ ³	0.21 (0.066)	0.19 (0.048)	0.10 (0.003)	0.50 (0.036)	0.43 (0.112)	0.38 (0.105)	0.81 (0.015)
% NKp46 ⁺ ³	0.55 (0.135)	0.22 (0.092)	0.55 (0.019)	1.33 (0.080)	0.42 (0.085)	0.17 (0.073)	0.59 (0.027)

694 ¹ % Peripheral Blood Mononuclear Cells

695 ² % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

696 ³ % of PBMC that were CD4, CD8 and NKp46 positive

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Table 6. Additive genetic correlations of immune-associated traits with production traits. Significant correlations (P<0.05) are given in bold.

Trait	% PBMC ¹ , ²	% Eosinop hils ²	% Lymphoc ytes ²	% Monocy tes ²	% Neutrop hils ²	% CD4 ⁺ ³	% CD8 ⁺ ³	CD4 ⁺ : CD8 ⁺ ³	% NKp46 ₊₃
Milk (kg)	-0.14 (0.196)	0.25 (0.251)	-0.23 (0.186)	0.02 (0.235)	0.07 (0.202)	-0.01 (0.205)	-0.05 (0.210)	0.18 (0.220)	-0.01 (0.195)
Fat (%)	0.33 (0.147)	0.15 (0.205)	0.36 (0.137)	0.11 (0.187)	-0.35 (0.149)	0.12 (0.159)	0.09 (0.156)	-0.13 (0.162)	0.09 (0.152)
Protein (%)	0.19 (0.151)	0.03 (0.204)	0.19 (0.143)	0.13 (0.187)	-0.18 (0.155)	0.04 (0.162)	-0.06 (0.166)	0.05 (0.175)	0.22 (0.153)
Feed intake (kg)	-0.16 (0.195)	0.19 (0.255)	-0.16 (0.189)	-0.03 (0.234)	0.09 (0.201)	-0.16 (0.193)	-0.06 (0.207)	-0.09 (0.215)	0.30 (0.184)
Dry matter intake (kg)	-0.18 (0.210)	0.25 (0.269)	-0.19 (0.203)	0.01 (0.255)	0.09 (0.217)	-0.23 (0.207)	-0.15 (0.223)	-0.02 (0.236)	0.29 (0.201)
Metabolizable energy intake (MJ)	-0.17 (0.214)	0.24 (0.276)	-0.19 (0.207)	0.01 (0.259)	0.09 (0.221)	-0.26 (0.208)	-0.15 (0.227)	-0.04 (0.239)	0.29 (0.205)
Empty body weight (kg)	-0.32 (0.172)	0.22 (0.215)	-0.26 (0.168)	-0.11 (0.205)	0.25 (0.175)	-0.02 (0.173)	0.33 (0.176)	-0.52 (0.172)	0.18 (0.160)
Live weight (kg)	-0.31 (0.172)	0.23 (0.215)	-0.25 (0.168)	-0.11 (0.205)	0.24 (0.175)	-0.02 (0.173)	0.33 (0.176)	-0.52 (0.172)	0.18 (0.161)
Body condition score (0-5)	-0.17 (0.205)	-0.04 (0.243)	-0.08 (0.194)	-0.21 (0.234)	0.18 (0.208)	0.22 (0.199)	0.22 (0.201)	-0.20 (0.209)	-0.03 (0.186)
Somatic cell count (x10 ³ /ml)	0.17 (0.233)	-0.13 (0.294)	0.09 (0.220)	0.48 (0.262)	-0.14 (0.230)	0.13 (0.226)	0.36 (0.232)	-0.31 (0.238)	-0.03 (0.213)
Feed intake:Milk (ratio)	0.25 (0.028)	-0.03 (0.149)	N.E.	-0.13 (0.166)	-0.19 (0.115)	0.07 (0.009)	0.14 (0.095)	N.E.	0.24 (0.103)
Dry matter intake:Milk (ratio)	N.E.	-0.02 (0.157)	0.33 (0.120)	-0.02 (0.144)	-0.24 (0.028)	0.08 (0.011)	0.06 (0.135)	N.E.	0.31 (0.020)

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¹ % Peripheral Blood Mononuclear Cells

703

² % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

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³ % of PBMC that were CD4, CD8 and NKp46 positive

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Table 7. Additive genetic correlations of immune-associated traits with fertility and health traits. Significant correlations (P<0.05) are given in bold.

