Genetic evaluation of differential somatic cell count in Italian Holstein cattle

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Abstract

Mastitis is a prevalent inflammatory condition affecting udder tissue in dairy cows. It leads to reduced milk production, increased veterinary costs and potential culling of affected animals, impacting both animal welfare and economic outcomes in dairy farming. Recently introduced as a supplementary measure to somatic cell count (SCC), the differential somatic cell count (DSCC) is an innovative indicator for intramammary infection. DSCC quantifies the proportion of polymorphonuclear neutrophils plus lymphocytes (PMN-LYM) within milk somatic cells, providing enhanced insights into udder health status and infection severity. The aim of this study was to estimate genetic parameters and develop a genetic evaluation of DSCC in Italian Holstein. An innovative, new categorical phenotype, named state of infection (SI), was created from each test-day, combining SCC and DSCC records. Values from 1 to 4 were assigned to the different test-day records based on two thresholds related to the parity order: 100,000 SCC and 60% DSCC for first parity cows; 200,000 SCC and 65% DSCC for later parity cows. Observations with both SCC and DSCC below the respective threshold were assigned a value of 1; SCC below and DSCC above were assigned to category 2; both above to category 3; SCC above and DSCC below to category 4. A multiple-trait repeatability linear animal model was applied to the two traits, with year-month-parity-region of recording, herd-parity of recording, parity-age at calving-year-region and parity-days in milk-year-region as fixed effects. Random effects included herd-test-day-parity of recording, herd-year-month-parity of calving, animal additive genetic, and permanent environment. The posterior mean (PM) for heritability was 0.13 for SCS (posterior standard deviation, PSD: 0.01) and 0.09 (0.01) for SI. The genetic correlation between SCS and SI was 0.94, highlighting the strong relationship between the two traits but also their differences. A SNPBLUP model was applied for estimating genomic breeding values (GEBV) using either a reference population composed of bulls or of both bulls and cows (mixed reference population). For the validation of GEBV, a three-year back cutoff date for phenotypes was used: the results highlighted the positive impact of a mixed reference population on dispersion and accuracy. The genetic trend based on bulls' GEBV indicates that the undergone selection for SCS indirectly improved the population also for SI. In conclusion, this study confirmed the possibility to select for SI in Italian Holstein population and provided the bases for the implementation of a routine genetic evaluation for this innovative udder health trait.

Key words: mastitis, dairy cattle, genomic selection, infection, health, genetic parameters

Introduction

Mastitis is one of the most relevant diseases in dairy farming, with negative consequences on farm net profit and animal welfare (Seegers et al., 2003): its subclinical form (subclinical mastitis, SCM), when there are no visible signs of inflammation, can lead to an undetectable spread of mastitis, resulting in significant economic loss (Halasa et al., 2007).

Early detection of an ongoing inflammatory process can strongly mitigate the adverse outcomes of mastitis. Historically, somatic cell count (SCC) or its log-transformation (somatic cell score, SCS) has been the main indicator of SCM, with 200,000 cell/ml as threshold (Sharma et al., 2011). SCS is used as a proxy for mastitis resistance due to: i) its high genetic correlation with clinical mastitis, ii) its higher heritability, and iii) its possibility to be routinely measured within the national milk recording system on a large scale and at a cost effective.

Nowadays, a novel indicator of inflammation is available, differential somatic cell count (DSCC) (Bobbo et al., 2018). DSCC is the percentage of polymorphonuclear neutrophils and lymphocytes (PMN-LYM) within milk somatic cells. A high level of DSCC indicates an active immune response in the mammary gland (Damm et al., 2017).

Using DSCC independently from SCC can be misleading; indeed, an animal with low DSCC but high SCC cannot be safely classified as healthy. For this reason, a new phenotype was analyzed in this study: the state of infection (SI), regarded as the relationship between DSCC and SCC, as proposed by Bobbo et al. (2020).

The objective of this study is to evaluate the feasibility of selecting for SI, the genetic trend occurring in Italian Holstein and the possibility of including females in the reference population for SNP effects estimation.

Materials and Methods

Data editing

Test-day data came from the official national milk recording system within the LEO project (PSRN mis 16.2, AIA, 2023).

Records from parity 1 to parity 5 and from 5 to 405 days in milk (DIM) were considered. Minimum age at first calving was set to 18 while the maximum admitted value for age at calving was 100 in parity 5.

Regarding TD records, the first recording of the lactation had to be within 60 DIM while the maximum allowed distance between consequent TD records was 70 days.

For the phenotypes, DSCC records out of the range 25 to 95% were deleted. SCC was log-transformed to somatic cell score (SCS) following Ali & Shook (1980) but adding 4 instead of 3 as in Martins et al. (2010), in order to have less records below 0: allowed values for SCS ranged from 0 to 10, with the lower bound not included in the range. From the relationship between SCC and DSCC a new phenotype was derived: two different parity-dependent thresholds were identified for both SCC and DSCC. For SCC, the thresholds were 100,000 cells/ml for first parity cows and 200,000 cells/ml for later ones. Regarding DSCC, the threshold for primiparae was 60% while for pluriparae was 65% (Bobbo et al., 2019a, Bobbo et al., 2019b, Zidi et al., 2019). Four categories were then identified, from best (1) to worst (4):

- Category 1, healthy: both parameters below the respective thresholds
- Category 2, at risk: SCC below while DSCC above the threshold
- Category 3, ongoing mastitis: both parameters above their respective thresholds
- Category 4, chronic: DSCC below while SCC above the threshold

The minimum number of contemporaries in contemporary groups (CG) was set to 5 and other constraints were applied in order to maintain a consistent numerosity per level of the fixed effects included in the model.

Finally, to be included in the analysis, TD records had to have both SCS and DSCC recorded.

The dataset after edits was composed of 8 million records.

Statistical model

A multiple trait repeatability linear animal model was applied, with SCS and SI as correlated dependent variables.

The model for both traits was the following:

$$Y_{ijklmnopqr} = htdp_i + hymp_j + S_k{*}Y_l + H_m + DIM_n{*}PARC_o{*}Y_l + AGEC\_PAR_p{*}Y_l + a_q + pe_q + e_{ijklmnopqr}$$

with $Y_{ijklmnopqr}$ as the r*th* phenotypic observation of DSCC or SI. Fixed effects were $S_k{*}Y_l$ as the crossed effect of season k by year l, H_m as the m*th* herd of recording, $DIM_n{*}PARC_o{*}Y_l$ as the n*th* days in milk class (10 classes of 40 days) by parity class o (3 classes: 1, 2, 3+) and year l, $AGEC\_PAR_p{*}Y_k$ as the p*th* age at calving by parity class (9 classes: 3 age at calving classes for every parity class) by year k. Random effects were $htdp_i$ as the i*th* contemporary group for herd-parity_class-date_of_recording, $hymp_j$ as the j*th* contemporary group for herd-parity_class-month_of_calving, a_q as the additive genetic effect of the q*th* animal, pe_q as the permanent environmental effect of the q*th* animal and $e_{ijklmnopqr}$ as the residual of observation r.

Variance components estimation, genetic and genomic evaluation

Variance components estimation was performed with the software THRGIBBS1F90 (Misztal et al, 2002) on a sample of 279,896 records on 26,168 animals located in 200 herds. The pedigree was traced back to 4 generations and was composed of 74,037 individuals. Convergence was assessed with R package BOA, Bayesian output analysis (Smith, 2007). Conventional estimated breeding values (EBVs) were obtained with MiX99 software (MiX99 Development Team, 2012). Genomic evaluation was performed with a SNPBLUP model using GS3 software (Legarra et al, 2011). For estimated deregressed proofs (EDPs), the method from Degano et al (2009) was applied. A conventional quality control was applied to SNP data. For the imputation process, PedImpute software was used (Nicolazzi et al, 2013).

Table 1. Results of variance components estimation.

	SCS	SI
SCS	0.13 (0.01)	0.94
SI	0.77	0.09 (0.01)

Genomic validation

Genomic validation followed the method described in Finocchiaro et al (2012) and Galluzzo et al (2022). Briefly, two datasets were used for EBVs estimation: one full (with records up to the 2404 run) and one reduced (with a 3-years back cutoff date). For both sets of EBVs, EDPs were calculated and used as pseudo-phenotypes for SNP effects estimation. Bulls with daughters in the full datasets but without in the reduced one were selected as validation bulls. Finally, a linear regression with EDPs of validation bulls from the full run as dependent variable and their direct genomic values (DGVs) from the reduced run as the independent one was fitted. The validation process was performed either using a training population composed of bulls only and of bulls and cows. Parameters considered for the comparison were the dispersion coefficient and the reliability of the linear regression model.

Results & Discussion

The dataset after edits was composed of 8 million records with phenotypes averaging 3.73% for SCS, 1.59% for SI, and 62.79% for DSCC, respectively. The results of variance components estimation are listed in Table 1. The posterior mean for heritability was moderate for both SCS and SI: the genetic correlation between the two traits was high reflecting also the phenotypic one.

Posterior means of heritability on diagonal with posterior standard deviations in parentheses, genetic correlation above diagonal and phenotypic correlation below.

The results of genomic validation for SI are listed in Table 2. Including females significantly increased the number of animals in the reference population and improved

reliability. Furthermore, its inclusion reduced the deviation of the dispersion coefficient from the expected 1. The mixed training population performed better than the one composed only of bulls for both parameters: dispersion coefficient and model reliability.

Table 2. Results of genomic validation.

	Training	Animals	b	r^2
SI	B	3,030	1.272	0.30
	M	136,763	0.950	0.60

B=bulls only; M=mixed; b=dispersion coefficient; r^2=model reliability.

Using a mixed reference population decreased the distance from 1 by 82% while doubling reliability. These results suggest that a mixed reference population composed by both bulls and cows would be beneficial for SI and thus was applied for the subsequent analyses.

The genetic trend of bulls' GEBVs by birth year is represented in Figure 1: an increasing trend is evident and may be due to the undergone selection for correlated traits like SCS.

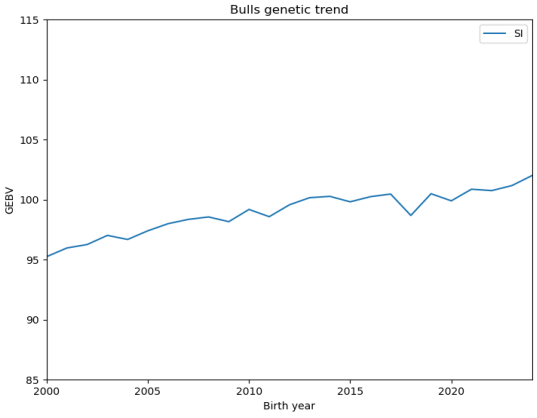

Figure 1. Bulls' genetic trend by birth year. GEBV=average GEBV.

Conclusions

In conclusion, this study demonstrated the possibility of genetically improving Italian Holstein for SI. It underscored the benefits of using a mixed reference population for SNP effects estimation. Moreover, selecting for correlated traits like SCS was effective to indirectly improve the population for SI. Based on these results, a routine genetic evaluation for SI in Italian Holstein will be developed and implemented. The SI is a powerful tool to help farmers make better decisions at the management and genetic level, thereby reducing the use of antimicrobials on the farm.

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