Trait	% PBMC ¹ ₂	% Eosinoph ils ²	% Lymphoc ytes ²	% Monocyt es ²	% Neutroph ils ²	% CD4 ⁺ ₃	% CD8 ⁺ ₃	CD4 ⁺ :C D8 ⁺ ₃	% NKp46 ₃
Calving interval (days)	0.07 (0.290)	0.37 (0.388)	-0.12 (0.281)	0.39 (0.332)	-0.14 (0.297)	-0.11 (0.300)	-0.42 (0.329)	0.40 (0.349)	0.18 (0.290)
Days to first heat (days)	0.24 (0.330)	0.32 (0.394)	0.09 (0.304)	0.61 (0.412)	-0.35 (0.355)	-0.36 (0.311)	-0.19 (0.324)	-0.07 (0.333)	-0.20 (0.291)
Days first last heat (days)	0.20 (0.586)	0.63 (0.779)	0.08 (0.531)	0.43 (0.836)	-0.36 (0.702)	-0.50 (0.718)	-0.84 (1.532)	0.87 (1.526)	0.07 (0.549)
Number of heats	0.20 (0.306)	0.40 (0.388)	0.10 (0.295)	0.42 (0.376)	-0.28 (0.315)	-0.10 (0.322)	-0.23 (0.359)	0.42 (0.386)	0.09 (0.304)
Days to first service (days)	0.22 (0.285)	0.28 (0.344)	0.06 (0.268)	0.56 (0.358)	-0.30 (0.297)	0.03 (0.286)	0.17 (0.286)	-0.08 (0.303)	-0.23 (0.264)
Days first last service (days)	0.15 (0.741)	0.72 (1.067)	0.01 (0.640)	0.39 (1.107)	-0.37 (1.062)	-0.63 (0.949)	N.E.	N.E.	0.57 (2.150)
Number of services	0.17 (0.295)	0.38 (0.375)	0.09 (0.284)	0.36 (0.352)	-0.25 (0.303)	-0.16 (0.309)	-0.28 (0.349)	0.40 (0.366)	0.17 (0.301)
Dystocia score (0/1)	-0.13 (0.237)	-0.06 (0.308)	-0.03 (0.231)	-0.14 (0.275)	0.11 (0.241)	-0.29 (0.234)	-0.22 (0.254)	-0.02 (0.264)	0.23 (0.243)
Stillbirth score (0/1)	-0.10 (0.330)	-0.67 (0.368)	-0.01 (0.310)	-0.42 (0.397)	0.27 (0.352)	0.22 (0.330)	0.44 (0.344)	-0.32 (0.369)	0.61 (0.278)
Mastitis	-0.07 (0.377)	0.63 (0.360)	-0.03 (0.379)	0.10 (0.498)	-0.27 (0.514)	-0.32 (0.355)	0.14 (0.399)	0.09 (0.457)	0.03 (0.362)
Lameness	0.02 (0.267)	-0.39 (0.283)	-0.01 (0.259)	0.01 (0.308)	0.10 (0.261)	-0.05 (0.277)	0.08 (0.267)	-0.08 (0.287)	0.06 (0.252)
Other condition	-0.53 (0.606)	N.E.	-0.38 (0.658)	-0.65 (0.728)	0.24 (0.598)	0.01 (0.666)	0.53 (0.642)	-0.47 (0.874)	-0.22 (0.660)
Mastitis episodes	0.05 (0.406)	0.23 (0.478)	-0.06 (0.369)	0.66 (0.571)	-0.12 (0.431)	-0.10 (0.395)	0.21 (0.401)	-0.08 (0.419)	0.05 (0.373)
Lameness episodes	-0.02 (0.236)	0.27 (0.337)	-0.06 (0.228)	0.05 (0.281)	-0.01 (0.236)	-0.15 (0.243)	-0.51 (0.261)	0.47 (0.266)	0.28 (0.235)

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¹ % Peripheral Blood Mononuclear Cells

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² % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

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³ % of PBMC that were CD4, CD8 and NKp46 positive

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715 **Table 8.** Phenotypic correlations of immune-associated traits with production, health and fertility traits. Significant
 716 correlations (P<0.05) are given in bold
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Trait	% Eosinophils ²	% Monocytes ²	% Neutrophils ²	% CD4 ⁺ ₃	CD4 ⁺ :CD8 ₊	% NKp46 ⁺ ₃
Milk (kg)	-0.04 (0.033)	0.08 (0.029)	-0.01 (0.032)	0.04 (0.039)	0.03 (0.040)	0.02 (0.037)
Empty body weight (kg)	0.08 (0.039)	-0.08 (0.034)	0.01 (0.038)	-0.08 (0.049)	-0.15 (0.049)	-0.02 (0.046)
Live weight (kg)	0.08 (0.039)	-0.08 (0.034)	0.01 (0.038)	-0.08 (0.049)	-0.16 (0.049)	-0.02 (0.046)
Body condition score (0-5)	0.06 (0.033)	-0.06 (0.029)	-0.03 (0.032)	-0.07 (0.040)	-0.11 (0.041)	-0.10 (0.037)
Somatic cell count (x10 ³ /ml)	0.04 (0.031)	-0.05 (0.028)	0.05 (0.031)	-0.00 (0.037)	-0.05 (0.038)	-0.01 (0.035)
Feed intake:Milk (ratio)	0.01 (0.018)	-0.01 (0.023)	-0.01 (0.018)	-0.01 (0.012)	-0.02 (0.000)	0.01 (0.018)
Dry matter intake:Milk (ratio)	0.01 (0.020)	-0.00 (0.020)	-0.01 (0.000)	-0.01 (0.000)	N.E.	0.02 (0.014)
Calving interval (days)	-0.05 (0.044)	0.10 (0.036)	0.01 (0.042)	-0.01 (0.053)	0.03 (0.055)	-0.00 (0.049)
Days first second service (days)	-0.04 (0.006)	N.E.	0.04 (0.035)	-0.14 (0.046)	-0.02 (0.050)	0.01 (0.044)
Lameness	0.03 (0.021)	0.01 (0.022)	-0.01 (0.021)	0.05 (0.021)	0.02 (0.020)	-0.02 (0.021)
Other condition	-0.04 (0.019)	-0.01 (0.020)	0.02 (0.020)	0.03 (0.019)	0.03 (0.018)	0.04 (0.019)

718 ¹ % Peripheral Blood Mononuclear Cells

719 ² % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

720 ³ % of PBMC that were CD4, CD8 and NKp46 positive